Inhibitory Effect of α-Glucosidase Inhibitors Varies According to Its Origin

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The inhibitory effect of α-glucosidase (AGH) inhibitors against its origins (baker's yeast and rat, rabbit, and pig small intestines) was investigated. All inhibitors used in this study showed quite different inhibitory activities according to AGH origins. Voglibose, acarbose and glucono-1,5-lactone strongly inhibited mammalian AGHs, whereas no or less inhibition was observed in yeast AGH. On the contrary, (+)-catechin, a good inhibitor against yeast AGH ($IC_{50} = 1.3 \times 10^{-1}$ mM) as well as voglibose ($IC_{50} = 2.6 \times 10^{-2}$ mM), did not retard the mammalian AGH activity. Subsequent inhibition study with various food components revealed that all of foods except for green (IC_{50} = 0.735 mg/mL) and oolong teas (IC_{50} = 1.34 mg/mL) showed no inhibitory activity against rat AGH, whereas they inhibited yeast AGH. Consequently, the magnitude of AGH inhibition was greatly affected by its origin, and more attention relating to AGH origin would be needed to evaluate in vitro AGH inhibitory effect.

Keywords: *α-Glucosidase; inhibition; non-insulin-dependent diabetes mellitus*

INTRODUCTION

A series of our studies concerning the prophylaxis on diabetes by food have been focused on the inhibition of α-glucosidase (AGH), exo-type α-D-glucoside *O*-linkage hydrolase (Chiba, 1997), to prevent excess glucose absorption at the small intestine (Matsui et al., 1996). We have already reported that *Bacillus licheniformis* alkaline protease hydrolysate derived from sardine muscle was one of the most appropriate food inhibitors, having a relatively high AGH inhibitory activity (IC_{50} = 0.487 mg/mL) as well as green and oolong teas (IC_{50} = 0.111 and 0.113 mg/mL, respectively). As a result of HPLC purifications of the hydrolysate, two peptides, Tyr-Tyr-Pro-Leu and Val-Trp (IC_{50} = 3.73 and 22.6 mM, respectively), were found to be involved in AGH inhibition (Matsui et al., 1999).

Similar attempts have been also done by other researchers, in which many natural (Miura et al., 1996; Watanabe et al., 1997; Nishioka et al., 1997) or synthetic compounds (Kameda et al., 1984) with in vitro AGH inhibitory activity have been already identified. However, most of these inhibition studies were done by using baker's yeast AGH, not mammalian small intestinal AGH. As Chiba (1997) has pointed out, AGH may be largely divided into two families, types I (baker's yeast) and II (mammals), on the basis of the difference in primary structure. This leads us to the assumption that the inhibitory effect against AGH would vary between both families, although no systematic studies have been performed.

Thus, in this study, we tried to clarify whether inhibition of a given inhibitor against AGH was affected by its origin or not.

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MATERIALS AND METHODS

Materials. α-Glucosidase (AGH, EC 3.2.1.20) from baker's yeast (type I) and intestinal acetone powder from rat, rabbit, and pig were purchased from Sigma Chemical Co. (St. Louis, MO). *p*-Nitrophenyl α-D-glucopyranoside (PNP-G) as a synthetic substrate was obtained from E. Merck (Darmstadt, Germany). Sardine muscle was supplied by Senmi Ekisu Co. (Ozu, Japan) and was subjected to hydrolysis by *B. licheniformis* alkaline protease (Novo Co., 2.4L, type FG, Chiba, Japan) (Matsui et al., 1996). Five foodstuffs used in this study (green tea, oolong tea, chicken essence, yogurt, unshu mikan (*Citrus unshu*), and fish sauce) were obtained from commercial sources. Voglibose (BASEN, 0.2 mg/tablet) and acarbose (Glucobay, 50 mg/tablet) as a synthetic AGH inhibitor were obtained from Takeda Medical Co. (Osaka, Japan) and Bayer Medical Co. (Leverkusen, Germany), respectively. Other reagents were of analytical grade and used without further purification.

Preparation of AGH Solution from Intestinal Acetone Powder. AGH solution was prepared by a partial modification of the procedure reported by Asano et al. (1996). Namely, 100 mg of intestinal acetone powder was added to 3 mL of 0.9% NaCl solution and homogenized with the sonication equipment for 30 s 12 times in ice bath. After centrifugation at 10000*g* for 30 min, the resulting supernatant was directly subjected to inhibitory assay.

Assay for AGH Inhibitory Activity and Kinetics Measurement. The AGH inhibitory activity was assayed according to the procedure described elsewhere, unless otherwise specified (Matsui et al., 1996). In the case of AGH prepared from intestinal acetone powder, slight modification was done: the substrate solution (0.1 M phosphate buffer), adjusted to pH 6.8, was used as a model of intestinal fluid described in the Japanese Pharmacopoeia (JP XII). In this study, the substrate concentration was set at about twice the apparent K_m value due to the variation of K_m value with the substrate used. Namely, the concentrations were 45 mM for sucrose, 6 mM for maltose, and 1.2 mM for PNP-G unless otherwise specified. The AGH activity was determined by monitoring the *p*-nitrophenol released from PNP-G at 400 nm or glucose liberated from maltose or sucrose by the glucose C II-Test Wako (Wako Pure Chemical Institute, Co., Osaka, Japan) or

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Table 1. Inhibitory Effect of Various Inhibitors against α-Glucosidase from Baker's Yeast and Mammalian Small Intestines

origin	IC_{50}[a] (mM) voglibose	acarbose	(+)-catechin	glucono-1,5-lactone
baker's yeast	2.6×10^{-2} [b]	NI[c]	1.3×10^{-1} [b]	NI
rat small intestine	7.3×10^{-5}	6.3×10^{-2}	NI	2.5
rabbit small intestine	1.4×10^{-4}	6.2×10^{-2}	NI	9.5×10^{-1}
pig small intestine	1.7×10^{-6}	8.7×10^{-2}	NI	1.4×10^{-1}

[a] IC_{50} value is defined as the concentration of α-glucosidase inhibitor to inhibit 50% of its activity under the assayed conditions, and 0.7 mM *p*-nitrophenyl-α-D-glucopyranoside (PNP-G) was used as a substrate in this assay. [b] Matsui et al. (1996). [c] NI, no inhibition.

F-kit D-glucose (Boehringer Mannheim GmbH, Mannheim, Germany). The concentration of AGH inhibitor required to inhibit 50% of the AGH activity under the assay conditions was defined as the IC_{50} value.

In the kinetic measurement, the ranges of final substrate concentrations were 0.25−2 mM for PNP-G, 2.5−40 mM for sucrose, and 0.5−5 mM for maltose. The experimentally obtained data (average values from three determinations) were processed in terms of Michaelis−Menten kinetics. The apparent K_m value was calculated from double-reciprocal Lineweaver−Burk plots 1/s versus 1/*v* (where *s* is the final substrate concentration and *v* the rate of enzymatic reaction).

RESULTS AND DISCUSSION

Primarily, the effect of AGH origin (baker's yeast and rat, rabbit, and pig small intestines) on the inhibition of four known inhibitors was investigated (Table 1). As summarized in Table 1, all of the inhibitors used in this study gave a variety of AGH inhibition behavior according to its origin. Voglibose and acarbose, which are known to be excellent in vivo AGH inhibitors (Bischoff, 1994; Okada et al., 1992), had 10^{-4} to 10^{-2} times lower or no ability to inhibit AGH from baker's yeast relative to that from small intestines. A similar result was obtained for glucono-1,5-lactone that was identified as a inhibitor against rabbit AGH by Cogoli and Semenza (1975), in which it inhibited only mammalian AGHs. The poor inhibitory effect against baker's yeast AGH compared to mammalian AGHs agreed with the results of Kameda et al. (1984), who found that aminocyclitol from *Streptomyces hygroscopicus* fermentation broth and its derivatives showed more potent inhibitory activity against pig AGH than against baker's yeast. Among the mammalian AGHs, pig AGH was strongly inhibited by voglibose and glucono-1,5-lactone; however, acarbose had a 20−100-fold higher sensitivity relative to the other AGHs. Takeuchi et al. (1990) reported that the apparent K_m value of pig AGH was 2-fold lower than that of rat AGH, indicating that the highly sensitive inhibitory effect of pig AGH obtained in this study would be responsible for the difference in the affinity of substrate with enzyme. On the contrary, although (+)-catechin was a good inhibitor against baker's yeast with an AGH inhibitory activity of $IC_{50} = 1.3 \times 10^{-1}$ mM as well as voglibose ($IC_{50} = 2.6 \times 10^{-2}$ mM) (Matsui et al., 1996), it showed no inhibitory action against mammalian AGHs. This indicates that (+)-catechin would have no physiological function relating to the prevention of glucose absorption at the small intestine within our experimental conditions. These findings, together with the fact that the oral administration of acarbose in non-insulin-dependent diabetes subjects was allowed to moderate the postprandial blood glucose level (Toeller, 1994), strongly suggested that the conventional AGH inhibitory study against baker's yeast may not give us any practical information concerning prevention of glucose absorption. Although pig AGH activity was potently retarded by inhibitors used in this study, our subsequent experiments were performed by using rat AGH, because of easy development for in vivo study by rat rather than pig AGH (Asano et al., 1996; Bischoff, 1994; Okada et al., 1992).

Table 2. Kinetic Constant and α-Glucosidase Inhibitory Activity in Rat α-Glucosidase

substrate	apparent K_m (mM)	IC_{50} (mM) voglibose	glucono-1,5-lactone	D-xylose	(+)-catechin
PNP-G[a]	0.6	6.8×10^{-5}	13.2	NI[b]	NI
maltose	3.0	1.3×10^{-5}	8.8		2.7
sucrose	22.8	1.3×10^{-5}	9.8	2.8	2.7

[a] PNP-G; *p*-nitorophenyl α-D-glucopyranoside. [b] NI, no inhibition.

Following this, effects of substrates on rat AGH inhibitions by voglibose, glucono-1,5-lactone for a competitive inhibitor, D-xylose (Asano et al., 1996) for an uncompetitive one, and (+)-catechin were investigated. Table 2 summarized the apparent Michaelis constants (K_m) calculated by Lineweaver−Burk plots in Figure 1 and the IC_{50} values of the inhibitors in each substrate. The apparent K_m value varied with substrate, the smallest value of 0.6 mM being obtained in PNP-G; in other words, PNP-G had as much as 40 times higher affinity for rat AGH than sucrose (K_m = 22.8 mm). In addition, different IC_{50} values of voglibose and glucono-1,5-lactone were obtained in each substrate. Thus, by considering that maltose is the specific substrate for maltase as well as sucrose for sucrase, the use of PNP-G would be valid for evaluating the overall inhibitory effect by food components against rat AGH composed of a variety of carbohydrases on the surface of small intestines. However, because D-xylose that inhibited sucrase activity uncompetitively (Asano et al., 1996) showed no inhibition in PNP-G, the use of PNP-G would not be appropriate for screening uncompetitive inhibitors. Furthermore, (+)-catechin showed inhibition against rat AGH using maltose and sucrose as a substrate, whereas no inhibition was observed when using PNP-G. Therefore, (+)-catechin might be an uncompetitive inhibitor like D-xylose.

Finally, the inhibition study of various foods being active for baker's yeast AGH (Matsui et al., 1996) was reinvestigated by using rat small intestinal AGH (Table 3). Apparently, the IC_{50} values of all foods used in this study for rat AGH were much higher than those for baker's yeast. Although green and oolong teas still inhibited both AGHs, their inhibitory activities against rat AGH decreased by $^1/_7$ and $^1/_{12}$, respectively, compared with those against baker's yeast AGH. Matsumoto and Hara (1992), however, have demonstrated that the increase in blood glucose level after a meal was significantly suppressed by administering green tea extract (60 mg) to rat. Thus, by considering our finding that the IC_{50}value of green tea was 0.735 mg/mL, the in vivo inhibitory effect against rat AGH might be comparable to the suppressing effect of the postprandial hyperglycemia in vivo. A remarkable loss of rat AGH inhibitory activity was observed in sardine muscle hydrolysate as

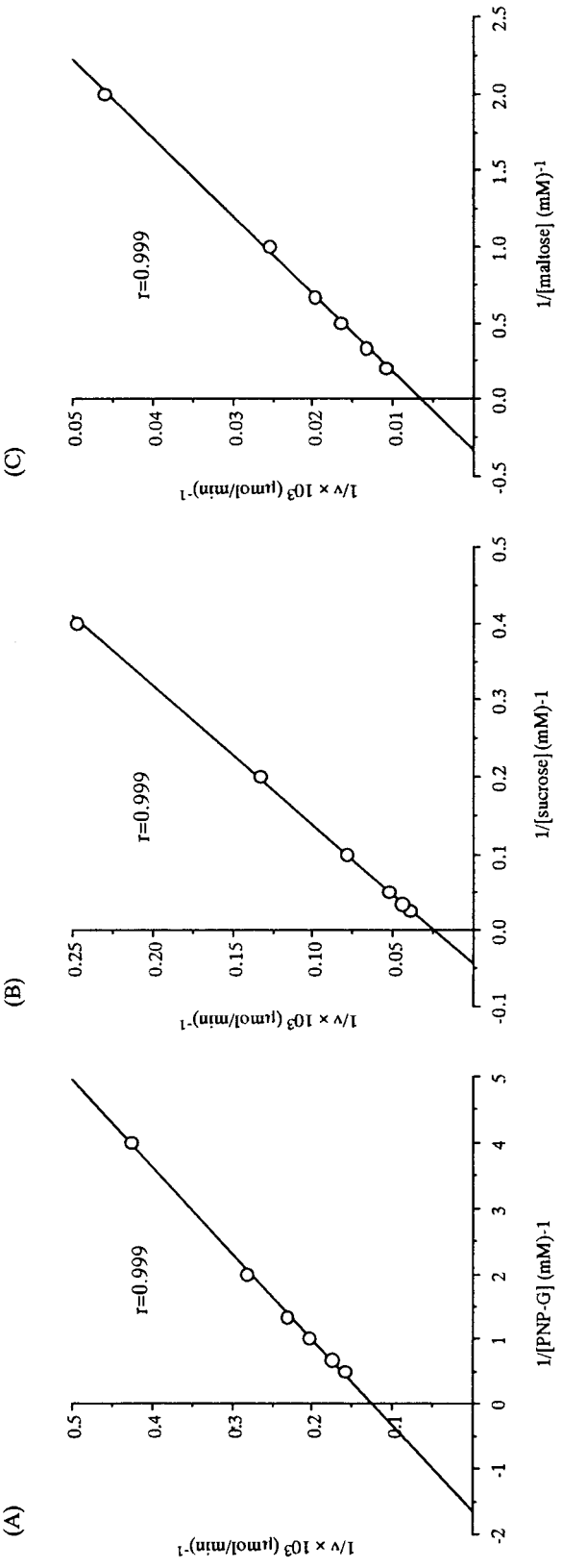

Figure 1. Lineweaver−Burk plots of activities of AGH from rat small intestine. The activities were measured by using (A) PNP-G, (B) sucrose, and (C) maltose as a substrate.

Table 3. Comparison of Inhibition by Various Foods against Baker's Yeast with Rat Small Intestine

	IC_{50}[a] (mg/mL)	
	baker's yeast (K_m = 0.36 mM)[c]	rat small intestine (K_m = 0.60 mM)
green tea	0.111[b]	0.735
oolong tea	0.113[b]	1.34
alkaline protease hydrolysate from sardine muscle	0.487[b]	3.45×10^2
yorgurt	5.20[b]	NI[e]
chicken essence	4.71[b]	NI
Citrus unshu	9.44[b]	NI
fish source	17.4[b]	NI
Val-Trp[d]	22.6 mM	NI
Tyr-Tyr-Pro-Leu[d]	3.73 mM	NI

[a] IC_{50} value was evaluateed by using *p*-nitrophenyl-α-D-glucopyranoside (PNP-G) as a substrate in this assay. [b] Matsui et al. (1996). [c] The K_m value was determined uisng PNP-G, ranging from 0.1 to 1.0 mM, with three replications. [d] These peptides were isolated from alkaline protease hydrolysate (Matsui et al., 1999). [e] NI, no inhibition.

well as other foods, indicating that the AGH inhibitory effect may be overestimated when baker's yeast AGH is subjected to the assay. On the basis of the unexpected result that the baker's yeast AGH inhibitory peptides, Tyr-Tyr-Pro-Leu (IC_{50} = 3.73 mM) and Val-Trp (IC_{50} = 22.6 mM) (Matsui et al., 1999), also did not retard the rat AGH activity, an AGH inhibition study should be done for mammalian or rat AGH to clarify AGH inhibitors with in vivo prophylaxis effect on diabetes.

ABBREVIATIONS USED

AGH, α-glucosidase; PNP-G, *p*-nitrophenyl α-D-glucopyranoside; HPLC, high-performance liquid chromatography.

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LITERATURE CITED

Asano, T.; Yoshimura, Y.; Kunugita, K. Sucrase Inhibitory Activity of D-Xylose and Effect on the Elevation of Blood Glucose in Rats. *J. Jpn. Soc. Nutr. Food Sci.* **1996**, *49*, 157−162 (in Japanese).

Bischoff, H. Pharmacology of α-Glucosidase Inhibition. *Eur. J. Clin. Invest.* **1994**, *24*, 3−10.

Chiba, S. Molecular Mechanism in α-Glucosidase and Glucoamylase. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1233−1239.

Cogoli, A.; Semenza, G. A Probable Oxocarbonium Ion in the Reaction Mechanism of Small Intestinal Sucrase and Isomaltase. *J. Biol. Chem.* **1975**, *250*, 7802−7809.

Kameda, Y.; Asano, N.; Yoshikawa, M.; Takeuchi, M. Valiolamine, A New α-Glucosidase Inhibiting Aminocyclitol Produced by *Streptomyces hygroscopicus*. *J. Antibiot.* **1984**, *37*, 1301−1307.

Matsui, T.; Yoshimoto, C.; Osajima, K.; Oki, T.; Osajima, Y. In Vitro Survey of α-Glucosidase Inhibitory Food Components. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 2019−2022.

Matsui, T.; Oki, T.; Osajima, Y. Isolation and Identification of Peptidic α-Glucosidase Inhibitors Derived from Sardine Muscle Hydrolyzate. *Z. Naturforsch.* **1999**, in press.

Matsumoto, N.; Hara, Y. Prophylaxis of Obesity and Effect of Suppressing Digestion by Tea Catechins. *Food Ind.* **1992**, *34*, 26−31 (in Japanese).

Miura, M.; Gomyo, T. Effect of Commercial Spices on the Activities of α-Amylase and α-Glucosidase. *J. Jpn. Soc. Food Sci. Technol.* **1996**, *43*, 157−163 (in Japanese).

Nishioka, T.; Watanabe, J.; Kawabata, J.; Niki, R. Isolation and Activity of *N*-*p*-Coumaroyltyramine, an α-Glucosidase Inhibitor in Welsh Onion (*Allium fistilosum*). *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1138−1141.

Odaka, H.; Miki, N.; Ikeda, H.; Matsuo, T. Effect of a Disaccharidase Inhibitor, AO-128, on Postprandial Hyperglycemia in Rats. *J. Jpn. Soc. Nutr. Food Sci.* **1992**, *45*, 27−31 (in Japanese).

Takeuchi, M.; Takai, N.; Asano, N.; Kameda, Y.; Matsui, K. Inhibitory Effect of Validamine, Valienamine and Valiolamine on Activities of Carbohydrases in Rat Intestine Brush Border Membranes. *Chem. Pharm. Bull.* **1990**, *38*, 1970−1972.

Toeller, M. α-Glucosidase Inhibitors in Diabetes: Efficacy in NIDDM Subjects. *Eur. J. Clin. Invest.* **1994**, *24*, 31−35.

Watanabe, J.; Kawabata, J.; Kurihara, H.; Niki, R., Isolation and Identification of α-Glucosidase Inhibitors from Tochucha. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 177−178.

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