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Fundamental studies on the inhibitory action of *Acanthopanax senticosus* Harms on glucose absorption

Kazuhiro Watanabe[a,*], Keiko Kamata[a], Juichi Sato[a], Tunehisa Takahashi[b]

[a] Hokkaido Pharmaceutical University School of Pharmacy, 7-1 Katsuraoka-cho, Otaru, Hokkaido 047-0264, Japan
[b] Yakuhan Pharmaceutical Co. Ltd., 27 Kitanosato, Kitahirosima, Hokkaido 061-1111, Japan

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ABSTRACT

Aim of the study: *Acanthopanax senticosus* Harms extract (ASE) is used as an ingredient of over-the-counter drugs and functional foods, such as health supplements, in Japan. ASE exhibits a hypoglycemic effect; however, the mechanism of the hypoglycemic effect is not clear. In the present study, we investigated whether ASE has a glucose absorption inhibitory action.
Materials and methods: We examined the effects of ASE on α-amylase and α-glucosidase activities, and on glucose uptake in Caco-2 cells. We also examined the effects of ASE oral administration on glucose tolerance in type 2 diabetes mellitus model db/db mice.
Results: The addition of ASE inhibited α-glucosidase activity but not α-amylase activity. The α-glucosidase inhibitory activity of ASE was approximately 1/13 of that of acarbose. The addition of ASE inhibited 2′-deoxy-D-glucose (DG) uptake in human intestinal Caco-2 cells, and the inhibitory activity of ASE was approximately 1/40 of that of phloretin. Kinetic analysis of glucose uptake indicated that ASE has no effects on DG uptake through passive diffusion, but that ASE inhibits intracellular DG uptake chiefly by inhibiting transport via a glucose transporter. In the glucose tolerance study, db/db mice orally administered ASE for 3 days showed significantly lower plasma glucose level than the control group 30 min after sucrose loading, without affecting plasma insulin levels. In addition, ASE oral administration significantly inhibited α-glucosidase activity in the small intestine mucosa extirpated from the mice.
Conclusion: These findings indicate that ASE may be useful as an ingredient of functional foods to improve postprandial hyperglycemia and prevent type II diabetes mellitus.

1. Introduction

Diabetes mellitus is a metabolic disease that is characterized by chronic hyperglycemia and a lack of the absolute or relative effects of insulin. Diabetes mellitus is classified into four types by etiology (Herman et al., 1984). Type II diabetes mellitus patients account for more than 90% of all diabetes mellitus patients in Japan, and their number is increasing rapidly. Type II diabetes mellitus is accompanied by hyperglycemia, glucose intolerance, obesity, lipid abnormality, hyperinsulinemia, and insulin resistance (DeFronzo, 1987). In addition to genetic factors, it is well known that lifestyle-related factors, such as overeating, obesity, lack of exercise, and stress, are associated with the onset of type II diabetes mellitus.

Diet and exercise are generally the first choice of treatment for type II diabetes mellitus. If diet and exercise treatments fail, antidiabetic agents are administered to control blood glucose level. As the antidiabetic agents (Mizuno et al., 2008) for type II diabetes mellitus, α-glucosidase inhibitors, sulfonylureas, biguanides, thiazolidinediones, and insulin formulations are available. On the other hand, various functional foods, including foods for specific health use approved by the Consumer Affairs Agency, Government of Japan, are used widely from the viewpoint of self-medication and primary care. Indigestible dextrin (Choi et al., 1998), guava tea polyphenol (Mai et al., 2007; Gutiérrez et al., 2008), wheat albumin (Kodama et al., 2005), L-arabinose (Seri et al., 1996), and Touchi extract (Fujita et al., 2001) are useful health foods for diabetes patients in Japan. These food products inhibit the absorption of monosaccharides in the small intestine, thereby suppressing the increase in blood glucose level after a meal.

Acanthopanax senticosus Harms (AS) is distributed in eastern Hokkaido, Korean Peninsula, northern China, and Siberia. Similar to Korean ginseng, AS belongs to Araliaceae. Isofraxidin, eleutherosides, senticosides, and chlorogenic acid are found in AS (Tokiwa et al., 2006). In China, ASE has been used ever since as a nutritional supplement and a sedative. In Japan, reports of the constituents (Deyama et al., 2001) and the pharmacological properties of ASE, such as antistress property, are available (Davydov and Krikorian, 2000). ASE used in this study is formulated as an

* Corresponding author. Tel.: +81 134 62 1849; fax: +81 134 62 1849.
E-mail address: watanabe@hokuyakudai.ac.jp (K. Watanabe).

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ingredient of over-the-counter (OTC) drugs and functional foods in Japan.

ASE exhibits a hypoglycemic effect as well (Heinonen et al., 2001; Bhathena and Velasquez, 2002). However, the mechanism underlying the hypoglycemic effect of ASE was not clear. Recently, the reports on a unique property of ASE were demonstrated. Liu et al. (2005) reported that oral administration of the aqueous extract of AS root at a dose of 150 mg/kg three times daily for 10 days to streptozotocin diabetic rats increased the responses to exogenous insulin. After the treatment with AS ethanol extract for 8 weeks in insulin-resistant ob/ob mice, plasma glucose and insulin levels were decreased, and insulin action in the liver was enhanced (Park et al., 2006). From these findings, the aqueous extract and ethanol extract of AS have the ability to improve insulin sensitivity and delay the development of insulin-resistant. On the other hand, it was reported that chlorogenic acid of specific constituent in ASE inhibited α-glucosidase activity (Ishikawa et al., 2007; Ma et al., 2008). Moreover, we reported that intestinal peptide transporter activity of nutrient transporter is suppressed by the addition of ASE (Takahashi et al., 2010). Therefore, we examined the effects of ASE on α-amylase and α-glucosidase activities in the present study. We also examined the effect of ASE on glucose uptake in the human small intestine cell line, Caco-2, and found that ASE inhibited glucose uptake as well as α-glucosidase activity in Caco-2 cells. Finally, we examined the effects of ASE oral administration on glucose tolerance and intestinal α-glucosidase activity in db/db mice, insulin-resistant type 2 diabetes mellitus model.

2. Materials and methods

2.1. Materials

Acarbose, 2′-deoxy-D-glucose (DG), α-amylase, amylase test Wako and glucose C-II test Wako were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). α-Glucosidase (from yeast) was purchased from Oriental Yeast, Ltd. (Tokyo, Japan). [^{3}H]DG ([1,2-^{3}H](N)-2′-deoxy-D-glucose; specific activity, 40 Ci/mmol, 1.48 TBq/mmol) was purchased from Moravek Biochemicals (Brea, CA, USA). N-(2-Hydroxyethyl)-piperazine-N′-2-ethanesulfonic acid (HEPES) and 2-(N-morpholino)ethanesulfonic acid (MES) were purchased from ICN Biomedicals Inc. (Costa Mesa, CA, USA). Dulbecco's modified Eagle's medium (DMEM), nonessential amino acids (NEAAs), penicillin G–streptomycin solution, and 0.25% trypsin/EDTA solution were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Invitrogen (Carlsbad, CA, USA) and inactivated at 56 °C for 30 min. Clear-sol II for radioactivity measurement was purchased from Nacalai Tesque Co., Ltd. (Kyoto, Japan). Mouse insulin ELISA kit (type U) reagent was purchased from Shibayagi (Gunma, Japan). All other chemicals used in the experiments were of the highest purity commercially available.

2.2. Preparation of ASE

AS stem bark was collected in eastern Hokkaido from July to September of 2007. AS extract (ASE) was prepared by Yakuhan Pharmaceutical Co., Ltd. (Hokkaido, Japan). Finely cut AS was extracted with hot water at 85–95 °C for 5 h. In order to remove impurities, the extract obtained was filtered and then concentrated under reduced pressure until it became as viscous as thick malt syrup (yield: about 10%). Afterwards, it was spray-dried in a spray drying facility to obtain a brown, powdery dry extract. Every 15 g of the finely cut AS yielded 1 g of the dry extract. The prepared ASE is used as an ingredient of OTC drugs in Yakuhan Pharmaceutical Co., Ltd. Chlorogenic acid, eleutheroside B, eleutheroside B_1, eleutheroside E and isofraxidin were detected as the specific constituents of ASE (Takahashi et al., 2010).

2.3. α-Amylase activity

α-Amylase activity was determined according to the method of Caraway (1959) with minor modifications. Briefly, the sample (ASE and acarbose) and the substrate (starch; 2 mg/mL) were incubated in 0.25 M sodium phosphate buffer (pH 7.0) for 5 min at 37 °C. Then, 40 μL of 0.2 unit/mL α-amylase dissolved in the buffer was added to the reaction mixture to make a total volume of 200 μL, and the whole was incubated for 15 min at 37 °C. After the incubation, the reaction was stopped by adding 200 μL of 1N HCl. To an aliquot (0.2 mL) of the mixture was added 10 μL of 0.01N I_2 and absorbance was measured at 690 nm.

2.4. α-Glucosidase activity

α-Glucosidase activity was determined according to the method of Nishioka et al. (1998) with minor modifications. Briefly, the sample (ASE and acarbose) and the substrate (sucrose or maltose; 100 mM) were incubated in 0.1 M potassium phosphate buffer (pH 7.0) for 5 min at 37 °C. Then, 20 μL of 1 unit/mL α-glucosidase dissolved in the buffer was added to the reaction mixture to make a total volume of 100 μL, and the whole was incubated for 10 min at 37 °C. After the incubation, the reaction was stopped by adding 200 μL of 2 M Tris–HCl buffer (pH 7.0). The amount of liberated glucose was determined by the mutarotase glucose oxidase method using a commercial reagent kit, glucose C-II test Wako.

2.5. Cell culture and DG uptake study

Caco-2 cells at passage 45 were obtained from RIKEN Gene Bank (Tsukuba, Japan). Cells at passages 65–75 were grown routinely in 75 cm^2 plastic culture flasks (Corning, NY, USA) filled with DMEM containing 10% FBS, 1% NEAAs, streptomycin (100 μg/mL), and penicillin G (100 U/mL) at 37 °C in 5% CO_2/95% air atmosphere. The medium was replaced every 3–4 days after inoculation. Subculture was carried out every 7 days using 0.25% trypsin–EDTA. For the uptake study, Caco-2 cells were seeded at a density of 1×10^5 cells/well on multiwell cell culture plates coated with rat tail collagen type I (12 wells, 3.8 cm^2, Becton Dickinson, Franklin Lakes, NJ, USA). Caco-2 cell monolayers on the 7th–9th day of culture were used in the experiments.

DG uptake study was performed according to the method of Bissonnette et al. (1996) with minor modifications. Briefly, the culture medium was aspirated and the monolayers were washed twice with incubation medium consisting of HEPES buffer [140 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, 4 mM glutamine, 10 mM HEPES (pH 7.2)]. After washing, the monolayers were incubated with 1.0 mL of HEPES buffer for 1 h at 37 °C. The buffer was aspirated after the incubation and the monolayers were incubated with 0.6 mL of fresh HEPES buffer containing [^{3}H]DG (0.25 μCi, 6.25 nM) for 10 min at 37 °C. The uptake was terminated by aspiration of the buffer, and the monolayers were washed twice with ice-cold HEPES buffer. Thereafter, 0.4 mL of 1N NaOH was added to each well to dissolve the cells. Then, 0.4 mL of 1N HCl was added to each well to neutralize the cell lysate. To measure radioactivity, 0.6 mL of the cell lysate was mixed with 3 mL of liquid scintillator, and radioactivity was measured with an LSC-6100 liquid scintillation counter (ALOKA, Tokyo, Japan). Protein concentration of the cell lysate was measured with a Bio-Rad Protein Assay Kit (Bio-Rad, CA, USA) with bovine serum albumin (BSA) as reference. To estimate the kinetic parameters for the saturable and nonsaturable components of DG uptake by Caco-2 cell monolayers, the uptake rate was fitted to Eq.

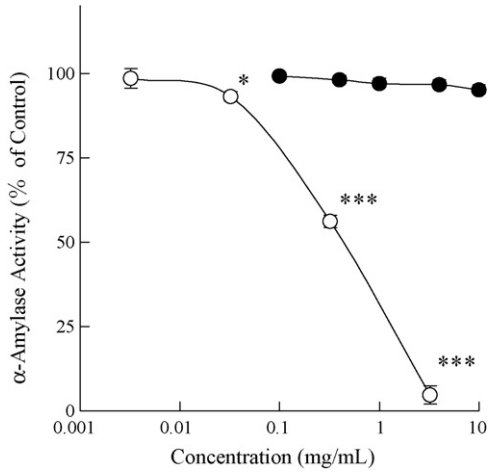

Fig. 1. Effects of ASE and acarbose on α-amylase activity. Each value represents the mean ± S.D. of four data ((●) ASE and (○) acarbose). Significantly different from the control ($^{*}p < 0.05$ and $^{***}p < 0.001$).

(1) that consists of both saturable and nonsaturable linear terms, using SigmaPlot (Ver. 8.0, SPSS, IL, USA):

$$V = \frac{V_{\max} \times [S]}{K_m + [S]} + K_d \times [S] \quad (1)$$

where [S]: DG concentration; $V_{\max}$: intracellular maximal uptake velocity; K_m: Michaelis constant; K_d: simple diffusion coefficient.

2.6. Animals, blood sampling, drug administration, oral sucrose tolerance test and intestinal homogenates

Animal studies were performed in accordance with "The Guidelines for the Care and Use of Laboratory Animals, Hokkaido College of Pharmacy." All animals were housed in individual cages in a room with controlled temperature (23 ± 1 °C), humidity (55 ± 5%), and light (06:00–18:00 h), and were maintained on a laboratory diet, MF (Oriental Yeast, Tokyo, Japan), and water *ad libitum*. Male db/db mice (BKS.Cg-m + Lepr^{db}/ + Lepr^{db}/Jcl, 9 weeks old) were purchased from Clea Japan (Tokyo, Japan). The mice used in the experiments were 10 weeks old.

For blood sampling, the tip of the tail vein was cut with a razor while the db/db mice were conscious and blood was collected into heparinized hematocrit tubes. Blood in the hematocrit tubes was transferred to 0.5 mL microfuge tubes. The tubes were centrifuged and the separated plasma was collected. The plasma glucose level obtained from the db/db mice was high as well as insulin-resistant type 2 diabetes mellitus model, KK-Ay mice (Ishikawa et al., 1998). Then, the plasma obtained from the db/db mice was diluted 5 vol. with saline. The diluted plasma was maintained on ice until the assay. Diluted plasma glucose and insulin concentrations were measured using commercial reagent kits.

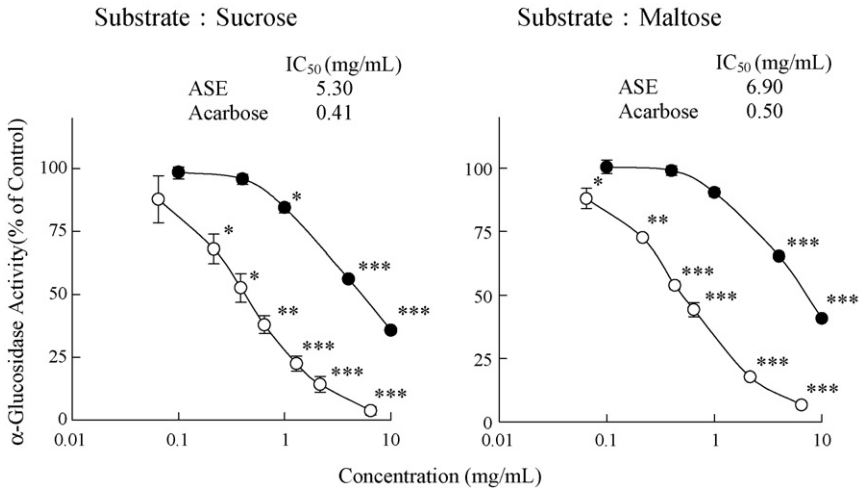

Fig. 2. Effects of ASE and acarbose on α-glucosidase activity. Each value represents the mean ± S.D. of four data ((●) ASE and (○) acarbose). Significantly different from the control ($^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$).

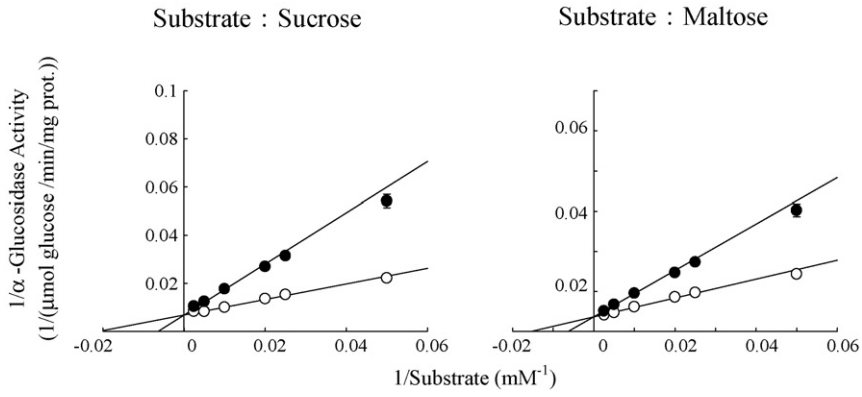

Fig. 3. Lineweaver–Burk plots for α-glucosidase. Each value represents the mean ± S.D. of four data ((○) control and (●) ASE 4 mg/mL).

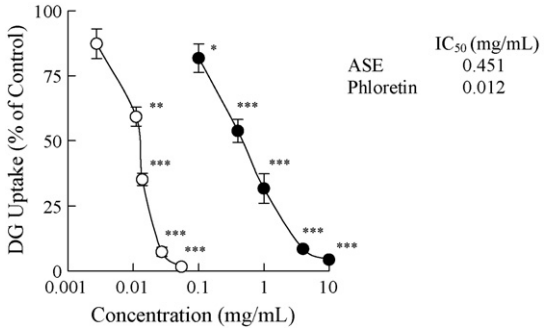

Fig. 4. Effects of ASE and phloretin on DG uptake in Caco-2 cell monolayers. Each value represents the mean ± S.D. of four monolayers ((●) ASE and (○) phloretin). Significantly different from the control (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

The db/db mice were randomly assigned to several groups having equivalent mean plasma glucose level and body weight. ASE 500 mg/kg (body weight) was orally administered to the mice twice a day (09:00 and 17:00) for 3 days. After an overnight fast (16 h), the mice received sucrose (2 g/kg) and ASE (final administration). Blood samples were taken 0, 30, 60 and 120 min after oral administration.

Intestinal homogenates from the mice were prepared according to the method of Lee et al. (1987). Intestinal segments were homogenized with 25 vol. of ice-cold 0.1 M potassium phosphate buffer (pH 7.4) in a Potter–Elvehjem homogenizer equipped with a Teflon pestle. The homogenates were centrifuged at 3000 × *g* for 15 min and the resulting supernatants were used to assay for α-glucosidase activity. The supernatant proteins were determined with a Bio-Rad Protein Assay Kit.

2.7. Statistical analysis

Values are shown as means ± S.D. and the difference between groups was tested with Stat Flex (Ver. 5.0, Artec, Osaka, Japan). To test the difference between two independent groups, the variance was examined with the *F*-test. If the variances were equal, Student's *t*-test was applied, while if the variances were not equal, Welch's *t*-test was applied. To test the differences among three independent groups, after the variance was examined by Bartlett's test, if the variances were equal, one-way analysis of variance (ANOVA) was performed. In the case that a statistically significant difference was noted and the sample number was the same among groups, Dunnett's multiple comparison test was performed, while if the sample number was different, Scheffe's multiple comparison test was applied. Also, after Bartlett's test, if the variances were not equal, Kruskal–Wallis's test was performed, and if a –significance was noted, Scheffe's multiple comparison test was applied. For each test, the significance level was set at $p < 0.05$.

3. Results

3.1. Effects of ASE addition on α-amylase and α-glucosidase activities

First, we examined the effects of ASE addition on α-amylase and α-glucosidase activities. Acarbose was used as the positive control. The concentration of ASE added was determined with the ASE concentration in a commercially available OTC drug (4.0 mg/mL) as a guide. Fig. 1 shows the effects of ASE addition on α-amylase activity. Whereas the addition of the positive control, acarbose, inhibited α-amylase activity significantly and dose-dependently at a concentration of 0.03 mg/mL or more, the addition of ASE did not change α-amylase activity.

Table 1
Effects of ASE addition on the kinetic parameters of α-glucosidase.

	Sucrose		Maltose	
	V_{max}[a]	K_m[b]	$Vmax$[a]	K_m[b]
Control	152.7 ± 6.6	51.5 ± 5.6	280.6 ± 5.8	67.9 ± 2.8
ASE 4 mg/mL	141.8 ± 5.3	145.4 ± 19.7***	268.1 ± 9.3	151.9 ± 18.8***

Each value represents the mean ± S.D. of four data.
[a] V_{max}: μmol glucose/min/mg prot.
[b] K_m: mM.
*** Significantly different from the control, $p < 0.001$.

Then, we examined the effects of ASE addition on α-glucosidase activity. The concentrations of sucrose and maltose, the substrates of α-glucosidase, were set at 100 mM based on the work of Khoursandi et al. (2004) as a guide. As shown in Fig. 2, the addition of the positive control, acarbose, inhibited α-glucosidase activity significantly and dose-dependently at a concentration of 0.22 mg/mL or more when sucrose was used as substrate, and at 0.065 mg/mL or more when maltose was used as substrate. On the other hand, the addition of ASE inhibited α-glucosidase activity significantly at 1 mg/mL or more when sucrose was used as substrate and at 4 mg/mL or more when maltose was used as substrate. The comparison of IC_{50} values revealed that the α-glucosidase inhibitory activity of ASE was approximately 1/13 of that of acarbose. Then, we examined the effects of adding 4 mg/mL ASE on α-glucosidase activity with sucrose or maltose at a concentration of 20–400 mM, respectively. The obtained results were plotted according to the Lineweaver–Burk equation (Fig. 3) to calculate K_m and V_{max} values. Table 1 shows the calculated kinetic parameters. The addition of 4 mg/mL ASE did not change the V_{max} value of α-glucosidase but significantly increased the K_m value.

3.2. Effects of ASE addition on DG uptake

We examined the effects of incubation time on DG uptake in Caco-2 cells. DG uptake increased almost linearly up to the incubation time of 10 min (data not shown). Accordingly, we performed an experiment with the incubation time set at 10 min. We examined the effects of ASE addition on DG uptake in Caco-2 cells using phloretin, a GLUT2 inhibitor (Walker et al., 2005), as the positive control. As shown in Fig. 4, DG uptake was inhibited significantly and dose-dependently by the addition of phloretin at 0.01 mg/mL or more, and the addition of ASE at 0.1 mg/mL or more. In addition, the comparison of IC_{50} values revealed that the DG uptake inhibitory activity of ASE was approximately 1/40 of that of phloretin. Then, we examined the effects of adding 4 mg/mL ASE on the uptake of DG at a concentration of 6.3 nM to 10 mM. DG uptake increased almost linearly at a concentration of up to 1 mM and nonlinearly at higher concentrations (Fig. 5). Accordingly, we analyzed the obtained results according to Eq. (1) described in Section 2. The dashed line and the dotted line represent the saturable component and the nonsaturable component of DG uptake calculated from the kinetic parameters, respectively (Fig. 5). Table 2 shows the calcu-

Table 2
Kinetic parameters for DG uptake in Caco-2 cell monolayers.

Parameter	Control	ASE 4 mg/mL
V_{max} (nmol/mg prot./10 min)	78.55 ± 1.92	26.25 ± 1.52***
K_m (mM)	0.47 ± 0.04	1.32 ± 0.23***
K_d (mL/mg prot./10 min)	1.82 ± 0.45	2.05 ± 0.26

Each value represents the mean ± S.D. of four monolayers.
*** Significantly different from the control, $p < 0.001$.

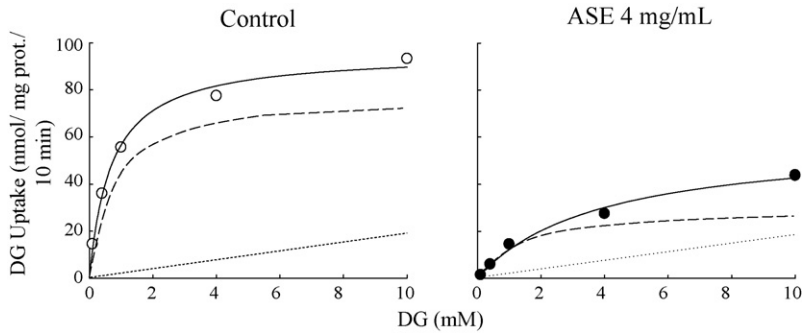

Fig. 5. Effects of ASE on the kinetic parameters of DG uptake in Caco-2 cell monolayers. Dashed and dotted lines represent the saturable and nonsaturable components of DG uptake calculated from the kinetic parameters, respectively. Each value represents the mean ± S.D. of four monolayers.

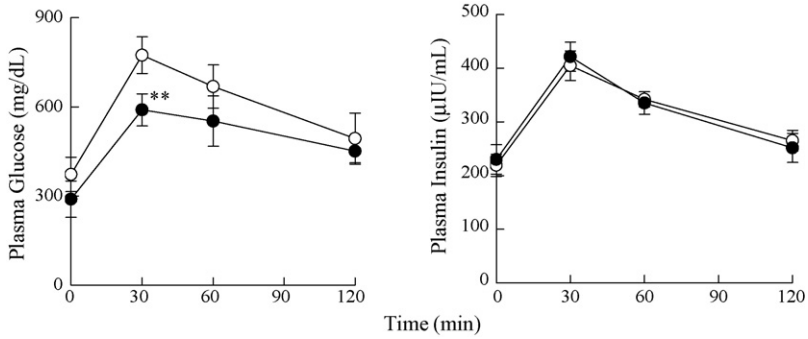

Fig. 6. Effects of ASE oral administration for 3 days on plasma glucose and insulin levels after oral administration of sucrose in db/db mice. ASE (500 mg/kg) was orally administered to db/db mice (10 weeks old) twice a day (09:00 and 17:00) for 3 days. After an overnight fast (16 h), ASE and sucrose (2 g/kg) were orally administered to the mice. Each value represents the mean ± S.D. of four mice ((○) control and (●) ASE). Significantly different between the groups (**$p < 0.01$).

lated kinetic parameters. The addition of ASE hardly changed the K_d value but caused a significant rise of the K_m value and a significant decline of the V_{max} value.

3.3. *Effects of ASE oral administration for 3 days on glucose tolerance and intestinal α-glucosidase activity in db/db mice*

Previously obtained experimental results have indicated the possibility that ASE could correct postprandial hyperglycemia and improve glucose tolerance. Accordingly, we examined the effects of ASE oral administration on glucose tolerance and intestinal α-glucosidase activity in db/db mice, an insulin-resistant animal model of type II diabetes mellitus. The ASE dose for the current study was set at 500 mg/kg, given that human daily ingestion of ASE is from 200 to 2000 mg.

db/db mice were orally administered ASE (500 mg/kg/time) twice a day (09:00 and 17:00) for 3 days and then fasted overnight. The next morning, we orally administered ASE and sucrose to the mice, collected blood from them at a certain time period, and measured plasma glucose levels and plasma insulin levels. As shown in Fig. 6, the ASE administration group showed lower plasma glucose levels before and after sucrose loading than the control group and a significantly lower plasma glucose level 30 min after the loading in particular. On the other hand, no significant difference in the plasma insulin level was seen between the two groups.

To examine the effects of ASE administration on intestinal α-glucosidase activity, the mice were orally administered ASE for 3 days in a manner similar to the previous study, following a 1-week washout period after the completion of the glucose tolerance study, and then fasted overnight. The next morning, ASE was administered to mice. The small intestine mucosa was extirpated 30 min after ASE

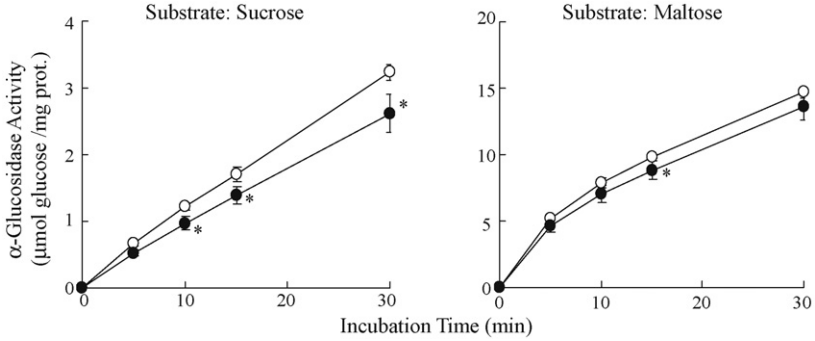

Fig. 7. Effects of ASE oral administration for 3 days on α-glucosidase activity in db/db mice. ASE (500 mg/kg) was orally administered to db/db mice (10 weeks old) twice a day (09:00 and 17:00) for 3 days. After ASE was orally administered to the mice that had been fasted for 16 h, intestinal α-glucosidase activities were determined. Each value represents the mean ± S.D. of four mice ((○) control and (●) ASE). Significantly different between the groups (*$p < 0.05$).

administration, and the homogenates were prepared to measure α-glucosidase activity. As shown in Fig. 7, ASE administration significantly inhibited α-glucosidase activity when sucrose was used as the substrate. On the other hand, ASE administration resulted in only a slight decline of α-glucosidase activity when maltose was used as the substrate.

4. Discussion

We conducted a literature search on the antidiabetic effects of *Acanthopanax senticosus* Harms and found reports of the hypoglycemic effects of ASE in an experiment that used an intestinal bacterial metabolite of a glycoside from *Acanthopanax senticosus* Harms (Heinonen et al., 2001; Bhathena and Velasquez, 2002). The mechanism of action, however, was not elucidated in detail.

Glucides ingested through meals are decomposed into monosaccharides by saliva, pancreatic juice α-amylase (Kimura, 2000), and disaccharidases (sucrase, maltase, etc.) of the small intestine mucosa, and are absorbed by small intestine epithelial cells. If α-amylase activity, disaccharidase activity, and glucose uptake in the small intestine are inhibited, intestinal absorption of the ingested glucide is expected to decline, resulting in an inhibition of the rapid rise of blood glucose level immediately after a meal. Accordingly, we conducted an *in vitro* study to examine the effects of ASE on glucide absorption related enzyme activities and glucose uptake in the small intestine. The results revealed that ASE had no effects on α-amylase activity (Fig. 1). α-Glucosidase (Kimura, 2000) significantly contributes to glucide absorption in the small intestine through the decomposition into monosaccharides of glucides that have been initially decomposed into disaccharides by α-amylase, etc. Accordingly, we examined the effects of ASE on α-glucosidase activity. The results revealed that ASE addition inhibited α-glucosidase activity significantly and in a dose-dependent manner (Fig. 2). In addition, kinetic analysis of this inhibition revealed no change in the V_{max} value but a significant increase in the K_m value for both substrates (Fig. 3 and Table 1). These findings suggest that the inhibition of α-glucosidase activity by ASE took place in a competitive manner.

The intracellular uptake of glucose in the small intestine is accomplished by passive diffusion and transport via a glucose transporter. Glucose transport via the transporter is performed by SGLT1 and GLUT2 (Thorens et al., 1990), with GLUT2 being primarily involved in glucose absorption at high concentrations as in immediately after a meal, rather than SGLT1 (Kellett and Helliwell, 2000; Kellett and Brot-Laroche, 2005). Accordingly, we studied the effects of ASE on glucose uptake in the small intestine by using DG as the substrate. As a glucose-like substance, DG is used as an index of glucose uptake in the small intestine because it is a specific substrate for GLUT2 and is not metabolized even after being taken up by cells (Olefsky, 1978). First, we examined the effects of ASE on DG uptake in human small intestine Caco-2 cells. The results revealed that ASE addition inhibited DG uptake in Caco-2 cells significantly and in a dose-dependent manner (Fig. 4). In a kinetic analysis performed to investigate in detail the effects of ASE addition on DG uptake in Caco-2 cells, no changes were found in the K_d value of simple diffusion coefficient (Fig. 5 and Table 2). Therefore, ASE has no effects on DG uptake through passive diffusion. On the other hand, while little change was observed in the K_m value, a significant decline was observed in the V_{max} value. From these findings, it is considered that ASE inhibits intracellular DG uptake chiefly by inhibiting transport via a glucose transporter.From the evidence of α-glucosidase activity inhibition by ASE, it was considered that ASE acts by inhibiting disaccharide (e.g., sucrose) digestion. Therefore, we used sucrose but not glucose in glucose tolerance study. Accordingly, given the ability of ASE to inhibit the rapid rise in blood glucose level immediately after a meal and to improve glucose tolerance, we subsequently examined the *in vivo* effects of ASE on db/db mice, an animal model of type II diabetes mellitus. The db/db mice employed in the current study are a diabetic model animal with characteristics of type II diabetes mellitus, such as hyperglycemia, obesity, and hyperinsulinemia (Surwit et al., 1991). In this study, we examined the effects of ASE oral administration twice a day for 3 days on glucose tolerance and intestinal α-glucosidase activity in db/db mice after sucrose loading. Medon et al. (1981) reported that *Eleutherococcus senticosus* extract 80 mg/kg by gastric intubation induced a hypoglycemic response by the 3rd or 4th day of the administration. We found there was no difference of the pharmacological action in the ASE oral administration for 3 days and 7 days in the preliminary experiment (data not shown). Therefore, ASE (500 mg/kg) was orally administered to the mice twice a day for 3 days. In the glucose tolerance study under sucrose loading (Fig. 6), the ASE administration group showed lower plasma glucose levels before loading than the control group. In addition, the ASE administration group showed significantly lower plasma glucose levels than the control group 30 min after sucrose loading. These findings revealed that ASE oral administration improved glucose tolerance. On the other hand, ASE oral administration had no effect on plasma insulin levels. This finding indicated that ASE and ASE-derived components are not likely to act on the pancreas to promote insulin secretion. In addition, we measured α-glucosidase activity using small intestine mucosa extirpated from db/db mice that were orally administered ASE for 3 days. The results revealed that ASE oral administration inhibited α-glucosidase activity slightly but significantly (Fig. 7). The above finding revealed that ASE oral administration inhibits glucose absorption in the small intestine. When we examined the effects of ASE addition on α-glucosidase and DG uptake activities *in vitro*, we used the positive control, acarbose and phloretin. Therefore, we consider that the comparative studies on the pharmacological actions of ASE and the positive control are necessary in the animal experiment. In a future study, we will report on the comparative study of pharmacological actions of ASE, acarbose, phloretin and other antidiabetic agents after the long time period administration by using the diabetes mellitus animal models.

Together, the experimental results revealed that ASE suppresses glucide absorption by the inhibition of intestinal α-glucosidase activity and glucose uptake at a concentration equivalent to that of a commercially available OTC drug. In this regard, ASE is expected to inhibit the rapid rise in blood glucose level immediately after a meal and to improve impaired glucose tolerance. In addition, the experiments using db/db mice, an animal model of type II diabetes mellitus, revealed that the oral administration of ASE for 3 days improved glucose tolerance and inhibited α-glucosidase activity in the small intestine after sucrose loading. The above findings indicate that ASE may be useful as an ingredient of functional foods to improve postprandial hyperglycemia and prevent type II diabetes mellitus. The known constituents of ASE include isofraxidin, eleutherosides, senticosides, and chlorogenic acid (Tokiwa et al., 2006). It was reported that chlorogenic acid inhibits α-glucosidase activity (Ishikawa et al., 2007; Ma et al., 2008). Future studies of the effects of those constituents, including chlorogenic acid, on α-glucosidase activity are highly awaited.

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