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Anti-hyperglycemic Effect of Diacylated Anthocyanin Derived from *Ipomoea batatas* Cultivar Ayamurasaki Can Be Achieved through the α-Glucosidase Inhibitory Action

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To clarify a postprandial glucose suppression effect of diacylated anthocyanin with α-glucosidase (AGH) inhibitory activity, a single oral administration study of it in male 8-week-old Sprague−Dawley rats was performed. The diacylated anthocyanin used in this study was peonidin 3-*O*-[2-*O*-(6-*O*-*E*-feruloyl-β-D-glucopyranosyl)-6-*O*-*E*-caffeoyl-β-D-glucopyranoside]-5-*O*-β-D-glucopyranoside isolated from storage roots of the purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki), which showed a potent maltase inhibitory activity with an IC_{50} value of 200 μM preferable to sucrase inhibition. When the diacylated anthocyanin (100 mg/kg) was administered following maltose (2 g/kg), a maximal blood glucose level (BGL) at 30 min was significantly decreased by 16.5% ($P < 0.01$) compared to vehicle. A minimum 10 mg/kg dose of the anthocyanin was necessary for the suppression of glycemic rise, and the ED_{20} (69 mg/kg) was estimated to be ∼30-fold lower than that of the therapeutic drug acarbose ($ED_{20} = 2.2$ mg/kg). A reduction of serum insulin secretion was also observed corresponding to the decrease in BGL. No significant change in BGL was observed when sucrose or glucose was ingested, suggesting that the anti-hyperglycemic effect of the anthocyanin was achieved by maltase inhibition, not by sucrase or glucose transport inhibition at the intestinal membrane.

KEYWORDS: α-Glucosidase; diacylated anthocyanin; anti-hyperglycemic effect; non-insulin-dependent diabetes mellitus

INTRODUCTION

Diabetic disease is known to be closely associated with the onset of diabetogenic failures such as kidney dysfunction, retinopathy, and neuropathy (*1*). Thus, an effective management of diabetes mellitus, in particular non-insulin-dependent diabetes mellitus (NIDDM), is to prevent an excess postprandial rise of blood glucose level (BGL) or to improve insulin resistance. The former is linked with a development of α-glucosidase (AGH, EC 3.2.1.20) inhibitors, used to delay carbohydrate digestions at the epithelium of the small intestine. In food scientific fields, many natural resources (*2*−*5*) have been examined with respect to an exertion of AGH inhibitory activity. Among them, D-xylose (*2*) as well as L-arabinose (*3*) was found to show an in vivo anti-hyperglycemic effect via sucrase inhibition, but not maltase inhibition. In contrast, by considering a postprandial BGL control of borderline NIDDM subjects, the inhibition of glucose production from maltose or isomaltose seems to be preferable to sucrase inhibition (*1*). However, there have been few reports on maltase inhibition or in vivo hypoglycemic effect of natural food components. Suzuki et al. (*6*) reported that an administration of banaba leaf extract with soluble starch to Wistar rats lowered the BGL through an action of maltase inhibition, but active components involved in the lowering effect remained unclear.

In a series of studies on AGH inhibition by food components (*7*−*11*), we found anthocyanins to be compatible with a suppression of glucose production from dietary carbohydrates (*10*, *11*). As a result of AGH inhibitory assay of the anthocyanins by using our proposed immobilized AGH assay system (*12*), diacylated anthocyanins showed a potent inhibitory activity against maltase with IC_{50} values of <200 μM, whereas no sucrase inhibition was observed (*11*). Of these anthocyanins, YGM-6 from *Ipomoea batatas* cv. Ayamurasaki [peonidin (Pn) 3-*O*-(2-*O*-(6-*O*-*E*-feruloyl(fer)-β-D-glucopyranosyl)-6-*O*-*E*-caf-

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feoyl(caf)-β-D-glucopyranoside)-5-*O*-β-D-glucopyranoside, IC_{50} = 200 μM] (*13*) as well as SOA-4 from *Pharbitis nil* cv. Scarlett O'Hara [pelargonidin (Pg) 3-*O*-(2-*O*-(6-*O*-(*E*-3-*O*-(β-D-glucopyranosyl) caf)-β-D-glucopyranosyl)-6-*O*-*E*-caf-β-D-glucopyranoside)-5-*O*-β-D-glucopyranoside, IC_{50} = 60 μM] was identified as a potent maltase inhibitor (*11*). Thus, with the aim of developing an anti-hyperglycemic food from the purple sweet potato, we attempted to evaluate in this study whether the maltase inhibitory anthocyanin, YGM-6, shows an in vivo BGL suppression effect.

MATERIALS AND METHODS

Materials. AGH from rat intestinal acetone powder was purchased from Sigma Chemical Co. (St. Louis, MO). Storage roots of the purple sweet potato cv. Ayamurasaki were obtained from the Kyushu National Agricultural Experiment Station in Miyazaki prefecture (Japan). Other reagents were of analytical grade and used without further purification.

Preparation of Anthocyanins. Crude anthocyanin pigments were extracted from the storage roots of the purple sweet potato cv. Ayamurasaki. Preparation procedures of the anthocyanin extract and YGM-6 were the same as reported previously (*10*, *13*). Namely, the roots (25 g) were ground and steeped in 1 L of 5% acetic acid (AcOH) solution overnight, and then the filtered extract was applied on an Amberlite XAD-2000 resin column (∅ 45 × 300 mm, Rohm and Haas) with elution by 5% AcOH in 70% EtOH. After the evaporation, the residue was dissolved in 50% AcOH in MeOH, followed by centrifugation. The supernatant was precipitated with excess diethyl ether, and the precipitate was collected to dryness in vacuo, to obtain the anthocyanin extract as a purple powder. The extract was dissolved in 0.1% trifluoroacetic acid (TFA)/EtOH 6:4 (v/v) and chromatographed on a PVP column (∅ 45 × 300 mm, polyclar AT, GAF Chemicals Co.) with the same solvent. Yield of the anthocyanin extract with purple color was 0.8% from the root, mainly containing six diacylated anthocyanins (*13*). From the anthocyanin extract, YGM-6 was prepared because of its high content of 67% and high AGH inhibitory activity (*11*). Isolation was done by applying the extracts on a preparative ODS-HPLC (L-6200 intelligent pump system, Hitachi Co.) on a column (Inertsil ODS 5, ∅ 20 × 250 mm, GL Sciences Inc.) with isocratic solvent system of A (15% AcOH)/B (15% AcOH, 30% CH_3CN) 70:30−50:50 (v/v) at 520 nm according to our reported procedure (*14*). The YGM-6 fraction was evaporated to dryness, dissolved in a minimum amount of TFA, and precipitated with the addition of excess diethyl ether; the precipitate was collected and dried in vacuo. YGM-6 was obtained as a purple powder of TFA salt.

Animal Experiments in SD Rat. Four male 7-week-old Sprague−Dawley (SD) rats (SPF/VAF Crj:SD, Charles River Japan, Kanagawa, Japan) in each rat experiment were fed a laboratory diet (MF, Oriental Yeast, Tokyo, Japan) and given water ad libitum. All rats were housed for 1 week at 21 ± 1 °C and 55 ± 5% humidity under controlled lighting from 8:30 a.m. to 8:30 p.m. Each rat (n = 4, 268.7 ± 3.7 g) was unfed for 16 h before a single oral administration of anthocyanin sample via a stomach sonde. At 5 min after the anthocyanin administration, 1 mL of a 2 g/kg substrate (maltose, sucrose, or glucose) solution was administered to each rat. Control rats (vehicle) were administered the same volume of substrate solution without sample. At each time point to 120 min, ∼20 μL blood samples were collected from tail vein, being immediately subjected to a BGL measurement by disposable glucose sensor (Glutest Pro, Sanwa Chemical Research, Co., Tokyo, Japan). Remaining blood (serum) sample was subjected to an insulin assay (Rat Insulin EIA Biotrak System, Amersham Pharmacia Biotech UK, Ltd., Little Chalfont Buckinghamshire, U.K.). All of the measurements were done at three replicates.

Data Analysis. Each result for the administration study is expressed as the mean of BGL (mg/dL of plasma) ± SEM (percent). Statistical differences of BGL in vehicle and anthocyanin groups at each administration time were evaluated by using a two-factor analysis of variance (ANOVA) followed by Dunnett'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).

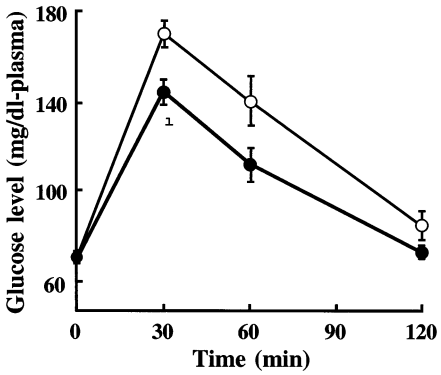

Figure 1. Effect of anthocyanin extract on BGLs after a single oral administration of 2 g/kg maltose in SD rats. One milliliter of a 400 mg/kg anthocyanin extract (●) was dosed in male 8-week-old SD rats. After 5 min, 1 mL of a 2 g/kg maltose solution was administered to each rat. Vehicle (○) was administered with the same volume of substrate solution without inhibitor. At each time point to 120 min, ∼20 μL blood samples were collected from the tail vein, being immediately subjected to a BGL measurement by disposable glucose sensor. Data are the mean (mg/dL of plasma) ± SEM. Significant differences between test and control groups were examined with Dunnett's t test (n = 4, $^{\dagger}P < 0.05$).

Table 1. Effects of Anthocyanin Extract[a] on the Postprandial Insulin Response after Maltose Ingestion in Sprague−Dawley Rats

	serum insulin (ng/mL)	
	30 min	60 min
vehicle[b]	2.85 ± 0.13	2.48 ± 0.19
extract[c]	1.15 ± 0.34**	0.80 ± 0.22**

[a] Anthocyanin extract (400 mg/kg) was administered to male 8-week-old SD rats (n = 4) with maltose (2 g/kg). Obtained serum sample was subjected to the EIA insulin assay. [b] Maltose without anthocyanin extract. Data are expressed as mean ± SEM (n = 4). [c] **, $P < 0.01$ vs vehicle at each time.

RESULTS AND DISCUSSION

Change in Blood Glucose Level after a Single Oral Administration of Anthocyanin Extract in Sprague−Dawley Rats. On the basis of the result that the anthocyanin extract from the purple sweet potato cv. Ayamurasaki showed a potent maltase inhibitory activity (IC_{50} = 0.36 mg/mL) (*11*), change in BGL after an administration of the anthocyanin extract with maltose was primarily examined in SD rats. As revealed in **Figure 1**, a significant lowering of glycemic response with a dose of 400 mg/kg in SD rats at 30 min ($BGL_{30min,extract}$ = 143.8 ± 4.2 mg/dL of plasma) was observed against control rats that ingested maltose ($BGL_{30min,vehicle}$ = 170.3 ± 4.6 mg/dL of plasma). Although the lowering effect turned out to be insignificant at 60 min after the administration, the extract rich in anthocyanins (*13*) was found to have a latent physiological function regarding a postprandial anti-hyperglycemic effect for the first time. In addition, the extract significantly ($P < 0.01$) reduced serum insulin secretions at 30 and 60 min in response to the maltose ingestion by a factor of 60−70% (**Table 1**), supporting the assumption that the anthocyanin extract did suppress the BGL rise in SD rats. According to a human study by Fujita et al. (*15*), even though a food (Touchi extract) showed a poor in vitro maltase inhibitory activity (IC_{50} = 1.1 mg/mL), 0.3 g of its ingestion following 200 g of cooked rice resulted in a 40% reduction of BGL rise in diabetic subjects. Therefore, much benefit would be present in developing the anthocyanin extract with anti-hyperglycemic effect against maltose or dietary carbohydrate intake. When sucrose was ingested, the incremental

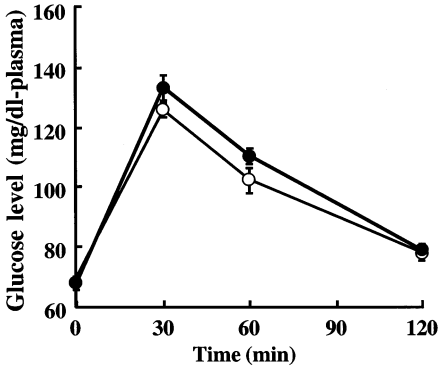

Figure 2. Effect of anthocyanin extract on BGLs after a single oral administration of 2 g/kg sucrose in SD rats. One milliliter of a 400 mg/kg anthocyanin extract (●) was dosed in male 8-week-old SD rats. After 5 min, 1 mL of a 2 g/kg sucrose solution was administered to each rat. Vehicle (○) was administered with the same volume of substrate solution without inhibitor. Other experimental conditions were the same as in **Figure 1**. Data are the mean (mg/dL of plasma) ± SEM. Significant differences between test and control groups were examined with Dunnett's *t* test ($n = 4$).

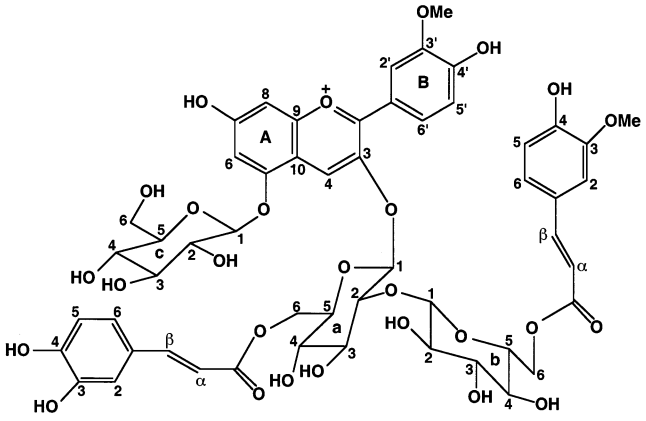

Figure 3. Strucuture of diacylated anthocyanin (YGM-6) used in this study.

BGL curve was not affected by the anthocyanin extract ingestion during the 120 min experiment (**Figure 2**), compatible with our proposed immobilized AGH inhibition study (*10*), in which the extract had no ability to inhibit sucrase activity.

Change in Blood Glucose Level after a Single Oral Administration of Diacylated Anthocyanin in Sprague−Dawley Rats. To clarify active compounds associated with the suppression of rat BGL, an anti-hyperglycemic effect of diacylated anthocyanins rich in the extract was then investigated. YGM-6 having a structure of Pn 3-*O*-[2-*O*-(6-*O*-*E*-fer-*β*-D-glucopyranosyl)-6-*O*-*E*-caf-*β*-D-glucopyranoside]-5-*O*-*β*-D-glucopyranoside shown in **Figure 3**, which we have already identified in a previous paper (*13*), was used in this study due to the highest content of 67% in the anthocyanin extract. **Figure 4** represents an incremental BGL curve following maltose ingestion (2 g/kg) when YGM-6 was orally administered to SD rats at a dose of 100 mg/kg. Acarbose at a dose of 3 mg/kg was used in this study as a positive control. Apparently, the BGL curve of YGM-6 was lower than that of the vehicle; significant BGL reductions of 28.1 and 21.3 mg/dL of plasma were observed at 30 and 60 min, respectively. The area under the curve ($AUC_{0-120min}$) of YGM-6 ingestion (93.6 ± 14.5 mg·h/dL of plasma) was also significantly lower than that of the vehicle (124.8 ± 9.9 mg·h/dL of plasma) by a factor of 25%. YGM-6 also significantly suppressed an increase in serum

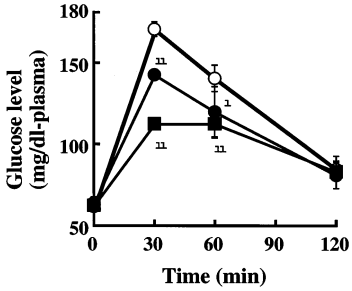

Figure 4. Effect of diacylated anthocyanin (YGM-6) on BGLs after a single oral administration of 2 g/kg maltose in SD rats. One milliliter of 100 mg/kg YGM-6 (●) was dosed in male 8-week-old SD rats. Acarbose (■) at the dose of 3 mg/kg was used as a positive control. After 5 min, 1 mL of a 2 g/kg of maltose or sucrose solution was administered to each rat. Vehicle (○) was administered with the same volume of substrate solution without inhibitor. At each time point to 120 min, ∼20 μL blood samples were collected from the tail vein, being immediately subjected to the BGL measurement by disposable glucose sensor. Data are the mean (mg/dL of plasma) ± SEM. Significant differences between test and control groups were examined with Dunnett's *t* test ($n = 4$, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$).

Table 2. Effects of YGM-6[a] on the Postprandial Insulin Response after Maltose Ingestion in Sprague−Dawley Rats

	serum insulin (ng/mL)	
	30 min	60 min
vehicle[b]	2.85 ± 0.13	2.48 ± 0.19
YGM-6[c]	1.62 ± 0.22**	1.09 ± 0.09**

[a] YGM-6, diacylated anthocyanin [peonidin 3-*O*-(2-*O*-(6-*O*-*E*-feruloyl-*β*-D-glucopyranosyl)-6-*O*-*E*-caffeoyl-*β*-D-glucopyranoside)-5-*O*-*β*-D-glucopyranoside]. YGM-6 (100 mg/kg) was administered to male 8-week-old SD rats ($n = 4$) with maltose (2 g/kg). Obtained serum sample was subjected to the EIA insulin assay. [b] Maltose without YGM-6. Data are expressed as mean ± SD ($n = 4$). [c] **, $P < 0.01$ vs vehicle at each time.

insulin level as shown in **Table 2**. This finding strongly suggested that a significant control or delay of postprandial BGL rise can be attributed to the diacylated anthocyanin, YGM-6, which is also effective in improving a deficient insulin secretion (*1*). Tsuda et al. (*16*) have already clarified that anthocyanins, in particular their aglycons such as pelargonidin, cyanidin, or delphinidin, had strong radical scavenging activities against hydroxyl and superoxide anion radicals, but the present study, in which the diacylated anthocyanin had the antihyperglycemic effect in vivo, also demonstrated an alternative physiological function of anthocyanins. However, with their poor absorption (*17*) taken into account, the function of diacylated anthocyanins would preferentially focus on a prevention of diabetic disease, because the effect was restricted at the epithelium of the brush border membrane without any consideration of its absorption.

Figure 4 also revealed that the BGL lowering effect of YGM-6 was still lower than that of a therapeutic drug, acarbose (BGL reduction vs vehicle = 58.4 mg/dL of plasma at 30 min). Thus, to clarify the lowering ability of YGM-6, dose-dependent experiments of YGM-6 and acarbose in SD rats after maltose ingestion were performed (**Figure 5**). As a result, YGM-6 suppressed a BGL rise ($AUC_{0-120min}$) dose-dependently as acarbose did. Then, from the linear relationship between the dose and reduction ratio of $AUC_{0-120min}$ of sample against that of the vehicle, an efficient dose required to achieve 20% suppression of BGL rise (ED_{20}) was interpolatively estimated. As shown in **Figure 5**, YGM-6 showed a much higher ED_{20} value of 69 mg/kg than that of acarbose (ED_{20} = 2.2 mg/kg) (extrapolated ED_{50} values: YGM-6, 220 mg/kg; acarbose, 3.1

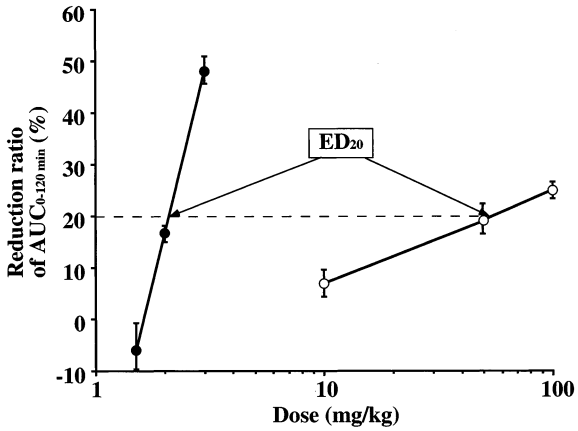

Figure 5. Dose dependency of YGM-6 (○) and acarbose (●) on the reduction of glycemic responses after maltose (2 g/kg dose) ingestion in SD rats. $AUC_{0-120min}$ is the area under the curve of incremental BGLs up to 120 min. The reduction ratio (percent) of $AUC_{0-120min}$ of YGM-6 or acarbose against $AUC_{0-120min}$ of vehicle was used for estimating either the ED_{20} or ED_{50} value. Each plot was the mean ± SEM ($n = 4$).

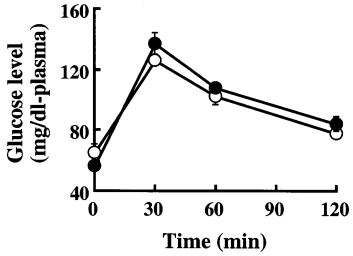

Figure 6. Effect of diacylated anthocyanin (YGM-6) on BGLs after a single oral administration of 2 g/kg sucrose in SD rats. One milliliter of 100 mg/kg YGM extract (●) was dosed in male 8-week-old SD rats. After 5 min, 1 mL of a 2 g/kg maltose or sucrose solution was administered to each rat. Vehicle (○) was administered with the same volume of substrate solution without inhibitor. Other experimental conditions were the same as in **Figure 4**. Data are the mean (mg/dL of plasma) ± SEM. Significant differences between test and control groups were examined with Dunnett's *t* test ($n = 4$).

mg/kg). This demonstrated that the BGL reduction power of YGM-6 in SD rats was ~30 times lower than that of acarbose. The ED_{20} value of acarbose in mice after starch ingestion was reported to be 1.0 mg/kg (*3*), the present result in SD rats being appropriate. Although there was no comparable study on a BGL reduction power of natural active components in rats given maltose or carbohydrates, the YGM-6 anthocyanin seems to have a weak anti-hyperglycemic potency compared to L-arabinose ($ED_{50} = 18.5$ mg/kg) in mice ingesting sucrose (*3*).

Figures 6 and **7** show glycemic responses in SD rats when sucrose or glucose was ingested, respectively. As a result, neither sucrose nor glucose ingestion with YGM-6 in rats affected the postprandial BGL curve. No significant difference between the curves with and without YGM-6 in rats ingesting sucrose was in fair agreement with the result in **Figure 2**, suggesting that YGM-6 did not possess a BGL lowering effect through sucrase inhibition. An interesting result that YGM-6 following glucose ingestion gave no influence on the postprandial BGL strongly suggested that the anti-hyperglycemic effect induced by the diacylated anthocyanin was achieved by the restrictive maltase inhibition, not by inhibiting a glucose transport in the small intestinal membrane via the Na^+/glucose co-transporter. Kobayashi et al. (*18*) reported catechins inhibited the glucose transport competitively in rat everted jejunal sacs experiment. They also

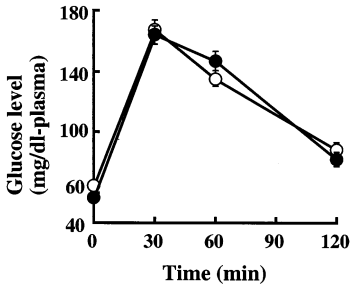

Figure 7. Effect of diacylated anthocyanin (YGM-6) on BGLs after a single oral administration of 2 g/kg glucose in SD rats. One milliliter of 100 mg/kg YGM-6 extract (●) was dosed in male 8-week-old SD rats. After 5 min, 1 mL of a 2 g/kg maltose or sucrose solution was administered to each rat. Vehicle (○) was administered with the same volume of substrate solution without inhibitor. Other experimental conditions were the same as in **Figure 4**. Data are the mean (mg/dL of plasma) ± SEM. Significant differences between test and control groups were examined with Dunnett's *t* test ($n = 4$).

concluded that the esterified galloyl group was a candidate responsible for the glucose transport inhibition. In the case of the diacylated anthocyanins with maltase inhibitory activity, we have already found that the deacylated moieties of the anthocyanins such as cyanidin-, Pn-, or Pg-3-*O*-(2-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranoside)-5-*O*-*β*-D-glucopyranoside became a no longer potent maltase inhibitor with the IC_{50} value of >4.6 mM (*11*). In combination with our previous finding that flavonoids such as luteolin and kaempferol did not show any reduction of BGL rise after maltose ingestion in SD rats (*19*), the diacylated moiety of YGM-6 (**Figure 3**) must be a candidate for exerting the anti-hyperglycemic effect in SD rats as shown in **Figure 4**. Further studies on which groups in YGM-6 are responsible for inhibiting maltase are now in progress.

In conclusion, the present study revealed for the first time that the diacylated anthocyanin as well as the anthocyanin extract possessed the postprandial anti-hyperglycemic effect in SD rats through the retardation of maltase activity and that the preparation would be useful in preventing hyperglycemia upon the intake of carbohydrates.

ABBREVIATIONS USED

AGH, α-glucosidase; Pg, pelargonidin; Pn, peonidin; SD, Sprague−Dawley; BGL, blood glucose level; AUC, area under the curve.

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