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Gastrointestinal Uptake of Nasunin, Acylated Anthocyanin in Eggplant

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We previously showed that nasunin, acylated anthocyanins in eggplant peel, comprises two isomers, *cis*-nasunin and *trans*-nasunin. In this study, gastrointestinal absorption of *cis*- and *trans*-nasunins was studied in rats. Orally administered nasunins were quickly absorbed in their original acylated forms and maximally appeared in blood plasma after 15 min. When the maximum plasma concentration and area under the plasma concentration curve were normalized by orally administered dose (micromoles per kilogram), there was no significant difference in the uptake efficiency between two isomers and both exhibited a plasma level almost identical to that of delphinidin 3-*O*-*β*-D-glucopyranoside. However, metabolites such as 4′-*O*-methyl analogues and extended glucuronides which were observed for delphinidin 3-*O*-*β*-D-glucopyranoside and cyanidin 3-*O*-*β*-D-glucopyranoside metabolisms were not detected in urine or blood plasma. Moreover, deacylated and glycolytic products of nasunins such as delphinidin 3-*O*-*β*-D-glucopyranoside or delphinidin (aglycone) were also not detected in blood plasma even after oral administration for 8 h. These results indicated that nasunins were absorbed in their original acylated forms and exhibit a bioavailability almost identical to that of nonacylated anthocyanins.

KEYWORDS: Acylated anthocyanin; *cis*-nasunin; *trans*-nasunin; delphinidin; absorption; antioxidant activity; eggplant; plasma concentration; isomerization

INTRODUCTION

The so-called "French paradox" has attracted attention to functional food factors, and thus, the health benefits of polyphenols, including flavonoids, have been widely discussed (*1*−*5*). The reddish pigments known as anthocyanins make up the family of flavonoids which are widely distributed in various types of colored fruits and vegetables (*6*−*8*). A large number of in vitro studies have characterized anthocyanins as powerful antioxidants capable of efficient scavenging of both reactive oxygen species (*9*, *10*) and nitrogen species (*11*, *12*). Recently, the apoptotic effect of anthocyanins on human leukemia cells (HL-60) has also been evaluated (*13*). These studies indicate that anthocyanin may play an important role in human health promotion. However, it is obvious that their ultimate antioxidant potential and their resulting bioactivities in vivo are dependent on their biological fate, including absorption, metabolism, distribution, and excretion.

Indeed, several studies on anthocyanin absorption and metabolism were carried out in both experimental animals and human subjects in an effort to assess the health benefit of anthocyanins (*14*−*16*). We also reported that delphinidin 3-*O*-*β*-D-glucopyranoside, a potent antioxidant in bilberry (*Vaccinium myrtillus* L.), is transformed to a 4′-*O*-methylated metabolite, and this metabolite is the major tissue distribution form of delphinidin 3-*O*-*β*-D-glucopyranoside (*17*, *18*). Further, we studied the metabolism of cyanidin 3-*O*-*β*-D-glucopyranoside and revealed that extended glucuronides (glucuronide of anthocyaninidin glycoside) were produced as the major metabolites that make up ∼40% of plasma cyanidin 3-*O*-*β*-D-glucopyranoside metabolites (*19*). These results suggest that these metabolites will play a critical role in anthocyanin functions in vivo, and thus, the uptake and metabolic studies are important for improving our understanding of the physiological function of anthocyanins.

The so-called "acylated anthocyanins", which carry acyl moieties in their structures, are the popular anthocyanins found in certain types of vegetables such as eggplant (*20*), red radish (*21*), and purple sweet potato (*22*). Nasunin is the one isolated from eggplant (*20*). It is generally accepted that acylated anthocyanins are relatively more stable than nonacylated anthocyanins (*23*), and some acylated anthocyanins have been reported to have unique physiological functions (*24*, *25*). Matsui et al. (*24*) reported α-glucosidase inhibitory activity of acylated

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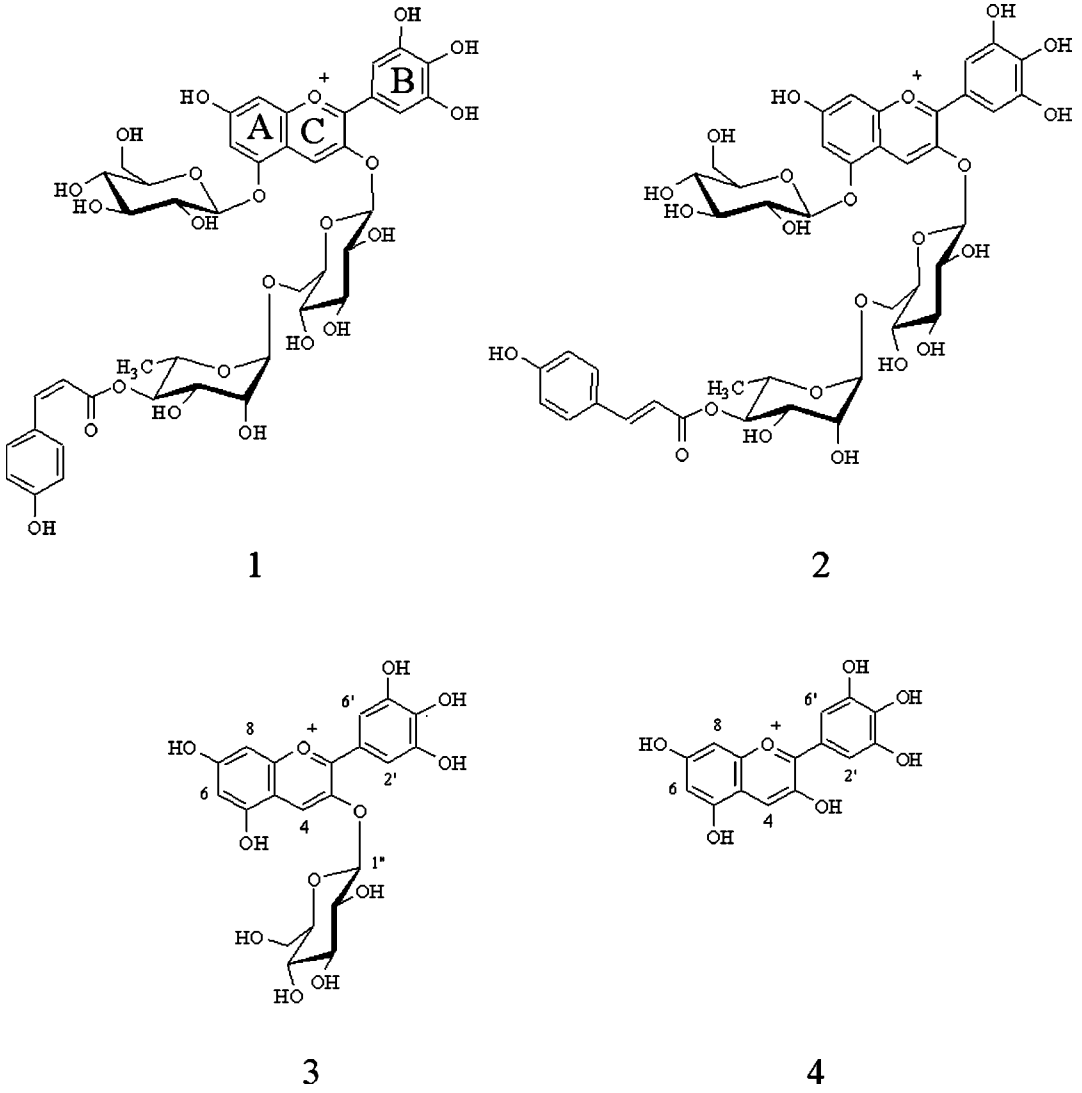

Figure 1. Chemical structures of eggplant anthocyanins: **1**, delphinidin 3-[4-(*cis-p*-coumaroyl)-L-rhamnosyl(1→6)glucopyranoside]-5-glucopyranoside (*cis*-nasunin); **2**, delphinidin 3-[4-(*trans-p*-coumaroyl)-L-rhamnosyl(1→6)glucopyranoside]-5-glucopyranoside (*trans*-nasunin); **3**, delphinidin 3-*O*-*β*-D-glucopyranoside; and **4**, delphinidin.

anthocyanins in vitro and showed that the acyl moiety plays an important role in the activity. Noda et al. (*25*) showed a strong superoxide radical scavenging activity of nasunin via an electron spin resonance (ESR) spin trapping method. They also studied the hydroxyl radical scavenging activity of nasunin and suggested that nasunin is not a direct scavenger of hydroxyl radical but chelates iron to inhibit the Fenton reaction (*25*). However, we showed recently that nasunin is a mixture of *cis*−*trans* isomers of delphinidin 3-[4-(*p*-coumaroyl)-L-rhamnosyl(1→6)-glucopyranoside]-5-glucopyranoside (*26*). Because there are few studies on the in vivo behavior of acylated anthocyanins such as their absorption and metabolism (*27*, *28*), it is interesting to study the uptake and metabolism of *cis*−*trans* isomers of nasunin found in our previous study. Thus, in this study, we focus on our aim of clarifying the absorption and metabolism of *trans*- and *cis*-nasunins (**Figure 1**) in rats together with their antioxidant properties in vitro.

MATERIALS AND METHODS

Chemicals. Bilberry extract (Bilberon 25) was generously donated by Tokiwa Phytochemical Company Ltd. An authentic sample of delphinidin 3-*O*-*β*-D-glucopyranoside was purified from bilberry extract (*V. myrtillus* L.) according to our previously described methods with a slight modification (*29*). Briefly, bilberry extract was separated on an open column packed with MCI gel (Mitsubishi Chemical Co.) (45 cm × 4.5 cm) by H_2O as an elution solution with an increased methanol concentration (0:1 to 1:0). The delphinidin 3-*O*-*β*-D-glucopyranoside-containing fraction thus obtained was further purified by semipreparative HPLC with Develosil ODS HG-5 column (250 mm × 20 mm, Nomura Chemical Co. Ltd., Aichi, Japan) using 0.5% TFA containing 13% acetonitrile as the elution solvent at a flow rate of 7 mL/min. The peak fraction was evaporated to dryness in vacuo and stored at −80 °C until it was used.

Peroxynitrite was synthesized by the method described by Beckman et al. (*30*) with slight modifications. Briefly, 0.6 M hydrogen peroxide (in 0.7 M hydrochloric acid) and 0.6 M sodium nitrite solutions were vigorously mixed in a quenched flow chamber. Excess hydrogen peroxide was removed by manganese dioxide. The concentration of peroxynitrite was determined by measuring the absorbance at 302 nm ($\epsilon = 1670\ M^{-1}\ cm^{-1}$). The typical yield of freshly prepared peroxynitrite ranged from 60 to 75 mM. The peroxynitrite was diluted in 0.3 M sodium hydroxide exactly before the experiment. 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) was obtained from Labotec Co. Ltd. All other reagents were purchased from Wako pure chemical Co. Ltd.

Preparation of the Nasunin Mixture. The nasunin mixture was obtained according to our previously described method (*26*). Briefly, eggplant peels were removed with a steel knife and immersed in 0.5% trifluoroacetic acid (TFA) containing methanol at 5 °C for 24 h. The

methanolic solution was evaporated to dryness in vacuo below 40 °C. The residue was redissolved in water and subjected to HP-20 (Mitsubishi Chemical Co.) column chromatography (90 cm × 7 cm). The column was washed with distilled water to remove water-soluble compounds, and the anthocyanin-containing band was eluted with 50% methanol. The anthocyanin-containing fraction was evaporated to dryness and redissolved in distilled water and subjected to LH-20 (Amersham Biosciences Co.) column chromatography (30 cm × 3.5 cm). The column was washed with distilled water and eluted with 30% methanol. The effluent was evaporated to dryness. The amount of eggplant extract obtained was 530 mg. From the HPLC analysis of nasunin mixture, the anthocyanin composition was as follows: 87% *trans*-nasunin and 13% *cis*-nasunin. The purity of anthocyanins in the extract was determined to be more than 98% based on a HPLC chromatogram using authentic *cis*- and *trans*-nasunin as references.

Purification of Authentic Eggplant Anthocyanins. Authentic nasunins for the purpose of quantitative evaluation were purified by semipreparative HPLC using the nasunin mixture described above according to our previously published method (*26*). Briefly, the eggplant extract obtained as described above was dissolved in a 0.1% aqueous TFA solution and further separated with a semipreparative HPLC system (Hitachi L-7200). HPLC was carried out on a Develosil ODS HG-5 column (Nomura Chemical, 150 mm × 1.0 mm) using 33% methanol containing 0.5% TFA at a flow rate of 6 mL/min. The eluted anthocyanin fractions were collected and evaporated to dryness in vacuo at a temperature no higher than 40 °C under dim light, and all the equipment, including the Eppendorf tubes, was covered with an aluminum sheet. The residue was redissolved in a small amount of 0.1% hydrochloric acid containing methanol, and diethyl ether was added to precipitate nasunin as the chloride salt. The structures of nasunins were identified by tandem TOF-MS and NMR spectrometry as described previously (*26*).

Animals and Diets. SPF male wistar ST rats (5 weeks old, 160 g body weight) purchased from Japan SLC Inc. (Hamamatsu, Japan) were individually housed in stainless steel wire-mesh cages at 23 ± 1 °C for conditioning with a 12 h light−dark cycle. The rats were allowed free access to tap water and a controlled diet for 7 days before the experiment. Rats were treated in accordance with the Guidelines of the Niigata University of Pharmacy and Applied Life Sciences.

Experimental Design and Sample Preparation. After adapting for 7 days, eight rats were cannulated with a polyethylene tube (PE-50) via a neck vein under anesthesia with diethyl ether according to our previously described method (*31*). Briefly, the neck vein was isolated, and a small hole was made by using the scissors to insert the polyethylene tube (PE-50). After the tube had been inserted, the vein and tube were occluded and the tube was guided out from the back of the rats. After the rats had been starved for 24 h, the nasunin mixture dissolved in 0.1% citric acid was orally (100 mg as anthocyanins/kg of body weight) administered to four rats. Delphinidin 3-*O*-*β*-D-glucopyranoside (100 mg/kg of body weight) was also orally administered to yet four other rats for the purpose of comparison. During the experiment, the rats were allowed to move freely in the wire-mesh floor cages. Blood samples were collected at 0, 15, 30, 60, 120, 240, and 480 min from the cannulated tube using a heparinized syringe. Donor blood was obtained from the inferior vena cava of other healthy rats using a needle containing sodium citrate (500 μL of 10% sodium citrate for 8 mL of blood) and a syringe under anesthesia with diethyl ether. After the blood (600 μL) had been withdrawn, the same volume of donor blood was injected through the cannulated vein tube. Each blood sample was immediately centrifuged at 3000*g* for 5 min at 4 °C to prepare plasma samples for HPLC analysis. The urine, which flew down through the wire-mesh floor, was collected into a plate set under the floor and then immediately acidified with a 1% aqueous TFA solution. The urine samples recovered during each time period (from 0 to 2 h, from 2 to 4 h, and from 4 to 8 h) were combined and kept in the dark for further analysis.

Assessment of Anthocyanins in Plasma. Extraction of anthocyanins was carried out using Sep-Pak C_{18} cartridge light (Waters) essentially according to our method previously reported (*31*). Briefly, the plasma (300 μL) samples were applied to Sep-Pak C_{18} cartridges light (Waters) conditioned with methanol (2 mL) and a 3% aqueous TFA solution (2 mL). After the sample had been applied, the cartridges were washed successively with 2 mL of a 3% aqueous TFA solution, dichloromethane, and benzene, and anthocyanins were eluted with 50% acetonitrile containing a 1% aqueous TFA solution. The eluent was evaporated to dryness in vacuo and dissolved in 150 μL of distilled water containing 0.5% TFA. The TFA solution was passed through a Centricut (0.45 μm, Kurabou Co. Ltd.) before HPLC injection. The HPLC conditions were like those in our previous report with slight modifications (*31*). Briefly, aliquots (100 μL) of these solutions were injected into a HPLC system (Hitachi 7200). For the separation of eggplant anthocyanins, HPLC was carried out on a Develosil ODS HG-5 column (Nomura Chemical, 1.0 mm × 150 mm). HPLC was carried out in linear gradient elution mode using 0.5% aqueous TFA (solvent A) and methanol containing 0.5% TFA (solvent B). The gradient conditions were as follows: 75% A/25% B (v/v) for 40 min and 75% A/25% B (v/v) to 30% A/70% B (v/v) for 15 min and then held at 30% A/70% B (v/v) for a further 10 min, at a flow rate of 0.1 mL/min. The elution profile was monitored at 520 nm with a UV−vis detector (Hitachi). Methanol (20%) containing 0.5% TFA was used for the analysis of delphinidin 3-*O*-*β*-D-glucopyranoside and its metabolite. The recovery of the sample by this method checked by spiking authentic anthocyanins into normal blood plasma was 85.7% for *trans*-nasunin, 85.3% for *cis*-nasunin, and 86.3% for delphinidin 3-*O*-*β*-D-glucopyranoside.

Conditions for Isomerization of *trans*-Nasunin. *trans*-Nasunin was dissolved in a 3% aqueous TFA solution and kept in daylight or irradiated in a quartz tube by UV at 290 nm using a fluorescence spectrophotometer (Hitachi Fluorescence meter 650-60) for a defined time period (10, 20, 30, and 60 min). The reaction solution was diluted and directly injected into the HPLC system equipped with a semi-micro column (Develosil ODS HG-5, 150 mm × 1 mm, Nomura Chemical) using 0.5% TFA containing 25% methanol as the elution solvent at a flow rate of 0.1 mL/min. The anthocyanin that eluted was monitored at 520 nm absorbance.

Superoxide Radical Scavenging Activity Measurement. The superoxide radical scavenging activity of anthocyanins was measured by the ESR spin trapping method as previously reported (*32*), using the hypoxanthine−xanthine oxidase system as the superoxide radical generator. The reaction mixture (total volume of 300 μL) containing 0.2 M phosphate buffer (pH 7.8), 1 mM diethylenetriaminepentaacetic acid monohydrate (DTPA), 0.5 mM hypoxanthine, various concentrations of anthocyanin samples or (+)-catechin as a reference antioxidant, 33 mM DMPO, and 0.1 unit/mL xanthine oxidase (XOD) was quickly transferred into a hematcrit capillary tube. Exactly 50 s after the addition of XOD, DMPO-OOH signals were determined with a JEOL model JES-TE 200 ESR spectrometer (X-band microwave unit). The spectrometer settings were as follows: microwave power of 8 mW, frequency of 8.9 GHz, modulation amplitude of 0.1 mT, time constant of 0.03 s, sweep time of 30 s, and center fields of 335 and 325 mT. The superoxide scavenging ability of the anthocyanins was expressed as the half-maximal inhibitory concentration (IC_{50}) for DMPO-OOH signals.

Inhibition of Peroxynitrite-Mediated Tyrosine Nitration by Anthocyanins. Inhibition of peroxynitrite-mediated tyrosine nitration was carried out by the HPLC method according to the previous report with slight modifications (*33*). Briefly, various concentrations (5−40 μM) of authentic antioxidant [anthocyanins and (+)-catechin] were added to 0.2 M phosphate buffer (pH 7) containing 300 μM tyrosine, and then a small volume of the $ONOO^-$ solution (final concentration of 50 μM) was added under vigorous mixing and the mixture left for 10 min at room temperature. An aliquot (50 μL) of the reaction solution was then injected into the HPLC system with an UV−visible detector. HPLC was carried out on a Develosil ODS HG-5 column (250 mm × 4.6 mm) using acetonitrile (2%) and 50 mM phosphate buffer (98%) as the elution solution at a flow rate of 1 mL/min, and the effluent was monitored at 420 nm. Then an aliquot of the sample (50 μL) was analyzed by HPLC using a Develosil ODS HG-5 column (250 mm × 4.6 mm) equipped with an UV−vis detector (Hitachi). The elution was performed using a solvent gradient system consisting of solvent A [50 mM phosphate buffer (pH 7)] and solvent B (acetonitrile). The gradient conditions were as follows: 98% A/2% B (v/v) from 0 to 9 min, linear

gradient from 75% A/15% B (v/v) to 40% A/60% B (v/v) from 9 to 14 min, and 98% A/2% B (v/v) from 14 to 15 min. The peroxynitrite scavenging ability of the anthocyanins was expressed as the half-maximal inhibitory concentration (IC_{50}) for 3-nitrotyrosine formation.

RESULTS

Absorption and Metabolism of Eggplant Anthocyanins in Rats. The plasma concentrations of *cis*- and *trans*-nasunins after oral administration were determined by HPLC; however, the plasma level of both isomers was quite variable from plasma sample to sample, when the samples were prepared for HPLC analysis without regard for light conditions, i.e., preparing it in the dark or in the light. Thus, the sample preparation conditions were checked carefully under both light-protected and unprotected conditions. **Figure 2** shows typical HPLC chromatograms of plasma samples (panel **B**, prepared under room light; and panel **C**, light-protected conditions) obtained after oral administration. The HPLC chromatogram of the original eggplant extract is given in **Figure 2A**. From the HPLC pattern, it was obvious that the ratio of *cis*- to *trans*-nasunin was varied, depending on the sample preparation condition.

The light-dependent isomerization of nasunin was confirmed in **Figure 3**, showing the generation of *cis*-nasunin after *trans*-nasunin (**A**) had been kept for 72 h under room light (**B**). To confirm the light-driven isomerization of nasunin, *trans*-nasunin (1% aqueous TFA solution) was irradiated with UV (290 nm) light and the time course change of each nasunin peak was followed by HPLC. Although the data are not shown, *trans*-nasunin converted to *cis*-nasunin in a time-dependent manner and reached an equilibrium state (40/60 for *cis/trans* ratio) within 10 min of UV irradiation. Therefore, the following studies were carried out under the light-protected condition.

When the *cis*-nasunin/*trans*-nasunin mixture was administered to rats (administered doses of 94.7 and 14.2 μmol/kg for *trans*- and *cis*-nasunin, respectively), both isomers were detected in blood plasma as shown in **Figure 4**. Moreover, the *cis*/*trans* ratio in the plasma was almost the same as in the original mixture that was administered. They reached their maximum level after oral administration for 15 min. The maximum plasma concentrations of nasunins were calculated as 0.29 μM for *trans*-nasunin and 0.04 μM for *cis*-nasunin (**Figure 4A**). Delphinidin 3-*O*-β-D-glucopyranoside studied for the purpose of comparison showed the maximum plasma level after oral administration for 15 min, and the maximum plasma level was approximately 0.3 μM, as we previously reported (*17*). To evaluate the uptake efficiency of nasunins, the area under the plasma concentration curve (AUC) and the maximum plasma concentration (C_{max}) were normalized to the orally administered dose (micromoles per kilogram), and the results are summarized in **Table 1** together with those of delphinidin 3-*O*-β-D-glucopyranoside.

Further, urinary metabolites after oral administration of nasunins were precisely analyzed by HPLC, but no newly detectable peak appeared in the chromatogram (data not shown).

Antioxidant Activity of Eggplant Anthocyanins in Vitro. To clarify the role of structural diversity in antioxidant activity, the O_2^- and $ONOO^-$ scavenging activities of eggplant anthocyanins were studied, and the results are listed in **Table 2** together with those of authentic delphinidin 3-*O*-β-D-glucopyranoside, delphinidin (aglycone), and (+)-catechin as reference antioxidants. Although the data are not shown, all antioxidants scavenged O_2^- in a concentration-dependent manner. The O_2^- scavenging activities expressed as IC_{50} values were in the following order: delphinidin (2.48 μM) > delphinidin 3-*O*-β-D-glucopyranoside (3.19 μM) > *trans*-nasunin (3.79 μM) > *cis*-

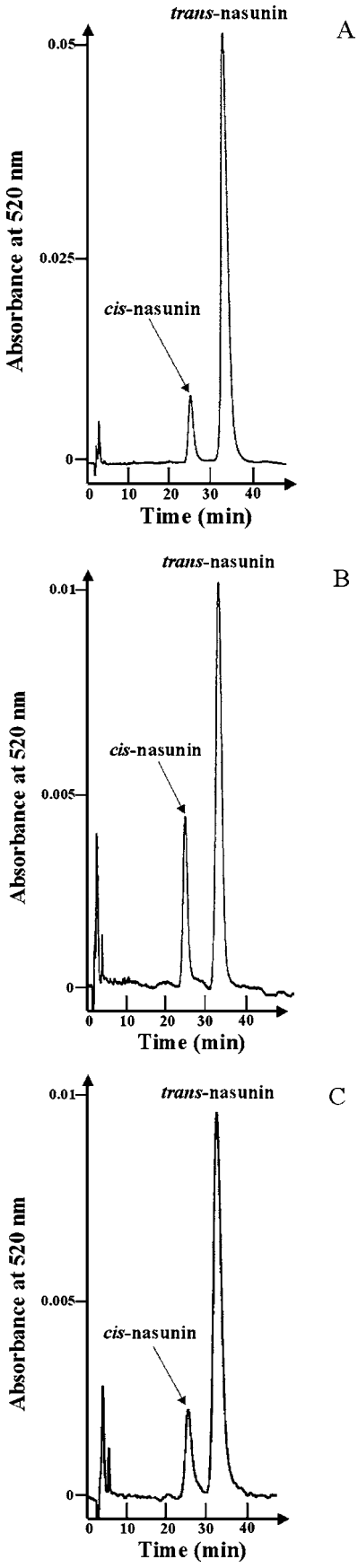

Figure 2. Typical HPLC chromatograms of rat blood plasma after oral administration of the nasunin mixture: (**A**) nasunin mixture, (**B**) plasma after oral administration for 15 min (under light), and (**C**) plasma after oral administration for 15 min (light-protected).

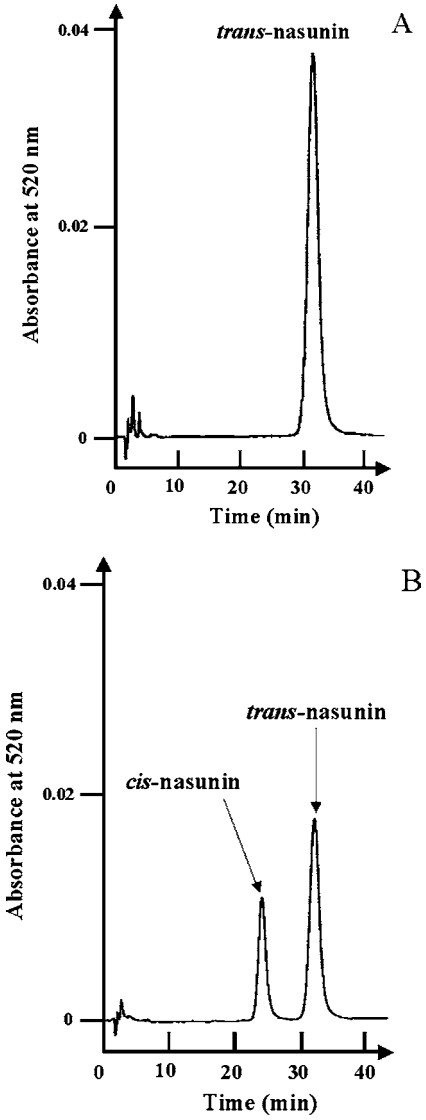

Figure 3. HPLC chromatogram of *trans*-nasunin before and after standing for 72 h under room light: (**A**) *trans*-nasunin and (**B**) *trans*-nasunin after 72 h under room light.

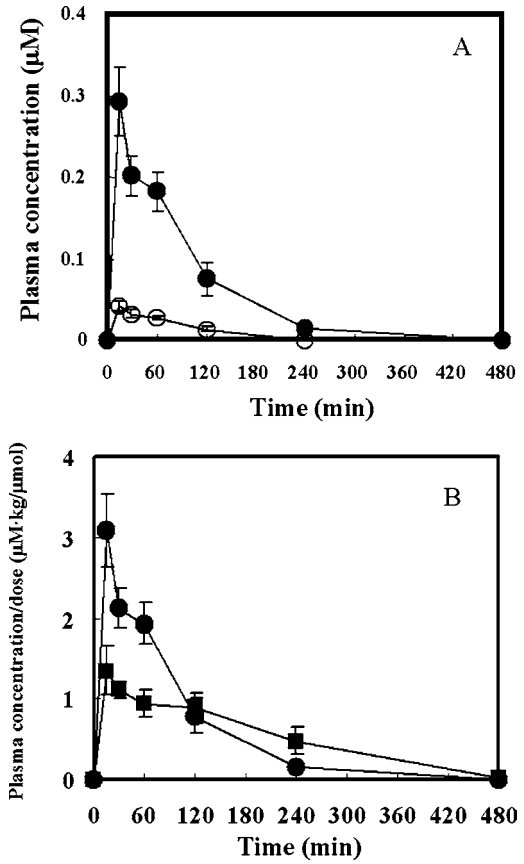

Figure 4. Plasma concentration profile of anthocyanins. (**A**) Eggplant anthocyanins: (●) *trans*-nasunin and (○) *cis*-nasunin. (**B**) Comparison of plasma concentration profiles of *trans*-nasunin and delphinidin 3-*O*-*β*-D-glucopyranoside normalized to the orally administered dose (micromoles per kilogram): (●) *trans*-nasunin and (■) delphinidin 3-*O*-*β*-D-glucopyranoside. Values are means ± the standard error of the mean of four rats.

Table 1. Plasma Uptake Parameters of Anthocyanins in Rats[a]

anthocyanin	C_{max} (μM)	dose (μmol/kg)	AUC (μM min)	C_{max}/dose (μM kg μmol^{-1})	AUC/dose (μM min kg μmol^{-1})
cis-nasunin	0.04	14.2	3.77	0.00281	0.2655
trans-nasunin	0.29	94.7	26.61	0.00316	0.2809
delphinidin 3-*O*-*β*-glucopyranoside	0.29	215.05	54.91	0.00139	0.2553

[a] Values are means of four rats.

Table 2. O_2^- and $ONOO^-$ Scavenging Activities of Anthocyanins[a]

	O_2^-	$ONOO^-$
delphinidin	2.48 ± 0.016	2.23 ± 0.017
delphinidin 3-*O*-*β*-D-glucopyranoside	3.19 ± 0.007	2.28 ± 0.020
trans-nasumin	3.79 ± 0.007	3.95 ± 0.029
cis-nasunin	3.91 ± 0.013	4.20 ± 0.132
(+)-catechin	9.37 ± 0.120	4.33 ± 0.033

[a] Values are means ± standard error of the mean of three experiments.

nasunin (3.91 μM) > (+)-catechin (9.37 μM). Both *cis*- and *trans*-nasunins exhibited almost the same scavenging activity as delphinidin 3-*O*-*β*-D-glucopyranoside against O_2^-, although they exhibited rather low activity versus that of the aglycone (delphinidin). However, the activity was 2 times higher than that of (+)-catechin.

The $ONOO^-$ scavenging activity of anthocyanins and (+)-catechin was also compared by the inhibition of 3-NT production in vitro. As shown in **Table 2**, both eggplant anthocyanins inhibited $ONOO^-$-mediated 3-NT production and the activity expressed as IC_{50} (3.95 μM for *trans*-nasunin and 4.20 μM for *cis*-nasunin) was similar to that of (+)-catechin (4.33 μM). Delphinidin and delphinidin 3-*O*-*β*-D-glucopyranoside, on the other hand, exhibited higher activity (2.23 and 2.28 μM, respectively) than nasunins.

DISCUSSION

Anthocyanins are reddish pigments having a variety of physiological functions, including antioxidant activity (*9*−*13*). Acylated anthocyanins share many features with naturally occurring anthocyanins, but few studies on the absorption of acylated anthocyanins have been reported (*27*, *28*). As we found previously, nasunin consists of *cis* and *trans* isomers (*26*) at the *p*-coumaroyl moiety; thus, the absorption and metabolism of *cis*- and *trans*-nasunins in rats were preciously examined in this study to predicate their availability in vivo. As we showed previously, *cis*−*trans* isomerization easily occurred under light irradiation (*26*); thus, all experiments were carried out under light-protected conditions.

The results that were obtained clearly showed that both nasunin isomers absorbed almost the same level in rats, indicating that the configuration difference in the *p*-coumaroyl moiety did not strongly affect the absorption process. One of the novel findings of this study is that the light-induced *cis*−*trans* isomerization in the *p*-coumaroyl moiety of nasunin sometimes leads to faulty conclusions about their absorption and metabolism data because *cis*−*trans* isomerization had easily occurred under room light conditions and thus the relative amounts of isomers were variable as shown in **Figure 3**. Thus, special care has to be sustained for the bioavailability study of other acylated anthocyanins.

An uptake study of other acylated anthocyanins, peonidin 3-caffeoylsophoroside-5-glucoside in PSP, reported elsewhere suggested the absorption efficiency was several times lower than that of the nonacylated anthocyanins (*27*). The maximum plasma level of PSP anthocyanins was ∼0.05 μM when the dose of 146.3 mg/kg (154.2 μmol/kg) was orally administered. In contrast, the study presented here reveals that nasunins were effectively absorbed from the gastrointestinal tract and appeared in blood plasma in their original acylated form. The maximum plasma levels of nasunins reached 0.29 μM for *trans*-nasunin and 0.04 μM for *cis*-nasunin when 94.7 μmol of *trans*-nasunin and 14.2 μmol of *cis*-nasunin per kilogram were orally administered, respectively.

In this study, absorption of delphinidin 3-*O*-β-D-glucopyranoside was also studied for the purpose of comparison, although its glycosylation pattern is relatively simple. The maximum plasma level of *trans*-nasunin (0.29 μM) after oral administration for 15 min was exactly the same as that of delphinidin 3-*O*-β-D-glucopyranoside when the dose of 100 mg/kg (215.05 μmol/kg) was orally administered. We previously checked the linearity between the administered dose and plasma anthocyanin concentration (unpublished data). It indicated no saturation occurred during the gastrointestinal uptake of anthocyanin up to 400 mg/kg; thus, both C_{max} and AUC were normalized to the orally administered dose. When the C_{max} was normalized to the administered dose (micromoles per kilogram), there was no significant difference between both eggplant anthocyanins, but the values were ∼3 times higher than that of delphinidin 3-*O*-β-D-glucopyranoside. However, when the AUC per dose was compared, both nasunins exhibited the same level as delphinidin 3-*O*-β-D-glucopyranoside (**Table 1**).

It is interesting to note that the AUC per dose of nasunins was approximately 30 times higher than that of PSP anthocyanin. Suda et al. explained the weak absorption of PSP anthocyanins was due to its high molecular weight (949). However, the molecular weight of nasunin (919) is also high; therefore, another reason has to be taken into account.

The major PSP anthocyanins consist of peonidin as aglycone and the caffeoyl moiety (*27*). We recently showed that mono- and di-*O*-methyl anthocyanins such as peonidin and malvidin glycosides were absorbed less effectively than delphinidin and cyanidin glycosides (*34*). This observation thus indicates that the aglycone structure is also the major determinant of acylated anthocyanin absorption. In addition, the structure of the attached acyl moiety is also different between nasunin and PSP anthocyanins (*p*-coumaroyl for nasunins but caffeoyl and feruloyl for PSP anthocyanins) (*27*). The effect of the acyl moiety could not be excluded as another factor modulating the absorption of acylated anthocyanins. Further study is required for the conclusion.

We previously showed that delphinidin 3-*O*-β-D-glucopyranoside was metabolized to 4′-*O*-methyldelphinidin 3-*O*-β-D-glucopyranoside in rats (*17*, *18*). We also reported the precise path of cyanidin 3-*O*-β-D-glucopyranoside metabolism in rats (*19*, *31*). In the study presented here, we analyzed the metabolites of *cis*- and *trans*-nasunins in urine and blood plasma. However, no metabolites were detected for both *trans*- and *cis*-nasunins by highly sensitive HPLC we previously developed (*31*). Thus, we concluded that delphinidin aglycone in nasunins was not modified by catechol-*O*-methyl transferase (COMT), probably because they were not taken up in hepatic cells where the O-methylation reaction mainly occurs. This was supported by another observation that extended glucuronides of nasunins which were expected to be produced in hepatic cells were also not detected in this study. This may partly explain the reason the level of nasunins remained high in the plasma. Further, the production of delphinidin 3-*O*-β-D-glucopyranoside and delphinidin, cleaved products of nasunins, was not detected in this study, indicating that neither deacylation nor glycolytic cleavage reactions occurred for nasunins in vivo.

Nasunin was reported to be a powerful antioxidant because it has two partial structures contributing to the antioxidant activity, that is, anthocyanidin (delphinidin) and acyl (*p*-coumaroyl) moiety (*25*). However, it was not conclusive because a comparative study has not yet been carried out for the antioxidant activity of acylated anthocyanin and the unacylated parent anthocyanin or the aglycone (anthocyanidin). Thus, in this study, the antioxidant activity of acylated anthocyanins (nasunins) was compared with those of delphinidin 3-*O*-β-D-glucopyranoside and the aglycone, delphinidin. However, it was revealed that the acyl moiety did not largely contribute to the antioxidant property of nasunins. Moreover, both *cis*−*trans* isomers showed no significant differences in antioxidant activity.

This study revealed that nasunins are taken up as their original acylated form in the same extent as delphinidin 3-*O*-β-D-glucopyranoside. This indicates the original acylated form functions as an antioxidant in vivo when nasunin is ingested. However, we cannot exclude some possibility that ring fission products of nasunin produced in small intestine might contribute to the in vivo function of nasunin. Further studies aimed at clarifying the antioxidant role of nasunins in vivo are required.

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