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Protocatechuic acid is not the major metabolite in rat blood plasma after oral administration of cyanidin 3-*O*-β-D-glucopyranoside

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Abstract

In this study, the formation of protocatechuic acid (PCA) as a degradation product of cyanidin 3-*O*-β-D-glucopyranoside (Cy3G) was evaluated in plasma after oral or intravenous administration of Cy3G in rats for better understanding of functionality of anthocyanins in vivo. Cy3G (100 mg/kg) was orally administered to rats and the blood was obtained time dependently. The plasma concentration of Cy3G and PCA was determined by high performance liquid chromatography. Cy3G was detected in rat blood plasma 15 min after oral administration and reached its maximum of 0.23 μM. Several metabolites reported in our previous studies were also observed at similar plasma levels. However, PCA was not detected in any rats at any time point, which indicates the formation of PCA from Cy3G is not a major pathway. Further, PCA was not detected in the blood plasma after intravenous administration of Cy3G. These results demonstrated that neither PCA was absorbed as the major intestinal metabolite of Cy3G after oral administration nor formed as the metabolite in liver and thus PCA does not exhibit high contribution to the functionality of Cy3G in vivo.

Keywords: Protocatechuic acid; Cyanidin 3-*O*-β-D-glucopyranoside; Ring fission product; Absorption; Metabolism

1. Introduction

Due to the recent studies on health benefits of active compounds in dietary food (Al-Awwadi et al., 2004; Arts & Hollman, 2005; Manach, Mazur, & Scalbert, 2005), flavonoids are generally accepted as functional food factors (Klinge et al., 2005; Lambert & Yang, 2003; Ono, Hasegawa, Naiki, & Yamada, 2004). The reddish pigment known as anthocyanins is a family of flavonoids widely distributed in colored fruits (Garcia-Viguera, Zafrilla, & Tomas-Barveran, 1998; Ichiyanagi, Hatano, Matsugo, & Konishi, 2004; Slimestad & Solheim, 2002) and vegetables (Ichikawa et al., 2001; Ichiyanagi et al., 2005; Terahara et al., 1999). Health beneficial properties of anthocyanins have also been reported both in human (Matsumoto et al., 2005; Nakaishi, Matsumoto, Tominaga, & Hirayama, 2000) and experimental animals (Kang, Hur, Kim, Ryu, & Kim, 2006; Mazza, Kay, Cottrell, & Holub, 2002). Vision improvement is one of the most famous functionalities of anthocyanins. Recently, Matsumoto, Nakamura, Tachibanaki, Kawamura, and Hirayama (2003) demonstrated that stimulating effect of anthocyanin on the regeneration of rhodopsion contributed to its function. On the other hand, we have demonstrated reactivity of anthocyanins towards both reactive oxygen (Ichiyanagi, Hatano, Matsugo, & Konishi, 2003, 2004a) and reactive nitrogen (Ichiyanagi, Hatano, Matsugo, & Konishi, 2004b) species in vitro and clarified structure-antioxidant

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activity relationship of anthocyanins (Rahman, Ichiyanagi, Hatano, Komiyama, & Konishi, 2006). Apoptotic effects of anthocyanidins and anthocyanins on cancer cell lines have also been reported both in vivo (Ding et al., 2006) and in vitro (Hou et al., 2003; Hou, Tong, Terahara, Luo, & Fujii, 2005). Thus, nutraceutical importance of anthocyanins has been extensively discussed because of their high consumption from daily diet (Wu et al., 2006).

Studies on biological behaviors such as absorption and metabolism of anthocyanin have also widely reported (Felgines et al., 2003; Ichiyanagi et al., 2004a, 2004b, 2005b; Ichiyanagi, Shida, Rahman, Hatano, & Konishi, 2005a; Kay, Mazza, Holub, & Wang, 2004; Matsumoto et al., 2001; Miyazawa, Nakagawa, Kudo, Muraishi, & Someya, 1999; Talavera et al., 2005) for further understanding of anthocyanin functionalities. Recently, we have reported absorption and tissue distribution of 14 types of anthocyanins contained in bilberry (wild type blueberry) in rats (Ichiyanagi, Shida, Rahman, Hatano, & Konishi, 2006) and clarified that both aglycone and attached sugar affect their bioavailability. We also reported that nasunin, typical acylated anthocyanin in eggplant, was absorbed to the same extent as that of non-acylated anthocyanins such as delphinidin 3-*O*-β-D-glucopyranoside (Dp3G) (Ichiyanagi, Terahara, Rahman, & Konishi, 2006). Taking our results together with others into consideration, it was concluded that anthocyanins are absorbed as their intact glycoside or acylated forms.

On the other hand, studies on flavonoid metabolism have indicated that glycolytic cleavage of sugar and degradation of flavonoid skeleton by intestinal bacteria plays an important role in their metabolism and absorption (Aura et al., 2002; Lin, Hsiu, Hou, Chen, & Lee Chao, 2003; Meng et al., 2002; Winter, Moore, Dowell, & Bokkenheuser, 1989). The ring fission products thus attract attention in relation to their physiological roles. It is known quercetin 3-glycoside incubated with human or rat intestinal microflora was degraded to phloroglucinol and 3,4-dihydroxyphenylacetic acid (Aura et al., 2002). Also, valerolactone has been reported as a ring-fission metabolite of epicatechin in both rats and human (Meng et al., 2002). Studies on intestinal degradation of anthocyanins have also reported (Aura et al., 2005; Perez-Vicente, Gil-Izquierdo, & Garcia-Viguera, 2002). In those studies, protocatechuic acid (PCA) was identified as a major ring fission product of cyanidin 3-*O*-β-D-glucopyranoside (Cy3G) when incubated with intestinal microflora (Aura et al., 2005; Perez-Vicente et al., 2002). A few studies indicated plasma appearance of PCA after oral administration of Cy3G but the results were not conclusive (Kay et al., 2004; Tsuda, Horio, & Osawa, 1999). However, this issue is quite important for discussing the physiological function of anthocyanins. Thus, in this study, we focused on our aim to assess whether PCA is detected as the major metabolite of Cy3G in rat blood plasma after oral and intravenous administration of authentic Cy3G.

2. Materials and methods

2.1. Chemicals

PCA was obtained from Sigma Co. Ltd. All other reagents including trifluoroacetic acid (TFA) were purchased from Wako Pure Chemical Industries Co. Ltd., Japan and used without further purification. Cy3G (Fig. 1) was purified from purple black rice (PBR) according to our previously described method with a slight modification (Ichikawa et al., 2001). Briefly, the peel was removed from PBR by a grinder and the colored peel was successively immersed in 3% trifluoroacetic acid (TFA) aqueous solution. The extract was filtered and evaporated to dryness *in vacuo* and re-dissolved in distilled water containing 1% TFA. The extract was washed with ethyl acetate to recover the anthocyanins in the water fraction. After the removal of residual ethyl acetate by evaporation, the water fraction was further separated by an open column packed with polyvinyl pyrrolidone (30 cm × 5.7 cm) (ISP, Japan). The column was washed with 1% TFA aqueous solution for the elution of water-soluble components and further Cy3G was eluted by 1% TFA containing 30% methanol aqueous solution. The Cy3G rich fraction was collected and evaporated to dryness in vacuo under 40 °C. Cy3G recovered was dissolved in a small amount of 0.1% hydrochloric acid containing methanol solution and diethyl ether was added to precipitate it as a chloride salt. The purity of Cy3G was checked by quantitative tandem time of flight mass spectrometry (Q-Tof Ultima, Micromass, Manchester, UK) and extensive 1D and 2D NMR (JEOL ECA 500) as described in our previous report (Ichiyanagi et al., 2004).

2.2. Quantification of plasma protocatechuic acid by previous method

The recovery and high performance liquid chromatographic (HPLC) separation of PCA was carried out

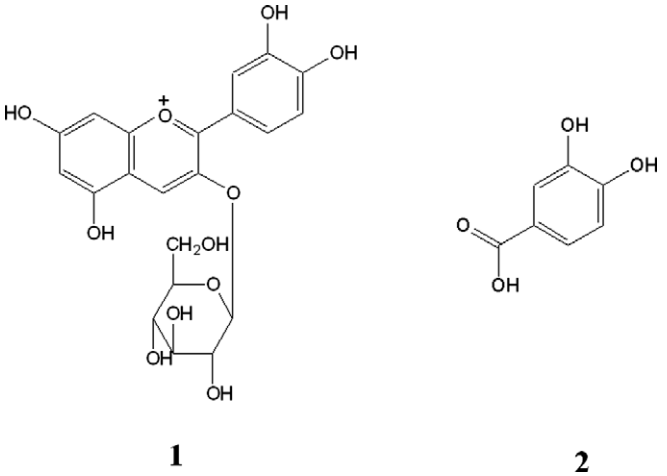

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Fig. 1. Chemical structure of cyanidin 3-*O*-β-D-glucopyranoside and protocatechuic acid. **1**: Cyanidin 3-*O*-β-D-glucopyranoside, **2**: protocatechuic acid.

according to the method previously described (Tsuda et al., 1999). Briefly, the plasma (400 μL) obtained from starved rats was mixed with an equal volume of 10 mM oxalic acid, and then the mixtures were subjected to Sep-Pak C18 cartridges (Waters, United States). After washing with 10 mM oxalic acid, anthocyanin and the metabolites were eluted with 1% TFA containing methanol. The effluent was evaporated to dryness in vacuo below 40 °C. The dried extract was dissolved using 100 μL of 15% acetonitrile containing 50 mM sodium phosphate (pH 1.7). An aliquot (20 μL) of this solution was injected into an HPLC system. HPLC was carried out on a Develsil ODS-HG-5 (Nomura Chemical Co. Ltd., 250 mm × 4.6 mm) using 15% acetonitrile containing 50 mM sodium phosphate (pH 1.7) as elution solvent at a flow rate of 1 mL/min. The effluent was monitored at 260 nm with a UV–vis detector (Hitachi, Japan). The recovery of through above method was checked by addition of authentic PCA with rat blood plasma.

2.3. Animals and diets

SPF male Wistar ST rats (5 weeks old, 100 g of average body weight) purchased from Japan SLC Inc. (Hamamatsu, Japan) were individually housed in stainless-steel wire-mesh cages at 23 ± 1 °C for conditioning under a 12-h light/dark cycle. The rats were allowed free access to tap water and control diet for 7 days before the experiment. Rats were treated in accordance with the Guidelines of Niigata University of Pharmacy and Applied Life Sciences.

2.4. Experimental design and plasma preparation

After 7 days of the conditioning period, carotid vein cannulation was carried out according to our previous method (Ichiyanagi et al., 2005b). Briefly, eight rats were cannulated with a polyethylene tube (PE-50) into a neck vein under anesthesia with diethyl ether. The neck vein was isolated and a small hole was made by the use of scissors to insert a polyethylene tube. After the insertion of the tube in the hole, the vein and the tube were occluded and the inserted tube was penetrated through the skin and guided out of the back of the rats. After starvation for 24 h, the rats were randomly assigned into two groups, and Cy3G (100 mg/kg body weight) dissolved in 0.1% citric acid was orally administered to four rats by direct stomach intubation. In the case of intravenous administration, Cy3G (2 mg/kg body weight) dissolved in physiological saline was injected to the neck vein of another four rats. Blood samples were collected via the cannulated tube using a heparinized syringe before and after the administration at 15, 30, 60, 120, 240, 480 and 720 min for the oral administration and 1, 15, 30, 60, 120 and 240 min for the intravenous administration of Cy3G. After the withdrawal of each blood sample (600 μL), the same volume of donor blood was injected through the tube. That donor blood was obtained from the inferior vena cava of other healthy rats using sodium citrate (500 μL of 10% sodium citrate for 8 mL blood). Each blood sample was centrifuged at 3000*g* for 5 min at 4 °C for HPLC analysis. The administered dose was determined for both orally and intravenously administered experiments based on the detection limit of the present HPLC method (Ichiyanagi et al., 2005a).

2.5. Determination of Cy3G in blood plasma

Sample preparation for HPLC analysis of Cy3G from plasma samples was followed according to our previous report (Ichiyanagi et al., 2005b) with a slight modification. Briefly, the obtained plasma samples (100 μL) were acidified with equal volume of 1% TFA aqueous and 150 μL were applied to Sep-Pak C_{18} cartridges light (Waters, USA) preconditioned with methanol (3 mL) and 3% TFA aqueous solution (3 mL). Then, the cartridges were washed successively with 1 mL of 3% TFA aqueous solution, and Cy3G and its metabolites were eluted with 1% TFA containing 50% acetonitrile aqueous solution. The recovered solution were evaporated to dryness in vacuo, dissolved in 150 μL of 0.5% TFA containing distilled water and then an aliquot (100 μL) of the solutions was injected into an HPLC system (Hitachi 7200). In the case of intravenously administered plasma samples, further dilution was carried out depending on the colors of the samples for the anthocyanin analysis. For the detection of anthocyanin and its metabolites, HPLC was carried out with Develosil ODS HG-5 column (Nomura Chemical Co. Ltd., Japan, 150 mm × 1.0 mm) using 0.5% TFA containing 18% methanol aqueous solution as the elution solvent at a flow rate of 0.1 mL/min, and the elution peaks were monitored at 520 nm with a UV–visible detector (Hitachi). The recovery of Cy3G through the above method was checked by addition of authentic Cy3G with rat blood plasma and it was determined as 82.4%. Calibration curves for Cy3G was made using authentic Cy3G.

2.6. Determination of PCA in blood plasma

Extraction of PCA was carried out essentially according to Hodgson, Morton, Puddey, Beilin, and Croft (2000) with modifications. Cy3G was administered to rats and the plasma of the rats (200 μL) was acidified with 200 μL of 3% TFA aqueous solution and then, 400 μL of ethyl acetate was added and vigorously mixed for 30 s. After centrifugation at 3000*g* for 1 min, ethyl acetate was collected in another tube. This procedure was repeated three times and combined ethyl acetate was evaporated to dryness in vacuo. The residue was re-dissolved in 0.5% TFA aqueous solution (300 μL) and then aliquot (100 μL) of the solutions was injected into an HPLC system. For the detection of PCA, HPLC was carried out with Develosil ODS HG-5 column (Nomura Chemical Co. Ltd., Japan, 150 mm × 4.6 mm) using 0.5% TFA containing 5% methanol aqueous solution as the elution solvent at a flow rate of 1 mL/min, and the elution peaks were monitored at 260 nm with a

UV–visible detector (Hitachi). The recovery of PCA through the above methods was checked by addition of authentic PCA with rat blood plasma and it was determined as 87.9%. Calibration curves for PCA were made using commercially available PCA.

3. Results

Cy3G was detected in the rat plasma both after oral and intravenous administration of Cy3G. The maximum plasma concentration of Cy3G reached its maximum of 0.23 μM at 15 min after oral administration and was almost the same level as we previously reported (0.19 μM) (Ichiyanagi et al., 2005b). Together with original Cy3G, several metabolites such as extended glucuronides of Cy3G, *O*-methyl Cy3G, cyanidin-glucuronide and *O*-methyl cyanidin-glucuronide were also detected in the blood plasma (data not shown) (Ichiyanagi et al., 2005a, 2005b).

In the present study, the conditions for separation and recovery of plasma PCA were checked at the beginning according to the method previously described by Tsuda et al. (1999). Fig. 2a showed typical HPLC chromatogram of rat blood plasma monitored at 260 nm using the same condition reported (Tsuda et al., 1999) and Fig. 2b showed HPLC chromatogram of PCA added rat blood plasma. However, we found that PCA cannot be separated completely from plasma-originated peaks under the HPLC conditions used by Tsuda's report (15% acetonitrile containing 50 mM phosphate buffer (pH 1.7)). The recovery of PCA was also evaluated using solid phase extraction, but the recovery was quite low (<60%) and varied depending on the quality and the condition of solid phase cartridges.

Thus, we reexamined the extraction method for PCA and HPLC separation conditions. Finally, we extracted PCA by ethyl acetate according to the method of Hodgson et al. (2000) with modifications. HPLC conditions for separating PCA from plasma-originated peaks with the same retention time have also been established as follows: 0.5% TFA containing 5% methanol aqueous solution as elution solvent at a flow rate of 1 mL/min. The recovery of PCA by the established method was 87.9% and the detection sensitivity was also enough (50 nM) for the quantitative purpose of protocatechuic acid in blood plasma.

Fig. 3a shows typical HPLC chromatogram of PCA added rat blood plasma (2 μM as final concentration of PCA) monitored at 260 nm. PCA was clearly separated

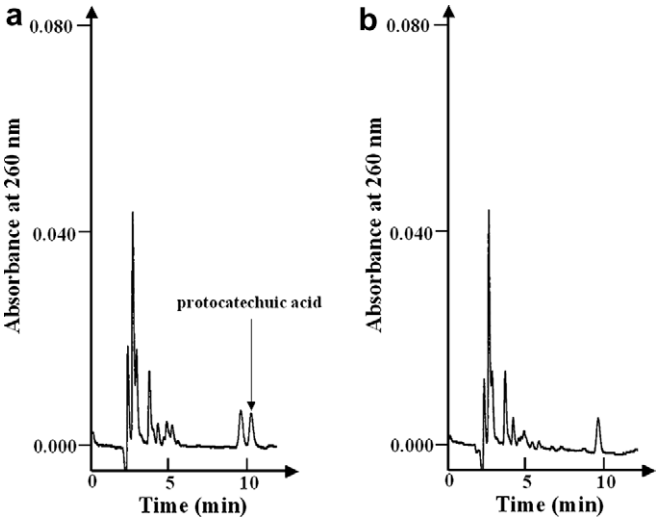

Fig. 3. Typical HPLC chromatogram of rat blood plasma by established method. (a) Rat blood plasma added with protocatechuic acid (0.2 μM for final concentration), (b) after 60 min of oral administration of cyanidin 3-*O*-β-D-glucopyranoside.

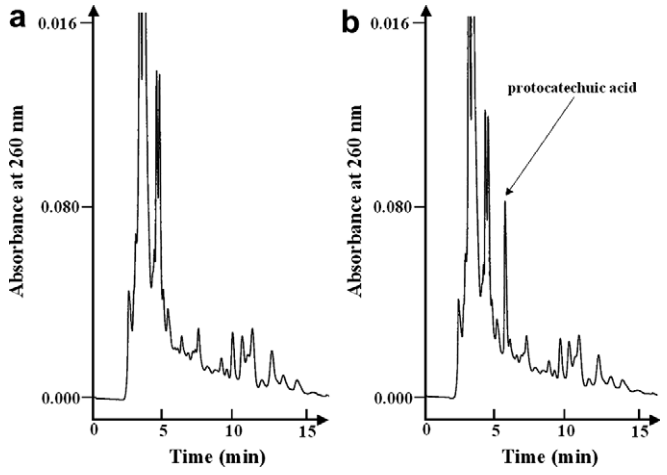

Fig. 2. HPLC chromatogram of rat blood plasma by the method previously reported. (a) Normal blood plasma, (b) rat blood plasma added with authentic protocatechuic acid (2 μM for final concentration). HPLC was carried out on a Develosil ODS HG-5 (4.6 mm × 250 mm) using 15% acetonitrile containing 50 mM sodium phosphate (pH 1.7) at a flow rate of 1 mL/min and detected at 260 nm.

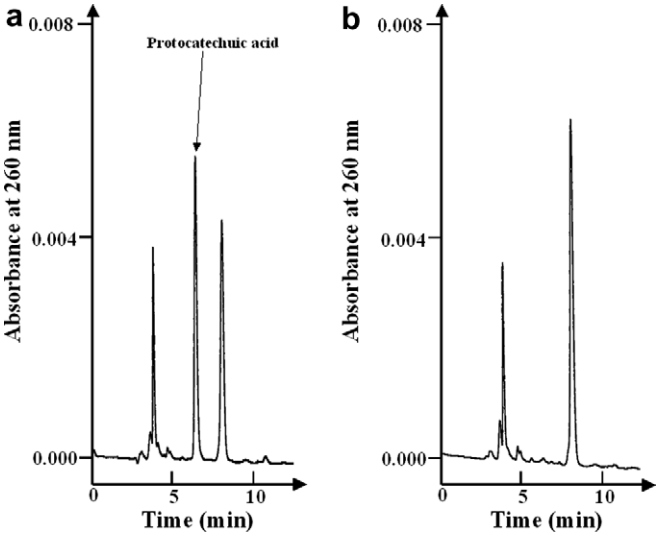

Fig. 4. Typical HPLC chromatogram of plasma samples prepared by the presently established extraction method and analyzed under the previously reported HPLC condition. (a) Rat blood plasma added with protocatechuic acid (2 μM for final concentration), (b) after 60 min of oral administration of cyanidin 3-*O*-β-D-glucopyranoside.

from back ground plasma peaks with retention time of around 10 min. Also, several plasma peaks observed in the original solid phase extraction procedure were disappeared from plasma samples. This established method was applied for the analysis of PCA in Cy3G administered rat blood plasma. Fig. 3b showed typical HPLC chromatogram of rat blood plasma 60 min after oral administration of Cy3G. It is clear that no PCA was detected on the HPLC chromatogram. PCA was not detected in Cy3G administered rat blood plasma in any rat at any time point even after 12 h. The same plasma sample was reevaluated by the HPLC condition reported by Tsuda et al. (1999), but PCA was not detected (Fig. 4a and b). Although the data were not shown, PCA also was not detected in the plasma after intravenous administration of Cy3G (2 mg/kg).

4. Discussion

Biological behavior of anthocyanins has been widely discussed both in human (Matsumoto et al., 2005; Mazza et al., 2002; Nakaishi et al., 2000) and experimental animals (Ding et al., 2006; Kang et al., 2006; Matsumoto et al., 2003) for the understanding of their functionalities. Recent results indicate that anthocyanins are absorbed in their original glycoside forms and the absorption rate is dependent on both the type of aglycone (Felgines et al., 2003; Ichiyanagi et al., 2004a, 2005b; Kay et al., 2004; Miyazawa et al., 1999; Talavera et al., 2005) and the type of the attached sugar (Ichiyanagi, Shida, et al., 2006; Ichiyanagi, Terahara, et al., 2006; Matsumoto et al., 2001). We further revealed that the metabolism of anthocyanins was also variably dependent on aglycone structure of anthocyanin, more correctly, B ring structure of aglycone (Felgines et al., 2003; Ichiyanagi, Shida, et al., 2006; Ichiyanagi et al., 2004a, 2004b, 2005a, 2005b).

On the other hand, it is generally accepted that the degradation of flavonoids in small intestine is another important metabolic pathway in vivo (Booth, Jones, & DeEds, 1958; Das & Griffiths, 1969; Nakagawa, Shetlar, & Wnder, 1965). For example, quercetin-glucoside was degraded to phrologlucinol and phenolic acid in small intestine (Nakagawa et al., 1965). Epicatechin, on the other hand, is metabolized to valerolacton as ring-fission metabolites (Meng et al., 2002). Recent study also indicates that rosmarinic acid, a major component in *Perilla frutescens*, is also degraded to give the ring fission products which function in vivo (Baba, Osakabe, Natsume, & Terao, 2004).

Anthocyanins are quite unstable under neutral pH and quickly degraded into ring fission product in vitro (Francis, 1989). Several researchers have reported the degradation of Cy3G when they incubated Cy3G with human or rat intestinal microflora (Aura et al., 2005; Perez-Vicente et al., 2002) and demonstrated that PCA is a major product in vitro. However, these studies have been carried out under aerobic conditions where oxidation of anthocyanin can easily occur. Plasma appearance of PCA after oral administration of Cy3G is still uncertain, although there are a few studies reported (Kay et al., 2004; Tsuda et al., 1999). Limited amount of purified anthocyanins restricted precise study on the ring-fission metabolism of anthocyanins. Thus, in this study, we focus our attention on whether PCA is absorbed into rat plasma as a major ring-fission metabolite after oral administration of authentic Cy3G. Further, the production of PCA in peripheral tissue such as liver after uptake into blood circulation was also studied by intravenous administration of Cy3G.

Orally administered Cy3G was appeared in blood plasma as its original glucoside form and reached its maximum concentration of 0.23 μM at 15 min after administration. Several metabolites reported in our previous studies (Ichiyanagi et al., 2005a, 2005b) were also detected in blood plasma (data not shown). Matsumoto et al. (2001) reported the maximum plasma concentrations were 0.85, 0.85 and 0.58 μM, respectively, for Cy3G, cyanidin 3-*O*-rutinoside and delphinidin 3-*O*-rutinoside with the dose of 400 mg/kg of purified samples in rats (Matsumoto et al., 2001). Taking these facts discussed above together into consideration, it was concluded that saturation on anthocyanin absorption from gastrointestinal tract does not occur up to 400 mg/kg. Tsuda et al. (1999) reported Cy3G was detected at maximum plasma level of 0.3 μM after oral administration of 400 mg/kg of purified Cy3G, although the dose was quite high compared with that from daily diet. However, this value is quite low compared with orally administered dose (400 mg/kg) of Cy3G. On the other hand, they (Tsuda et al., 1999) have reported PCA (2.4 μM) appeared in the plasma with approximately 8 times higher concentration than original Cy3G after oral administration of Cy3G and concluded that PCA is a major functional metabolite produced from Cy3G in vivo.

On the other hand, Kay et al. (2004) have reported that only a trace of PCA was detected in human urine when 20 g of chokeberry extract (1.3 g for cyanidin 3-glycoside and 899 mg as Cy3G) was orally ingested. However, the conclusion is left uncertain whether PCA detected in their study was metabolically produced directly from Cy3G in the intestinal tract. Artificial production of PCA in the extract during a processing step or degradation of Cy3G during retention in urine bladder must be taken into account because of the low stability of Cy3G in neutral pH. This lead to fault result, thus, in the present study, we especially focused on plasma samples and did not analyze PCA accumulation in urine.

Previous experimental procedure reported by Tsuda et al. was found inadequate to separate PCA from background plasma components (Fig. 2). Thus at first, we reestablished the extraction method and HPLC separation conditions as described in the result section. By the revised method, PCA was successfully separated from plasma-originated peaks with the detection limit of 50 nM with 87.9% recovery. Using this established method, plasma uptake of PCA was evaluated after oral administration of Cy3G. However, no detectable PCA was observed in rat

blood plasma even after 1 h of oral administration of Cy3G (100 mg/kg) (Fig. 3b). Finally, any detectable amount of PCA was not observed in any rat plasma at any time point (up to 12 h).

Tsuda et al. (1999) described in their report that plasma sample was acidified and stored at −80 °C until use. Under such condition, Cy3G will be oxidized and degraded during sample storage or during further sample preparation procedures, especially at thawing step. Several studies have also demonstrated that the production of PCA from Cy3G after fermentation with intestinal microflora in vitro under aerobic conditions (Aura et al., 2005; Perez-Vicente et al., 2002). Keppler and Humpf (2005) have reported the production of PCA when Cy3G was incubated under anaerobic conditions. However, the production of PCA was only 20% of original Cy3G added in the system indicating PCA is not the sole ring-fission metabolite formed in small intestine. On the other hand, we have previously reported uptake and metabolism of Dp3G in rats (Ichiyanagi et al., 2004a). In that study, we found intestinal contents were almost colorless at 4 h after oral administration. This indicates rapid degradation of Dp3G under physiological condition. Wu, Pittman, and Prior (2006) also reported that Cy3G was less stable than other glycosides of cyanidin such as cyanidin 3-rutinoside and cyanidin 3-sambubioside. Taking all these previous and present results together into consideration, it will be reliable to conclude that PCA might be produced in small intestine for certain extent after oral administration of Cy3G but the estimated amount was considerably low compared with that of orally administered original Cy3G. Thus, detectable amount of PCA was not absorbed from gastrointestinal tract into blood plasma. In the present study, intravenous administration of Cy3G was also carried out to confirm production of PCA in peripheral tissues such as liver, although it was deduced that PCA might be mainly produced in gastrointestinal tract after oral administration of Cy3G. However, no PCA was detected in the plasma even after 4 h of intravenous injection (data not shown).

From the results obtained in the present and previous studies, expected metabolic fate of orally administered Cy3G in small intestine was proposed in Fig. 5. Orally administered Cy3G is primarily absorbed directly from small intestine as its intact glucoside form (Kay et al., 2004; Matsumoto et al., 2001; Miyazawa et al., 1999). Some part of Cy3G is hydrolyzed by β-glucosidase and the released aglycon is subjected further conjugation by UDP-glucuronyl transferase to form cyanidin-glucuronide (Ichiyanagi et al., 2005b). On the other hand, part of cyanidin produced is degraded into ring fission product but the production of PCA is thought to be a minor, although we cannot avoid the possibility that Cy3G is directly degraded into unidentified ring-fission metabolites and absorbed into blood plasma.

Conclusion is that PCA is not the major metabolite of orally administered Cy3G in the plasma and thus does not contribute significantly to the biological function of Cy3G. Identification of other colorless metabolites of anthocyanins which contribute to their in vivo functionalities are now in progress.

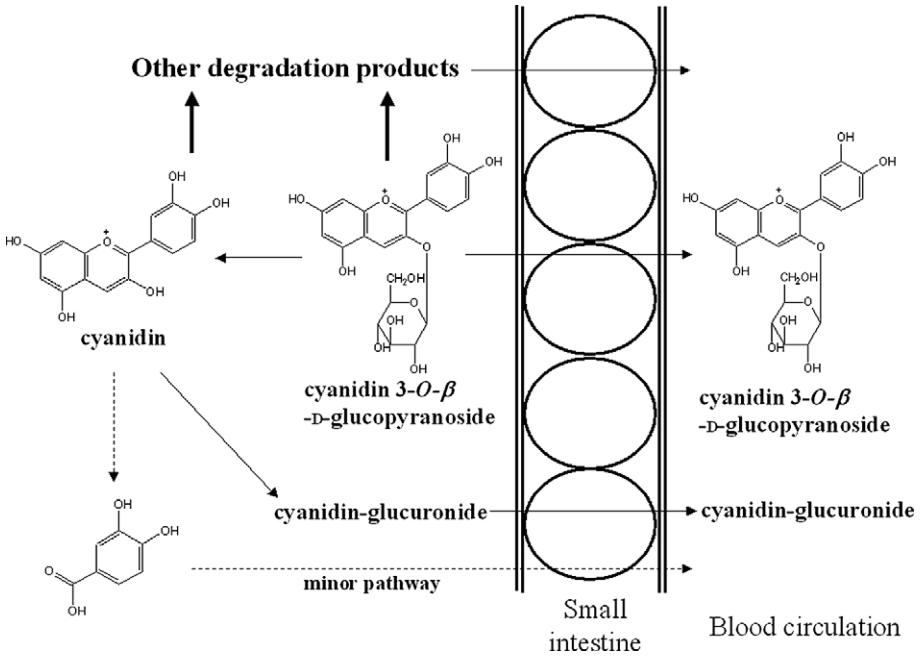

Fig. 5. Proposed fate of orally administered cyanidin 3-*O*-β-D-glucopyranoside in rat small intestine. (◀- - - -) minor pathway, (◀——) major pathway.

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