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REVIEW ARTICLE

Bioavailability of anthocyanins

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Abstract

Anthocyanins are a subgroup of flavonoids responsible for the blue, purple, and red color of many fruits, flowers, and leaves. Consumption of foods rich in anthocyanins has been associated with a reduced risk of cardiovascular disease and cancer. The fate of anthocyanins after oral administration follows a unique pattern rather different from those of other flavonoids. Anthocyanins could be absorbed from the stomach as well as intestines. Active transporters may play a role in the absorption of anthocyanins from the stomach as well as in their transfer within the kidney or liver. Anthocyanins such as cyanidin-3-glucoside and pelargonidin-3-glucoside could be absorbed in their intact form into the gastrointestinal wall; undergo extensive first-pass metabolism; and enter the systemic circulation as metabolites. Phenolic acid metabolites were found in the blood stream in much higher concentrations than their parent compounds. These metabolites could be responsible for the health benefits associated with anthocyanins. Some anthocyanins can reach the large intestine in significant amounts and undergo decomposition catalyzed by microbiota. In turn, these decomposition products may contribute to the health effects associated with anthocyanins in the large intestine. This review comprehensively summarizes the existing knowledge about absorption, distribution, metabolism, and elimination of anthocyanins as well as their decomposition within the gastrointestinal lumen.

Abbreviations: Cy: cyanidin; **De**: delphinidin; **Pt**: petunidin; **Pn**: peonidin; **Pg**: pelargonidin; **Ma**: malvidin; **glc**: glucose; **gal**: galactose; **ara**: arabinose; **rut**: rutinose; **rham**: rhamnose; **xyl**: xylose; **PCA**: protocatechuic acid

Keywords

Absorption, anthocyanin, bioavailability, cyanidin-3-glucoside, distribution, excretion, first-pass metabolism, metabolism

History

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Introduction

Over the past 20 years, there has been increasing interest in the health benefits of plant-derived polyphenols. Anthocyanins are a subgroup of flavonoids responsible for the blue, purple, and red color of many fruits, flowers, and leaves. Consumption of foods rich in anthocyanins has been associated with a reduced risk of cardiovascular disease (Cassidy et al., 2011; Jennings et al., 2012; McCullough et al., 2012; Mink et al., 2007) and cancer (Touvier et al., 2012; Zamora-Ros et al., 2012). Food intervention studies have shown that anthocyanins can improve oxidative and inflammatory biomedical indices in patients with various health conditions (Basu et al., 2010a; Biedermann et al., 2013; Dohadwala et al., 2011; Stull et al., 2010). The health benefits of anthocyanins have been the subject of many excellent review articles (Basu et al., 2010b; Chen & Chen, 2013, Del Rio et al., 2013; Giacalone et al., 2011; Kay et al., 2012; Tsuda 2012).

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Anthocyanin content varies widely in food in both type and concentration (Bhagwat et al., 2013; Eldridge et al., 2003; Wu et al., 2006). As a result, intake of anthocyanins also varies widely in different regions and seasons and among individuals with different social, cultural, and educational backgrounds (Beking & Vieira, 2011, Perez-Jimenez et al., 2011; Zamora-Ros et al., 2011). Fruits and vegetables with high anthocyanin content can be readily identified by their blue, purple, and red colors. Detailed flavonoid composition can be found in the food composition databases published by the U.S. Department of Agriculture (Bhagwat et al., 2013; Eldridge et al., 2003; Holden et al., 2005). High intake of anthocyanins can be achieved with regular consumption of selected fruits, such as blueberries, blackberries, raspberries, strawberries, red grapes, and saskatoon berries.

The fate of anthocyanins after oral administration follows a unique pattern rather different from those of other flavonoids. While there are many review articles on the disposition of flavonoids and other antioxidants, most of them only dedicate a section of the review to anthocyanins (Crozier et al., 2010; Del Rio et al., 2013; Hribar & Ulrih, 2014; Kay, 2006; Kroon et al., 2004; Manach et al., 2005; Prior & Wu, 2006). This paper offers a comprehensive review of the disposition of anthocyanins.

Terminology related to bioavailability

Bioavailability is defined by the Food and Drug Administration (FDA) as ''rate and extent to which the active ingredient or moiety is absorbed from a drug product and becomes available at the site of action.'' This definition is given in the context of bioequivalence requirements used for approval of new generic products and takes into consideration not only the extent but also the rate of absorption. In pharmaceutical sciences textbooks, bioavailability is often calculated as the extent of absorption. Bioavailability seems to have adopted a broader meaning in the community of researchers on flavonoids. For the purpose of starting a discussion, here is an attempt to define terms related to the absorption of xenobiotics based on previous discussions in the literature (Shargel et al., 2012; Studdert et al., 2012):

Bioavailability: The extent to which a xenobiotic can be used by the body.

Systemic availability: The proportion of the dose of a xenobiotic that reaches the systemic circulation intact after oral administration.

Apparent systemic bioavailability (apparent bioavailability): For xenobiotics that undergo extensive first-pass metabolism, apparent bioavailability is the proportion of the dose that reaches the systemic circulation intact after oral administration.

Total systemic bioavailability (total bioavailability): The proportion of the dose of a xenobiotic that is absorbed through the gastrointestinal wall and enters the systemic circulation both in its original form and as metabolite(s) produced by first-pass metabolism.

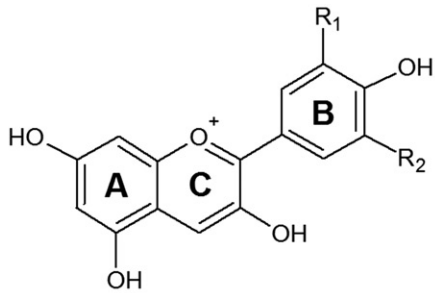
Disposition: The process of getting a xenobiotic or its active metabolite(s) to their site(s) of action(s) in the body in appropriate concentration(s).

With these definitions, bioavailability and disposition are suitable terms to cover the complex processes involved in the fate of anthocyanins within the gastrointestinal tract and in the body.

Chemistry of anthocyanins

About 17 anthocyanidins have been identified, but only six of them are ubiquitously distributed: cyanidin (Cy), delphinidin (De), petunidin (Pt), peonidin (Pn), pelargonidin (Pg) and malvidin (Ma) (Castaneda-Ovando et al., 2009) (Figure 1). They occur mainly as glycosides of their respective aglycone anthocyanidin-chromophores. Glucose (glc), galactose (gal), arabinose (ara), rutinose (rut), rhamnose (rham), and xylose (xyl) are the most common sugars that are bonded to anthocyanidins as mono-, di-, or trisaccharide forms. The sugar moieties mainly attach to the 3-position on the C-ring or the 5, 7-position on the A-ring.

The chemical stability of anthocyanins is of considerable interest given their health benefits and increasing applications as artificial colorants (Castaneda-Ovando et al., 2009). Anthocyanins vary widely in stability. Some are highly instable. *In vitro* studies with pelargonidin, cyanidin, and delphinidin showed that increased B-ring hydroxylation is associated with decreased stability (Woodward et al., 2009). Their stability can also be affected by factors such as pH, temperature, concentration, light, solvents, presence of oxygen, enzymes, other flavonoids, proteins, and metallic ions.



Name	R_1	R_2
Pelargonidin (Pg)	H	H
Cyanidin (Cy)	OH	H
Delphinidin (De)	OH	OH
Peonidin (Pn)	OCH_3	H
Petunidin (Pt)	OH	OCH_3
Malvidin (Ma)	OCH_3	OCH_3

Figure 1. Structures of anthocyanidins.

In aqueous solution, anthocyanins undergo structural re-arrangements in response to changes in pH in four molecular structures: the flavylium cation, quinoidal base, carbinol and chalcone forms (Figure 2). Anthocyanins are most stable in acidic solutions (pH 1–3) where they exist primarily as flavylium cations. At pH above 4, anthocyanins adopt the forms of the carbinol and chalcone. Chalcone can then undergo chemical degradations to produce phenolic acids.

Absorption and first-pass metabolism

Anthocyanins can be absorbed intact despite having different molecular sizes and types of sugar or acylated groups attached (Kurilich et al., 2005; Matsumoto et al., 2001; Mazza et al., 2002; Stalmach et al., 2012). The rate and extent of absorption of anthocyanins are affected by the glycone, sugar moiety, and acylated groups (Tian et al., 2006; Wu et al., 2004, 2005). The extent of absorption may be decreased for the complex anthocyanins (Kurilich et al., 2005).

The maximal plasma concentration is attained within 0.5–2 hours after consumption of anthocyanin-rich fruits. The systemic bioavailability of anthocyanins is estimated to be 0.26–1.8% in animal studies (Borges et al., 2007; Felgines et al., 2002, 2003; Ichiyanagi et al., 2006; Marczylo et al., 2009; Matsumoto et al., 2006). Maximum plasma levels of total anthocyanins are in the range of 1–100 nmol/l following consumption of berries or grapes in human studies (Prior & Wu, 2006). The absorbed anthocyanins are cleared from the circulation rapidly.

Absorption from stomach

Anthocyanins were found in the blood stream within minutes of consumption in humans (Milbury et al., 2002), suggesting that anthocyanins can be quickly absorbed from the stomach. Indeed, anthocyanin urine concentrations were

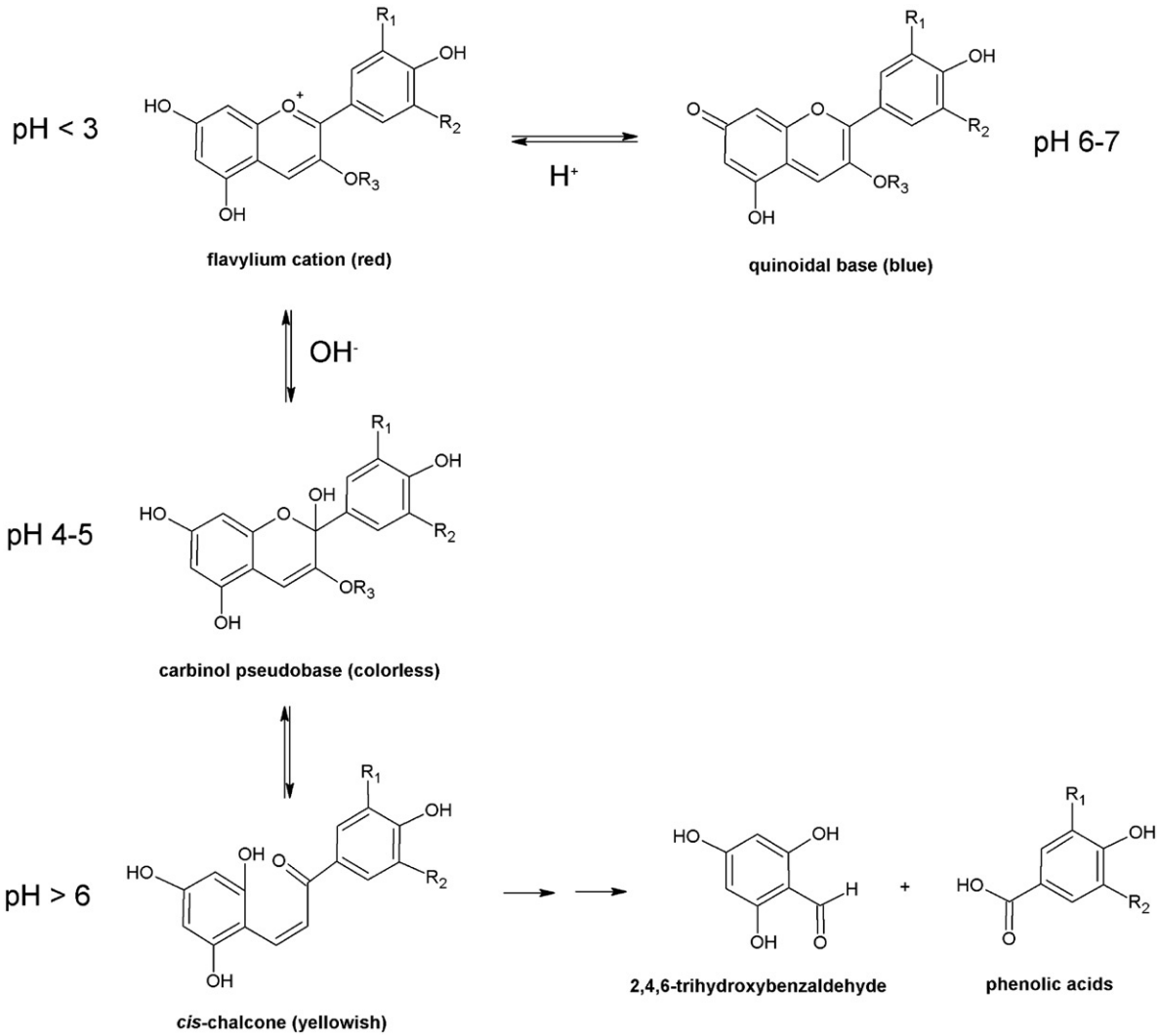


Figure 2. pH-dependent chemical forms and degradation reactions (Cabrita et al., 2014; Castaneda-Ovando et al., 2009). More than one chemical forms could be present at different pH and contribute to the overall color of the solutions.

fivefold higher when introduced through nasal tubes into the stomach as opposed to the jejunum in two patients (Cai et al., 2011).

Efficient absorption of anthocyanins from the stomach was confirmed in animal studies. In an *in situ* gastric perfusion study in rats, high plasma anthocyanin concentrations were found in the portal vein and systemic circulation (Passamonti et al., 2003, 2005b; Vanzo et al., 2008). In another study, bilberry anthocyanins in the gastric fluid were decreased by 19–37% following 30 min *in situ* gastric absorption (Talavera et al., 2003). These decreases were attributed to absorption because anthocyanins did not degrade in simulated acidic gastric juice (Bermudez-Soto et al., 2007; Stalmach et al., 2012; Talavera et al., 2003). It was suggested that anthocyanins permeate the gastric mucosa through a bilitranslocase-mediated mechanism (Passamonti et al., 2009).

Absorption from small intestine

Anthocyanins were absorbed when introduced through nasal intubation directly into the jejunum in humans (Cai et al., 2011). Anthocyanins were absorbed efficiently after *in situ* perfusion of the jejunum and ileum in rats (Talavera et al., 2004). The absorption was influenced by the chemical structure of the anthocyanin and varied from 10.7% (Ma-3-glc) to 22.4% (Cy-3-glc).

Using an Ussing chamber mounted with mouse intestine sections, the highest absorption of anthocyanins occurred with jejunum tissue ($55.3 \pm 7.6\%$) (Matuschek et al., 2006). Minor absorption occurred with duodenal tissue ($10.4 \pm 7.6\%$), and no absorption was detected from the ileum or colon. Two peaks of Dp-3-glc were observed in the plasma of rats at 15 and 60 min after oral administration of Dp-3-glc (Ichiyanagi et al., 2004). It is possible that the two peaks appeared in relation to the time at which the anthocyanins were absorbed from the stomach and the jejunum.

About 7.5% of ingested anthocyanins were found in the small intestine tissue in their native form 2 hours following administration of black raspberries to rats (He et al., 2009). In another study, the jejunum contained 605 nmol/g tissue of Cy-3-glc and its methylated and glucurono-conjugated metabolites when rats were administered an anthocyanin-enriched diet for 15 days (Talavera et al., 2005). It was also demonstrated that anthocyanins can permeate cultured Caco-2 cell monolayers (Faria et al., 2009; Steinert et al., 2008; Yi et al., 2006). Thus, the permeability of anthocyanins

across the gastrointestinal mucosa is quite high. The high anthocyanin concentrations in intestinal tissues were in great contrast to their low concentrations in blood. This lends additional support to the notion that anthocyanins undergo extensive first-pass metabolism before entering the systemic circulation as metabolites.

Anthocyanins in gastrointestinal tissues can achieve μM concentrations similar to those used in *in vitro* studies on the biomedical effects of anthocyanins (Basu et al., 2010b, Chen & Chen, 2013; Giacalone et al., 2011; Kay et al., 2012; Tsuda, 2012). Thus, anthocyanins could achieve pharmacologically relevant concentrations in the gastrointestinal wall and exert their protective effects locally (Mallery et al., 2011; Jakesevic et al., 2013; Jurgonski et al., 2013).

Metabolism of anthocyanins

Cy-3-glc

A total of 35 metabolites were identified after [^{13}C]-Cy-3-glc was administered to humans (de Ferrars et al., 2014). By labelling Cy-3-glc on both A-ring and B-ring, the authors were able to ascertain the origin of the metabolites. Among the metabolites identified, 17 were found in the circulation, 31 in urine, and 28 in feces (Figure 3).

Plasma concentrations of Cy-3-glc declined very rapidly following intravenous administration (Vanzo et al., 2011). Its methylated product peonidin 3-glucoside was detected 15 seconds after intravenous administration indicating the rapid redistribution, metabolism, or decomposition of Cy-3-glc. The major metabolites of anthocyanins recovered in urine were glucuronidated and/or methylated conjugates (Felgines et al., 2005; Kay et al., 2005; Tian et al., 2006; Wu et al., 2002). Urinary excretion of anthocyanins and their metabolites was found to be 0.26–2.67% of the anthocyanins ingested (Felgines et al., 2002, 2003; Matsumoto et al., 2006). Enzymes responsible for the biotransformation are located in the small intestine, liver, and kidney.

Unlike a few other flavonoids, Cy-3-glc is not a substrate of cytosolic β-glucosidase (Berrin et al., 2002) or lactase-phlorizin hydrolase (Nemeth et al., 2003). In fact, high concentrations of intact Cy-3-glc were found in the intestinal tissues of rats following oral administration (He et al., 2009; Talavera et al., 2005). Furthermore, only the glycosides, not their metabolites, were recovered in the perfusion solution when a number of anthocyanins were perfused in the rat intestinal lumen (Talavera et al., 2004). Thus, Cy-3-glc and probably some other anthocyanins could be absorbed intact into the gastrointestinal wall. They would then undergo extensive first-pass metabolism and enter the systemic circulation as metabolites.

The mechanism of cleavage of the sugar moiety of Cy-3-glc is not clear. It is of interest to clarify whether mechanisms other than chemical breakdown is responsible for this reaction. It is important to note that cleavage of the sugar moiety is not a prerequisite for the further chemical breakdown of anthocyanins (Figure 2) (Fleschhut et al., 2006; Woodward et al., 2009).

In addition to chemical breakdown, human liver microsomes were found to be responsible for the further breakdown of the anthocyanin aglycones. It was found that human liver microsomes can metabolize Cy to PCA, which is further metabolized to form three glucuronide conjugates (Woodward et al., 2011).

Pg-3-glc

Strawberry Pg-3-glc was found to be metabolized to 4-hydroxybenzoic acid in humans (Azzini et al., 2010). In *in vitro* studies, human liver microsomes can metabolize Pg-3-glc to 4-hydroxybenzoic acid (Woodward et al., 2011). 4-Hydroxybenzoic acid is further metabolized to form two glucuronide conjugates. These liver metabolic activities may play an important role in the first-pass metabolism of anthocyanins.

When pure Pg-3-glc was orally administered to rats, one pelargonidin monoglucuronide and three Pg-3-glc-monoglucuronides (glucuronides of the glucoside) were identified together with intact Pg-3-glc in both blood plasma and urine samples (Ichiyanagi et al., 2013). The two dominant metabolites were elucidated as pelargonidin-3-glucuronide (Pg-3-GlcA) and pelargonidin-3-glucoside-glucuronide (Pg-3-glc-glcA) (Figure 4).

De-3-glc, Pt-3-glc, Ma-3-glc

De-3-glc, Pt-3-glc, and Ma-3-glc were found in blood or urine in their native forms after the administration of Concord grape juice in humans (Frank et al., 2003; Mazza et al., 2002; Stalmach et al., 2012). Delphinidin-glucuronide, petunidin-glucuronide, and malvidin-glucuronide were identified as their respective major metabolites in urine (Stalmach et al., 2012).

Gallic acid, a fragmentation product of delphinidin glycosides, was not detected in the urine of volunteers administered bilberry–lingonberry puree (Nurmi et al., 2009). Only a small amount of syringic acid, a potential metabolite from malvidin glycosides, was detected in the same study. It is possible that the corresponding phenolic acid metabolites produced from delphinidin, malvidin, or petunidin were further degraded, resulting in their low concentrations in urine. This is in great contrast to Cy-3-glc and Pg-3-glc where high concentrations of their phenolic acid metabolites are found in plasma.

Bioavailability and first-pass effect

The systemic bioavailability of anthocyanins was found to be only 0.26–1.8% in animal studies when intravenous administrations were used as reference for comparisons (Borges et al., 2007; Felgines et al., 2002, 2003; Ichiyanagi et al., 2006; Marczylo et al., 2009; Matsumoto et al., 2006). The percentage of intact anthocyanins excreted in urine was estimated to be less than 0.1% in humans. This indicates that anthocyanins undergo extensive metabolism in the body before being excreted in the urine (Figure 5).

However, systemic bioavailability of the intact anthocyanins is probably not the best way to estimate the degree of absorption of anthocyanins (Fang, 2014). First-pass metabolism plays an important role in the disposition of some anthocyanins such as Pg-3-glc and Cy-3-glc. High plasma concentrations of the phenolic acid metabolites have been found following administration of fruits containing

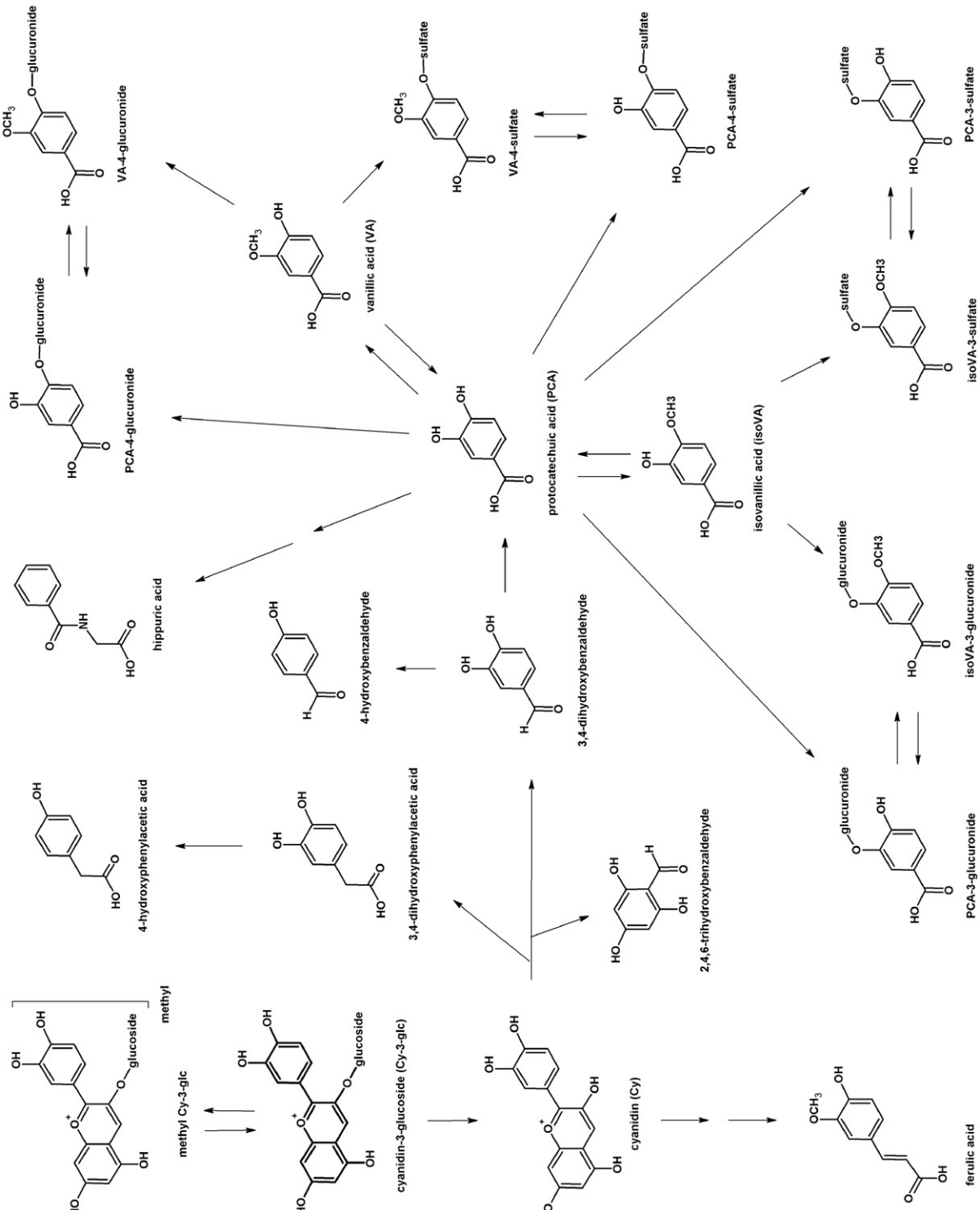


Figure 3. Major metabolic pathways of cyanidin-3-glucoside (Aura et al., 2005; Azzini et al., 2010; Czank et al., 2013; Dacre & Williams, 1968; de Ferrars et al., 2014; Felgines et al., 2003; Miyazawa et al., 1999; Woodward et al., 2011; Wu et al., 2002).

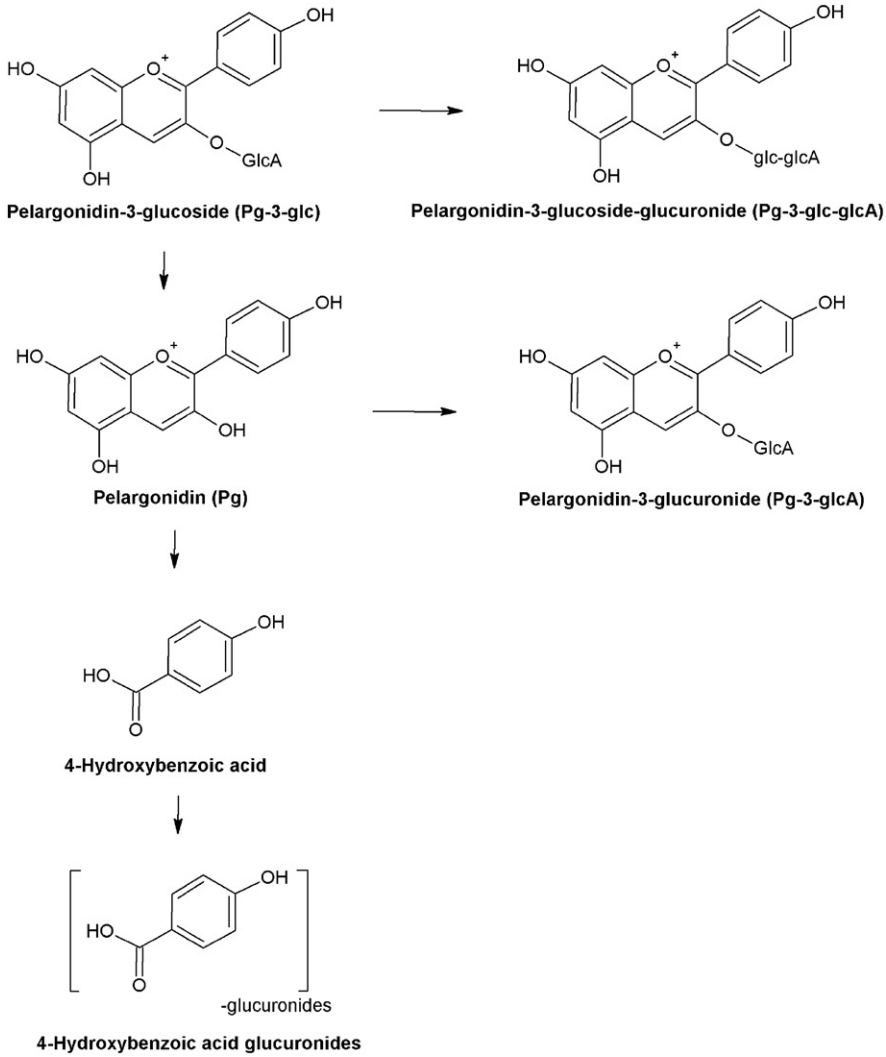


Figure 4. Metabolic pathways of pelargonidin-3-glucoside (Azzini et al., 2010; Ichiyanagi et al., 2013; Woodward et al., 2011).

anthocyanins. Between 30% and 44% of consumed Cy-3-glc was found as protocatechuic acid (PCA) in human plasma following consumption of blood orange juice (Vitaglione et al., 2007) and black raspberries (Chen et al., 2012) (Figure 3). Maximum concentration of PCA was found to be about 0.5 μM following the administration of 71 mg Cy-3-glc in humans. In the meanwhile, maximum concentration of Cy-3-glc was found to be 1.9 nM (Vitaglione et al., 2007). A recent study using [^{13}C]-Cy-3-glc revealed that PCA was extensively further metabolized to numerous metabolites such as vanilic acid, hippuric acid, ferulic acid, and 4-hydroxybenzaldehyde (de Ferrars et al., 2014). 12.4% of ^{13}C-label was recovered from urine and breath following oral consumption in humans (Czank et al., 2013). There was a 42-fold higher abundance of [^{13}C]-labeled metabolites relative to [^{13}C]-Cy-3-glc in plasma (Czank et al., 2013). Similarly, plasma 4-hydroxybenzoic acid, a metabolite of Pg-3-glc, accounted for 54–56% of strawberry Pg-3-glc ingested by volunteers (Azzini et al., 2010). A maximum of 2.5 μM 4-hydroxybenzoic acid was found following administration of strawberries. Thus, the total systemic bioavailability of anthocyanins could be rather high for some anthocyanins such as Cy-3-glc and Pg-3-glc.

Since phenolic acid such as PCA can be absorbed after oral ingestion (Chen et al., 2012; Guo et al., 2008; Russell et al., 2009), it was suggested that the phenolic acids could be produced within the gastrointestinal lumen before being absorbed (Hribar & Ulrih, 2014). However, Pg-3-glc and Cy-3-glc are rather stable in the upper gastrointestinal tract (Gonzalez-Barrio et al., 2011; Woodward et al., 2011), especially in the stomach (Bermudez-Soto et al., 2007; Stalmach et al., 2012; Talavera et al., 2003). Enzymatic degradation is also unlikely for Cy-3-glc because it is not a substrate of cytosolic β-glucosidase (Berrin et al., 2002) or lactase-phlorizin hydrolase (Nemeth et al., 2003). Thus, most plasma 4-hydroxybenzoic acid and PCA were probably produced during first-pass metabolism in the intestinal wall or liver (Figure 5). These observations on Pg-3-glc and Cy-3-glc could be extended to other anthocyanins which are stable in the upper gastrointestinal tract, chemically and enzymatically.

While most anthocyanins are stable in the stomach, some anthocyanins such as De-3-glc are somehow unstable in the small intestine (Talavera et al., 2004). It is perceivable that chemical degradation could play a role in the formation of their phenolic acid metabolites. However, De-3-glc was found in the blood stream in its native form (Frank et al., 2003; Mazza et al., 2002; Stalmach et al., 2012). And, 40.7% of De-3-glc was found to remain in ileal fluid in ileostomists-administered blueberries (Kahle et al., 2006).

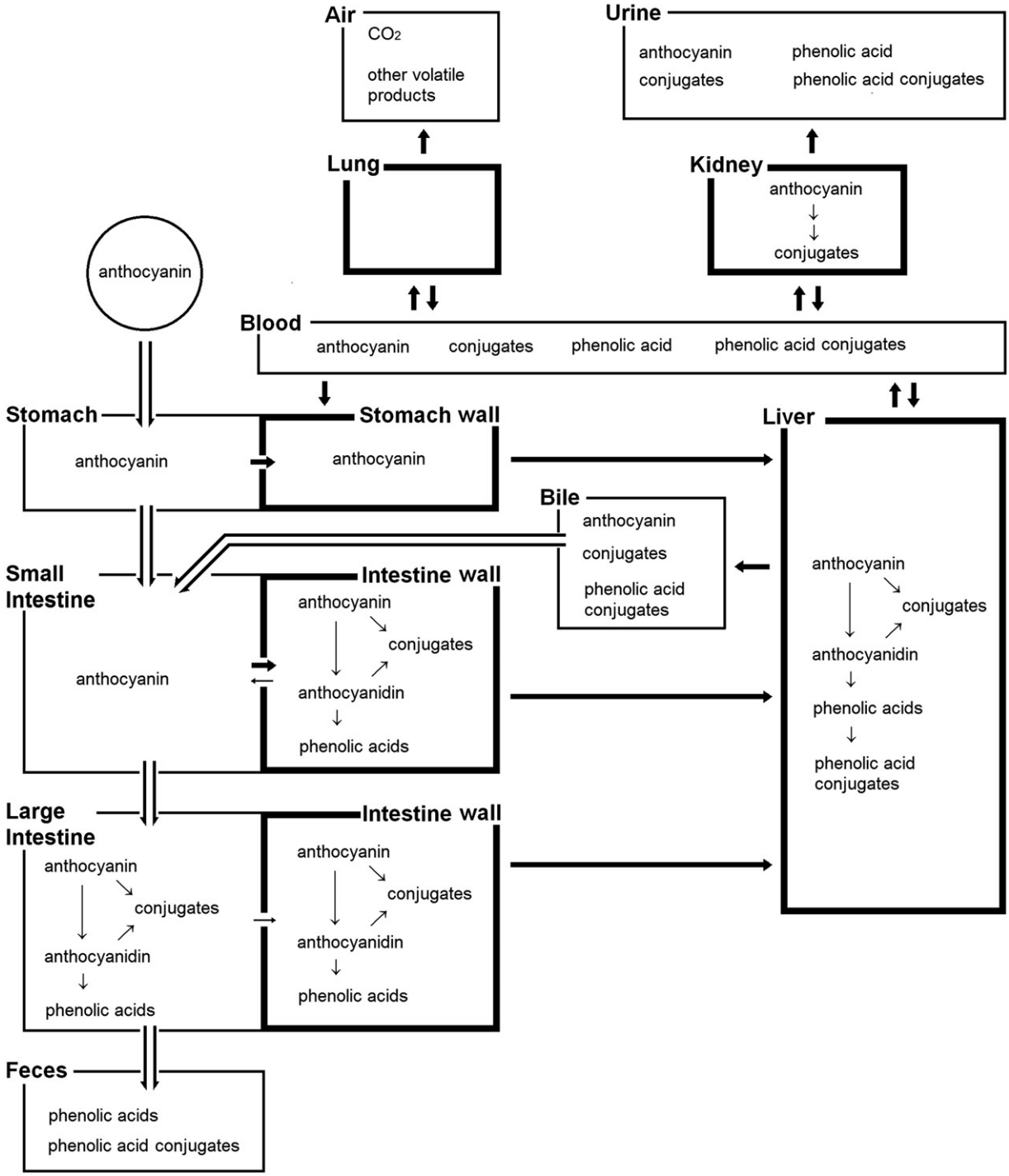


Figure 5. Schematic diagram of the absorption, first-pass metabolism, and further disposition of Cy-3-glc following its ingestion (Andlauer et al., 2003; Dreiseitel et al., 2009; Talavera et al., 2005; Tsuda et al., 1999). Solid arrows: transmembrane transfer; open arrows: direct transfer; small arrows: minor pathways. Abbreviations. Cy: cyanidin; Cy-3-glc: cyanidin 3-glucoside; PCA: protocatechuic acid.

Effects of dietary components on anthocyanin absorption

Substances with small systemic bioavailability are often susceptible to influences in their absorption, because a small change in their bioavailability would lead to major differences in their plasma concentrations. For example, an increase of systemic bioavailability from 0.1% to 0.2% (an increase of 0.1%) would double their plasma concentrations.

Certain dietary components can alter anthocyanin absorption (Nielsen et al., 2003; Walton et al., 2009). Milk is reported to reduce the absorption of anthocyanins and diminish the effect of blueberries to increase plasma antioxidant capacity (Hassimotto et al., 2008b; Mazza et al., 2002; Serafini et al., 2009). Coadministration of sucrose with elderberry juice led to a delay and a reduced amount of anthocyanins in urine (Mulleder et al., 2002). A carbohydrate-rich diet may delay absorption by prolonging the transit of

anthocyanins through the gastrointestinal tract but not necessarily affect the extent of absorption (Nielsen et al., 2003; Walton et al., 2009). Interestingly, Cy-3-glc was found to be more stable when present in blackberry extract (0.69% decomposed) than in its pure form (2.3% decomposed) when incubated in a simulated intestinal buffer (pH 6.6) at 37 °C (Talavera et al., 2004).

The presence of other flavonoids may also interfere with the absorption of anthocyanins. For example, e*x vivo* studies using mouse jejunum mounted in Ussing chambers indicated that the flavonol quercetin-3-glucoside can significantly inhibit the absorption of Cy-3-glc (Walton et al., 2006).

Decomposition of anthocyanins in lower GI tract

Some anthocyanins can reach the large intestine intact. Using ileostomy patients, the amount of polyphenols reaching the colon was determined (Kahle et al., 2006). Ileostomy effluent was collected after the consumption of polyphenol-rich apple juice or anthocyanin-rich blueberries. Percentages remaining in ileal fluid relative to food content are in the following order: Ma-3-ara (85.1%) > Pt-3-ara (73%) > Pt-3-gal (59.1%) > Ma-3-gal (54.4%) > Pt-3-glc (47.5%) > De-3-gal (45.3%) > Cy-3-ara (44.6%) > Ma-3-glc (42.8%) > De-3-glc (40.7%) > De-3-ara (37.8%) > Cy-3-gal (36.8%) > Pn-3-glc (29.9%) > Cy-3-glc (28.3%) (Kahle et al., 2006). In another study with ileostomists, the recovery of anthocyanins are in the following order: cyanidin-3-O-(2″-O-xylosyl) rutinoside (93%) > Pg-3-glc (75%) > pelargonidin-3-O-sophoroside (59%) ≥ pelargonidin-3-O-(2″-O-glucosyl)rutinoside (57%) ≥ Cy-3-rut (54%) ≥ cyanidin-3-O-(2″-O-glucosyl) rutinoside (53%) > cyanidin-3-O-sophoroside (40%) > Cy-3-glc (5.9%) (Gonzalez-Barrio et al., 2010). Little intact anthocyanins was found in feces, thus most anthocyanins entering the large intestine would be degraded (Gill et al., 2010; Jimenez-Giron et al., 2013).

It is well-established that the microbiota in the large intestine can facilitate the decomposition of anthocyanins (Aura et al., 2005; Forester & Waterhouse, 2008, Gill et al., 2010; Gonzalez-Barrio et al., 2011; Hanske et al., 2013; He et al., 2005; Hidalgo et al., 2012; Jimenez-Giron et al., 2013; Keppler & Humpf, 2005; Sanchez-Patan et al., 2012; Stalmach et al., 2012). It was shown that the cyanidin-based anthocyanins are degraded into a number of phenolic metabolites following incubation of anthocyanins in fecal slurries. Anthocyanidin glycosides can be hydrolyzed by the intestinal microflora within 20 min to 2 hours of incubation (Keppler & Humpf, 2005). The liberated anthocyanidin aglycones are unstable at neutral pH and degradation products were detected within 20 min of incubation. The major stable products of anthocyanin degradation are the corresponding phenolic acids descending from the B-ring of the anthocyanin skeleton (Fleschhut et al., 2006). Different strains of colon microbiota show remarkable differences in their abilities to metabolize anthocyanins (Avila et al., 2009). It is perceivable that anthocyanins such as Ma-3-ara, Pt-3-ara and cyanidin-3-O-(2″-O-xylosyl)rutinoside would enter the large intestine and be degraded to produce large quantities of metabolites (Hassimotto et al., 2008a, Hidalgo et al., 2012; Sanchez-Patan et al., 2012).

Chemical degradation could also be important for anthocyanins which are unstable in the gastrointestinal tract (Talavera et al., 2004). It was observed that degradation of Ma-3-glc is entirely dependent on the presence of microbiota while De-3-glc degradation is bacterial-independent (Jakesevic et al., 2013).

It is perceivable that large amounts of phenolic acids would be produced from those anthocyanins which reach the large intestine in quantities (Borges et al., 2013; Gonzalez-Barrio et al., 2010; Kahle et al., 2006; Stalmach et al., 2012). However, it is hard to determine what compounds are actually generated from anthocyanins rather than from other food components *in vivo*. Recently, [^{13}C]-Cy-3-glc was used to evaluate fecal recovery of Cy-3-glc, its degradation products, and derived metabolites in humans. A total of 28 [^{13}C]-labelled compounds were detected in feces accounting for 32% of the dose 48 hours after administration of [^{13}C]-Cy-3-glc (Czank et al., 2013). Ferulic acid and PCA were the most abundant metabolites recovered in feces (de Ferrars et al., 2014) (Figure 3). The decomposition products could play significant roles in modulating the microbial and chemical environment and contribute to the health effects of anthocyanins within the large intestine (Russell & Duthie, 2011; Vendrame et al., 2011).

Are the gut microbiota decomposition products extensively absorbed and contribute to the high blood concentrations of the phenolic acid metabolites? Literature indicates that the amount of Cy-3-glc entering the large intestine is similar to the amount excreted in feces. Only 5.9% to 28.3% of administered Cy-3-glc was excreted in ileal fluid from ileostomists-administered blueberries, grapes, or raspberries (Gonzalez-Barrio et al., 2010; Kahle et al., 2006; Stalmach et al., 2012). In separate studies, fecal recoveries were 32.1% and 44.5% of administered [^{13}C] or [^{14}C] Cy-3-glc in humans (Czank et al., 2013) and mice (Felgines et al., 2010), respectively. It is therefore suggested that most Cy-3-glc entering the large intestine is excreted in feces. In other words, the degradation products of Cy-3-glc may not be extensively absorbed from the large intestine (Fang 2014). This is also consistent with the observation that high plasma PCA and 4-hydroxybenzoic acid concentrations were achieved (Azzini et al., 2010; Vitaglione et al., 2007) when most of the administered blood orange juice is still in the stomach and small intestine (Kahle et al., 2006). The postulated poor absorption of phenolic acids in the large intestine should be confirmed by introducing anthocyanins or their phenolic acid metabolites directly into the large intestine in human or animal studies.

Distribution

Since the early observations of the effects of blueberry on cognitive performance, several studies have found that anthocyanins can reach the brain (Passamonti et al., 2005b, Talavera et al., 2005). It was found that intact Cy-3-glc concentrations reached 0.21 nmol/g of brain tissue in rats fed a blackberry anthocyanin-enriched diet for 15 days (Talavera et al., 2005).

Cy-3-gal, Cy-3-glc, Cy-3-arab, Mv-3-gal, Mv-3-glc, Mv-3-ara, Pn-3-ara, and Dp-3-gal were identified in the brain of

aged rats fed a 2% blueberry diet for 10 weeks (Andres-Lacueva et al., 2005). There was a significant correlation between the total amount of anthocyanins found in the cortex and Morris water maze performance (a measure of spatial learning and memory).

Pigs were used to examine the deposition of anthocyanins in the liver, eye, and brain tissue (Kalt et al., 2008). Pigs were fed diets supplemented with blueberries for 4 weeks and then fasted for 18–21 hours prior to euthanasia. Eleven intact anthocyanins were detected in the liver, eye, cortex, and cerebellum despite the fact that no anthocyanins were detected in the plasma or urine of the fasted animals. This suggests that anthocyanins were retained in tissues rather than being in rapid equilibrium with blood circulation. The mechanism of this retention is not clear, but may involve localization in subcellular components.

In another study, rats were fed a blackberry anthocyanin-enriched diet for 15 days. The stomach tissues contained only native blackberry anthocyanins (Cy-3-glc and cyanidin-3-pentose), while other organs (jejunum, liver, and kidney) contained native as well as methylated and conjugated anthocyanidins (cyanidin and peonidin monoglucuronides) (Talavera et al., 2005). Proportions of anthocyanin derivatives differed among organs. The liver presented the highest proportion of methylated forms while jejunum and plasma also contained aglycone forms.

Excretion of anthocyanins

Anthocyanins can be excreted in urine and bile in their intact form or as metabolites. Volatile metabolites produced from [^{13}C]-Cy-3-glc have also been found in large quantities in breath.

Excretion in urine

In humans administered [^{13}C]-Cy-3-glc, 5.4% of ^{13}C-label was recovered from urine following oral ingestion (Czank et al., 2013). In another study where [^{14}C]-Cy-3-glc was administered to mice, 3.3% of the radioactivity was detected in urine 3 hours after oral administration (Felgines et al., 2010). In studies where no isotopes were used, urinary excretion of anthocyanins and their metabolites were found to be 0.26–2.67% of the anthocyanins ingested (Felgines et al., 2002, 2003; Matsumoto et al., 2006).

The urinary excretion of Pg-3-glc seems to be higher than that of Cy-3-glc (Carkeet et al., 2008; Felgines et al., 2003; Mullen et al., 2008). It was suggested that this may have more to do with the stability of Pg-3-glc than its high absorption.

Bile excretion and enterohepatic recycling

Anthocyanins likely undergo extensive bile secretion in their original form or as their metabolites (Figure 5). In mice administered [^{14}C]-Cy-3-glc, a higher concentration of radioactivity was found in bile (7·81 Bq/mg tissue) than in liver (0·35 Bq/mg tissue). This suggests an important role for biliary excretion in the disposition of Cy-3-glc (Felgines et al., 2010). Following intra-peritoneal injection in rats, 13% of PCA was found to be excreted in bile (Dacre & Williams, 1968), probably as its conjugates (Woodward et al., 2011). Extensive bile secretion of De-3-rut and its 4′-methylated metabolite was also observed in rats (Matsumoto et al., 2006).

In human studies, entero-hepatic recycling of a xenobiotic could be revealed by a second peak on the plasma concentration versus time curve. The second peak could be interpreted to be due to the secretion of the xenobiotic accumulated in bile into the duodenum and their subsequent reabsorption (Figure 6). This phenomenon can be observed in literature for several anthocyanins. For example, a second peak can be identified for De-3-glc and petunidin-3-glucoside in volunteers administered Concord grape juice (Stalmach et al., 2012). A similar pattern in the plasma concentration versus time curve can be observed in humans for Ma-3-glc following ingestion of red wine (Bub et al., 2001) and purple carrot juice (Charron et al., 2009).

Two peaks were also observed in the plasma concentration versus time curve in rats administered De-3-glc at 15 and 60 min after ingestion (Ichiyanagi et al., 2004). The first peak was attributed to gastric absorption (Ichiyanagi et al., 2004). This explanation for two peaks in plasma concentration curve is valid only when the first peak appears very early on in the plasma concentration when most anthocyanins are still in stomach.

In addition to the intact anthocyanins, bile can also excrete their phase II metabolites. For example, a second peak was visible on the plasma concentration versus time curve for phase II metabolites of PCA (Czank et al., 2013). This could also be due to entero-hepatic recycling where conjugates of PCA were secreted into duodenum and underwent cleavage to release the free PCA which was reabsorbed (Figure 6). The cleavage could take place either within the intestinal lumen or during first-pass metabolism.

Expiration in air

Volatile metabolites or auto-oxidation products were found to be expired into the air following oral administration of [^{13}C]-Cy-3-glc in humans (Czank et al., 2013). This elimination pathway accounted for 6.9% of the administered dose.

The role of transporters

Many anthocyanins were shown to be inhibitors of transporters such as bilitranslocase (Passamonti et al., 2002), glucose transporters (Faria et al., 2009), breast cancer resistance protein (BCRP) (Dreiseitel et al., 2009), and multidrug resistance protein 1 (MDR1) (Dreiseitel et al., 2009).

It was suggested that anthocyanins permeate the gastric mucosa through a bilitranslocase mediated mechanism (Passamonti et al., 2009). In a cell culture study, anthocyanins were found to be able to cross MKN-28 cell monolayers (differentiated adenocarcinoma stomach cells) (Fernandes et al., 2012). Kinetic studies suggest that the absorption of Cy-3-glc through the MKN-28 cell line barrier was saturable although saturation was not achieved at the highest concentration used (2 mM). More research is needed to confirm the involvement of a saturable transporter mechanism.

Direct evidence for carrier-mediated membrane transport of Ma-3-glc was reported in cultured HepG2 liver cells

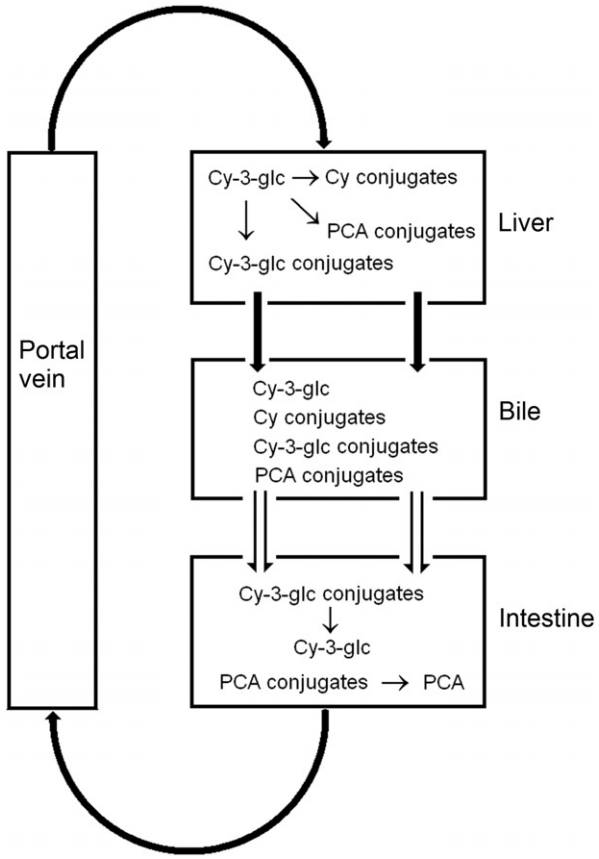


Figure 6. Schematic diagram of the entero-hepatic recycling of cyanidin-3-glucoside (Cy-3-glc). Solid arrows: transmembrane transfer; open arrows: direct transfer.

(Passamonti et al., 2005a), and cultured human aortic primary endothelial cells (Maestro et al., 2010), where transport was inhibited by the antibodies of bilitranslocase. The organic anion carrier bilitranslocase is expressed in the gastric epithelium (Nicolin et al., 2005), at the vascular domain of the hepatocyte plasma membrane (Baldini et al., 1986), and in the basolateral membrane of renal proximal tubules (Elias et al., 1990). Transport of Mv-3-glc was also reported using Caco-2 cells (Faria et al., 2009). Interestingly, pretreatment of Caco-2 cells with anthocyanins was found to significantly increase their own transport.

In another study, seven of 16 anthocyanins were identified as potential substrate of BCRP because they were shown to stimulate the BCRP ATPase. These anthocyanins are malvidin, petunidin, Ma-3-gal, malvidin-3,5-diglucoside, Cy-3-gal, Pn-3-glc, and Cy-3-glc (Dreiseitel et al., 2009).

Conclusion

The disposition of anthocyanins after oral administration follows a unique pattern rather different from those of other flavonoids. Anthocyanins can be absorbed from the stomach as well as the intestines. Some anthocyanins can reach the large intestine in significant amounts and undergo decomposition catalyzed by microbiota. Decomposition products may contribute to the health effects of anthocyanins in the large intestine. Some anthocyanins undergo extensive presystemic metabolism and the resultant metabolites likewise, may have beneficial properties.

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Declaration of interest

JF wrote the paper and has primary responsibility for the final content. The author has no conflicts of interest. This work is supported by Saskatchewan Ministry of Agriculture, Canada (Agriculture Development Fund #20110138).

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