Absorption and metabolism of proanthocyanidins

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Proanthocyanidins
Absorption
Microbial metabolism

ABSTRACT

Proanthocyanidins are found in fruits, tree nuts, cereals, legumes, wine, and chocolate. They affect nutritional value, appearance, taste, and texture of these foods and promote better health by preventing cardiovascular diseases, cancers, urinary tract infections, and other aging-related metabolic complications. The bioavailability of proanthocyanidins is largely influenced by their degree of polymerization. The absorption rate of proanthocyanidin dimers is 5–10% of that of (+/−)-epicatechin. Trimmers and tetramers had lower absorption rates than dimers. Absorbed intact dimers, trimers, and tetramers underwent limited phase II metabolism in the intestine and liver in rats compared with (+/−)-epicatechin. Proanthocyanidins with a degree of polymerization over 4 (DP > 4) are not absorbable because of their large molecular size and gut barrier. Depolymerization of proanthocyanidins in the gastrointestinal tract was negligible. The majority of proanthocyanidins reaches the colon intact and is degraded into phenylvalerolactones and phenolic acids by colon microbiota. These microbial metabolites may contribute to the health promoting properties of proanthocyanidins in vivo. Future research of proanthocyanidin bioavailability will likely focus on identification of new microbial metabolites and investigation of how proanthocyanidins influence human health by affecting the composition of human gut microbiota.

1. Introduction

Proanthocyanidins, also known as condensed tannins, are oligomers or polymers of flavan-3-ols [e.g. (+)-epicatechin or (+)-catechin] linked through interflavan bonds. B-type proanthocyanidins are linked by C4 → C8 and/or C4 → C6 bonds. A-type proanthocyanidins contain an additional ether bond between C2 → O7. The molecular size of proanthocyanidins is described as degree of polymerization (DP). Proanthocyanidins with a degree of polymerization of 1, 2, 3 or 4 are called monomers, dimers, trimers, or tetramers, respectively. The flavan-3-ol units at the end of the proanthocyanidins are terminal units. All flavan-3-ols above the terminal units are extension units. Flavan-3-ol has two aromatic rings (A and B) and a heterocyclic ring C (Fig. 1). In this review, we define proanthocyanidins with DP 2–4 as oligomers, >4 as polymers, and >10 as high polymers, respectively. Proanthocyanidins exist in berries, cereals, nuts, legumes, chocolates, and wines (Gu et al., 2003). At an estimated average intake of 95 mg/day (Wang, Chung, Song, & Chun, 2011), proanthocyanidins represent a major class of flavonoids ingested from the diet.

Most foods contain exclusively B-type proanthocyanidins. A small number of foods, such as cranberries, plum, and peanuts, contain A-type proanthocyanidins (Gu et al., 2003). Polymers are the predominant proanthocyanidins in many foods that contain proanthocyanidins. For instance, >75% of proanthocyanidins in cranberries, blueberries, strawberries, brown sorghum, grapes, and pinto beans are polymers (Gu et al., 2004; Hellstrom, Torronen, & Mattila, 2005). About 40% of the ingested proanthocyanidins are absorbable monomers and oligomers. The rest are the non-absorbable polymers with a DP above 4. Proanthocyanidins consisting exclusively...
of (+)-catechin and (−)-epicatechin are procyanidins. Proanthocyanidins containing afzelechin or galloatechins are propelargonidins or prodelphinidins, respectively. Many common foods such as cocoa, apples, pears, grape seeds, blueberries, and cranberries contain exclusively procyanidins. The structures of several prodelphinidin, propelargonidin, and procyanidin oligomers are depicted in Fig. 1. A fraction of proanthocyanidins in strawberries and pinto beans are prodelphinidins. Some proanthocyanidins in grape skins are prodelphinidins. The rest are procyanidins (Gu et al., 2003). The majority of absorption and metabolism studies of proanthocyanidins were done on procyanidins due to their ubiquitous occurrence.

Proanthocyanidins from various sources have been reported to possess a variety of physiological activities such as antioxidant and disease prevention capacities. For example, proanthocyanidins were more effective than resveratrol or ascorbic acid in scavenging free radicals (Maldonado, Rivero-Cruz, Mata, & Pedraza-Chaverri, 2005). A recent study suggested cranberry procyanidins may help to prevent lung cancer by inducing rapid cancer cell apoptosis and growth arrest (Kresty, Howell, & Baird, 2011). Cranberry procyanidins may also serve as a chemoprevention agent against esophageal cancer by inducing apoptosis and inhibiting proliferation (Kresty, Howell, & Baird, 2008). Procyanidins from peanut skin decreased the production of inflammatory cytokines, tumor necrosis factor-α, and interleukin-6 in cultured human monocyte THP-1 cells in response to lipopolysaccharide (Tatsuno et al., 2012). Administering grape seed procyanidins reduced lung inflammation and decreased IL-4, IL-5, and IL-13 expression in a mouse model of acute or chronic asthma (Lee et al., 2012). Procyanidins from cocoa were found to inhibit growth of human breast cancer and colon cancer cells (Carnésecchi et al., 2002; Ramljak et al., 2005).

Fig. 1 – Structure of prodelphinidin B3, a propelargonidin A-type dimer, procyanidin dimers B2, B5, procyanidin A2, and an A-type trimer from cranberries (Foo, Lu, Howell, & Vorsa, 2000).
The bioactivities of proanthocyanidins are often confounded by their seemingly low bioavailability. Bioavailability of proanthocyanidins closely resembles that of flavan-3-ol monomers. Proanthocyanidins used in many bioavailability studies were a mixture of monomers, oligomers, and polymers. This review will focus on the current knowledge on the absorption and metabolism of proanthocyanidin oligomers and polymers. The absorption of monomers [i.e. (+)-catechin or (−)-epicatechin] will be briefly mentioned to facilitate the discussion of oligomers. A detailed review of these flavan-3-ol monomers can be found in a separate review (Lambert, Sang, & Yang, 2007).

2. Absorption of proanthocyanidin oligomers

Proanthocyanidins in a solid food matrix are not available for absorption. Only solubilized proanthocyanidins in the aqueous phase are bioaccessible for the enterocyte surface of the small intestine. No transporters have been identified for proanthocyanidins. Proanthocyanidins are absorbed through passive diffusion. Proanthocyanidins are not likely to pass the lipid bilayer via the transcellular pathway due to its large number of hydrophilic hydroxyl groups. Paracellular diffusion was thought to be a preferential absorption mechanism (Deprez, Mila, Huneau, Tome, & Scalbert, 2001). The major organs that involved in the absorption and metabolism of proanthocyanidins are shown in Fig. 2.

The absorption and metabolism of flavan-3-ol monomers was investigated extensively. Both human and animal studies indicated that (+)-catechin and (−)-epicatechin were rapidly absorbed from the upper portion of the small intestine. A maximum level of (+)-catechin at 76.7 nmol/L was detected in humans at 1.4 h after intake of 121 μmol (+)-catechin in dealcoholized red wine (Bell et al., 2000). A peak plasma (−)-epicatechin level of 260 nmol/L was achieved within 2 h in humans after the consumption of 557 mg of procyanidins containing 137 mg of (−)-epicatechin from a procyanidin-rich chocolate (Rein et al., 2000). Upon absorption, (epi)catechin undergoes extensive phase II metabolism in the intestine and liver to form glucuronidated, sulfated, and/or methylated conjugates. These metabolites are present in blood and tissues. Major conjugates of (−)-epicatechin in human plasma, bile, and urine were (−)-epicatechin 3′-O-sulfonate and (−)-epicatechin 3′-O-β-glucuronide after ingestion of 50 mg of (−)-epicatechin by volunteers (Romanov-Michailidis et al., 2012).

An early study suggested that proanthocyanidin oligomers (trimers to hexamers) were depolymerized into mixtures of (−)-epicatechin and dimers in simulated gastric fluid (pH 2.0, 37 °C) (Spencer et al., 2000). These authors also detected (−)-epicatechin as a major metabolite after ex vivo perfusion of rat small intestines with procyanidin dimer B2 or B5 extracted from cocoa (Spencer et al., 2001). A study in humans demonstrated that depolymerization did not occur and procyanidins were stable during gastric transit (Rios et al., 2002). A later study confirmed that procyanidin dimers and trimers were highly stable under gastric and duodenal digestion conditions (Serra et al., 2010). Oligomeric procyanidins in grape seed extract or sorghum were not depolymerized in the gastrointestinal tract releasing monomeric flavan-3-ols after ingestion by rats (Gu, House, Rooney, & Prior, 2007; Tsang et al., 2005). A recent study compared the plasma concentration of (−)-epicatechin in human blood and urine after volunteers were given (−)-epicatechin, cocoa procyanidin monomers [predominantly (−)-epicatechin] through decamers, or cocoa procyanidins dimers through decamers. It was found that all absorbed (−)-epicatechin in blood or urine were from ingested (−)-epicatechin. No (−)-epicatechin was derived from ingested oligomers and polymers (Ottaviani, Kwik-Uribe, Keen, & Schroeter, 2012).

One study detected procyanidin B2 and (−)-epicatechin in rat plasma and urine after administration of purified B2. It was suggested that a portion of dimer was degraded into...
The cleavage of interflavan bond likely occurred in the large intestine by microbiota. Catabolism of purified procyanidin B2 with human fecal microbiota in a static in vitro culture model caused less than 10% of dimers to be converted to (−)-epicatechin (Baba, Osakabe, Natsume, & Terao, 2002). In contrast, scission of interflavan bond was not observed in vivo in rats (Gu et al., 2007; Tsang et al., 2005). It can be concluded from these studies that depolymerization of proanthocyanidins to monomers is negligible in the gastrointestinal tract in vivo.

Results from in vitro and in vivo models demonstrated that proanthocyanidin oligomers with a degree of polymerization lower than 5 are absorbable. Déprez et al. observed that (+)-catechin, procyanidin dimer and trimer had similar permeability coefficients to that of mannitol, a marker of paracellular transport, on the human intestinal epithelial Caco-2 monolayers. Procyanidin polymers with a degree of polymerization of 7 were not permeable (Déprez et al., 2001). We also showed a 3.0% transport rate of procyanidin B2 in the same model (Ou, Percival, Zou, Khoo, & Gu, 2012). A study employing in situ perfusion of rat small intestine with the procyanidin B2 from grape seeds unraveled that procyanidin B-type dimer was absorbed from the small intestine. But the absorption rate was only 5–10% of that of (−)-epicatechin (Appeldoorn, Vincken, Gruppen, & Hollman, 2009). Spencer et al. also demonstrated that procyanidin dimer B2 or B5 from cocoa transferred from the lumen of isolated rat small intestines to the serosal side of enterocytes but only to a very small extent (<1% of the total transferred flavanol-like compounds) (Spencer et al., 2001). Shoji et al. gave apple procyanidins to rats using a dose of 1 g/kg and found that the concentration of (−)-epicatechin, procyanidin dimer B2, and trimer C1 in the plasma reached C\text{\text{max}} of 1.3 μM, 0.4 μM, and 0.14 μM respectively using HPLC-mass spectrometry. Dimer and trimer concentration peaked at 2 h after administration, whereas monomer concentration peaked 1 h after administration. Using a mass spectrometer and the Porter method, they also detected free nonconjugated procyanidin dimers to tetramers in rat plasma after oral administration of purified oligomers to rats at a dose of 1 g/kg (Shoji et al., 2006). Pentamers were detected using the Porter method only (Shoji et al., 2006), but these data should be interpreted cautiously because Porter method lacks the specificity and accuracy to detect procyanidins in blood (Gu, 2012).

The concentration of procyanidin B1 [epicatechin-(4β–8)catechin] in human serum was 10.6 nmol/L 2 h after intake of 2.0 g of grape seed extract (Sano et al., 2003). The C\text{\text{max}} of procyanidin dimer B2 and (−)-epicatechin in human plasma was 41 nmol/L and 5.9 μmol/L, respectively, 2 h after consumption of 0.375 g cocoa/kg body weight. The plasma concentration of dimer was only 3% of (−)-epicatechin (Holm et al., 2002). Serum concentration of (−)-epicatechin, procyanidin dimers, and trimers reached C\text{\text{max}} of 2.5 nM, 0.57 nM, and 0.10 nM, respectively, 1 h after a grape seed extract was gavaged to rats using 1 g/kg body weight. The absorption rate of dimers and trimers was calculated as 1.69% and 0.04%, respectively (Serafini et al., 2003). These findings suggested that protein may negatively impact the absorption of proanthocyanidins; however, additional research is needed to confirm this.

3. Microbial metabolism of proanthocyanidins

After ingestion, a small amount of flavan-3-ols or proanthocyanidins oligomers are absorbed in the small intestine. The majority of them reach the colon. In 1962, Griffith et al. detected 3-(3′-hydroxyphenyl)propionic acid in the urine of rats fed a diet with (+)-catechin, and first suggested the formation of this metabolite could depend on the action of intestinal microflora (Griffiths, 1962). Their subsequent work confirmed this by comparing metabolites from rats fed a (+)-catechin diet with and without added antibiotics (Griffiths, 1964). There is resurgence in research on the microbial degradation of polyphenols due to the recognition of the importance of human microbiota in the metabolism of proanthocyanidins and human health in general.

The role of gut microflora in the catabolism of proanthocyanidins was often explored using a static anaerobic incubation system in which proanthocyanidins were fermented with freshly collected human colonic fecal bacteria. Using this system, Déprez showed that polymeric proanthocyanidins from willow tree were completely degraded after 48 h of incubation. Major catabolites included 3-(3′-hydroxyphenyl)propionic acid, sulfated metabolites of dimers were not detected in biological fluids after intestinal perfusion in rats (Appeldoorn, Vincken, Aura, Hollman, & Gruppen, 2009). The representative structures of phase II metabolites of procyanidin dimer B2 are depicted in Fig. 3. One study detected methylated B-type dimer and trimer, but not glucuronide forms, in rat plasma using a mass spectrometer. The amount of methylated metabolites was not determined but appeared to be low compared with that of intact oligomers (Shoji et al., 2006). The extent of phase II metabolism of oligomers remained unclear in humans. Procyanidin A-type dimers, trimers and tetramers in cranberry traversed across Caco-2 cell monolayers with transport ratios of 0.6%, 0.4%, and 0.2%, respectively (Ou et al., 2012). An in situ perfusion study showed that A-type procyanidin dimer A1 [epicatechin-(4β–8, 2β–O–7)-epicatechin] and A2 were absorbed in the small intestine of rats with absorption rates higher than B-type dimer. This was the first study that detected A-type procyanidin in blood (Appeldoorn et al., 2009). Procyanidin A2 was recently detected in human plasma and urine using LC-MS/MS after large volumes of samples were extracted (Zampariello, Mckay, Dolnikowski, Blumberg, & Chen, 2012). A-type trimers or tetramers have not been detected in biological fluids.
4-hydroxyphenylacetic acid, 3-(4′-hydroxyphenyl)propionic acid, and 3-phenylpropionic acid (Déprez et al., 2000). The microbial degradation of B-type dimers was investigated extensively due to its abundance in the human diet and the availability of commercial standards. The microbial catabolism pathways of procyanidin dimer B2 (1) are summarized in Fig. 4. In the early stage of microbial catabolism, bacteria cleave the C-ring of epicatechin at C2 position to form (2). Bacteria may also cleave the C-ring and/or oxidize the A-ring to form metabolites (3) and (4). Twenty-four ‘dimeric’ metabolites with a molecular weight greater than 290 were detected after procyanidin B2 was incubated with human fecal microflora (Stoupi et al., 2010a). Microflora may also cleave the interflavan bond to convert procyanidin B2 into two (−)-epicatechin (5). This pathway was proven by the detection of (−)-epicatechin after procyanidin B2 was incubated with human fecal flora for up to 12 h (Stoupi et al., 2010a). However, this route appeared to be slow and accounted for less than 10% of procyanidin B2 (Appeldoorn et al., 2009). Dimer (1), its initial degradation products (2, 3, 4), and monomeric metabolites (5, 6) were further degraded by gut microflora into 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (7A). Metabolite 7A can be methylated by catechol-O-methyltransferase in vivo to form 5-(3′-methoxy, 4′-dihydroxyphenyl)-γ-valerolactone (7B). Microflora can also remove a hydroxyl group from 7A to form

Fig. 3 – Representative structures of phase II metabolites of procyanidin dimer B2. Methylation occurs on catechol moieties on B-ring of flavan-3-ols. Glucuronidation and sulfation can take place on multiple hydroxyl groups.

Fig. 4 – Microbial catabolism of procyanidin dimer B2. Adapted from Stoupi et al. (2010a, 2010b), Engemann et al. (2012).
Table 1 – Microbial metabolites of epicatechin, catechin, or procyanidin dimers.

<table>
<thead>
<tr>
<th>Microbial metabolites</th>
<th>Substrate</th>
<th>Epicatechin</th>
<th>Catechin</th>
<th>Procyanidin B2</th>
<th>Procyanidin A2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skeleton</strong></td>
<td><strong>Structures</strong></td>
<td><strong>Names</strong></td>
<td><strong>Substrate</strong></td>
<td>Epicatechin</td>
<td>Catechin</td>
</tr>
<tr>
<td>Phenylvalerolactone</td>
<td><img src="image" alt="Phenylvalerolactone" /></td>
<td>5-(3',4'-Dihydroxyphenyl-γ-valerolactone) R1 = OH, R2 = OH, R3 = H</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-(3' or 4'-Hydroxyphenyl-γ-valerolactone) R1 = OH or R2 = OH, R3 = H</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-Phenyl-γ-valerolactone R1 = H, R2 = H, R3 = H</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><strong>C6-C5</strong></td>
<td><img src="image" alt="C6-C5" /></td>
<td>5-(3',4'-Dihydroxyphenyl)valeric acid R1 = OH, R2 = OH</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-(3' or 4'-Hydroxyphenyl)valeric acid R1 or R2 = OH</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><strong>C6-C3</strong></td>
<td><img src="image" alt="C6-C3" /></td>
<td>3-(3',4'-Dihydroxyphenyl)propionic acid R1 = OH, R2 = OH</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-(3' or 4'-Hydroxyphenyl)propionic acid R1 = OH, R2 = H</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-(4'-Hydroxyphenyl)propionic acid R2 = OH, R1 = H</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-Phenyl propionic acid R1 = H, R2 = H</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><strong>C6-C2</strong></td>
<td><img src="image" alt="C6-C2" /></td>
<td>2-(3',4'-Dihydroxyphenyl) acetic acid R1 = OH, R2 = OH</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-(3'-Hydroxyphenyl) acetic acid R1 = OH, R2 = H</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-(4'-Hydroxyphenyl) acetic acid R1 = H, R2 = OH</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phenyl acetic acid R1 = H, R2 = H</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><strong>C6-C1</strong></td>
<td><img src="image" alt="C6-C1" /></td>
<td>3,4-Dihydroxybenzoic acid R1 = OH, R2 = OH</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-Hydroxybenzoic acid R1 = OH, R2 = H</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-Hydroxybenzoic acid R1 = H, R2 = OH</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><strong>C6</strong></td>
<td><img src="image" alt="C6" /></td>
<td>Phloroglucinol</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>
form 5-(3′-hydroxyphenyl)-γ-valerolactone 7C. Microbial dehydroxylation of metabolites occurs favorably at the C4′ position and the aliphatic side chains. Phenylvalerolactones were slowly degraded into phenylvaleric acids (9) after dehydroxylation on the side chain. Progressive shortening of the aliphatic chain by α- and β-oxidations further generate the phenylpropionic acids (10), phenylacetic acid (11) and benzoic acid derivatives. When incubating proanthocyanidins with human fecal bacteria, phenylvalerolactones were generated during the first 10 h of fermentation whereas side chain shortening and dehydroxylation dominated the later phase of incubation (10–48 h) (Sánchez-Patán et al., 2012).

Procyanidin B2 was degraded by human fecal flora twice as fast as (−)-epicatechin. 5-(2′,4′-dihydroxy) phenyl-2-ene valeric acid and 5-(3′,4′-dihydroxyphenyl) valeric acid were tentatively identified after in vitro anaerobic incubation of procyanidin B2 with human fecal bacteria but not from (−)-epicatechin. They were likely unique metabolites of dimers (Stoupi et al., 2010a). Microbial metabolites derived from (−)-epicatechin, (+)-catechin, procyanidin B2 or A2 are summarized in Table 1. It should be noted that the ability of bacteria to catabolize proanthocyanidins in the gut decreases with an increase of molecular size. The yield of phenolic acids in rat gut was 10% and 7% for monomers and dimers, whereas it decreased to 0.7% and 0.5% for trimers and polymers, respectively (Gonthier et al., 2003). These results suggested that bioavailability of proanthocyanidins decrease drastically with the increase of molecular size in the form of intact proanthocyanidins or microbial metabolites.

A-type proanthocyanidins had a rigid interflavan linkage due to an additional C2–O7 covalent bond. This linkage is more stable than B-type proanthocyanidins (Gu et al., 2003). Their unique activity on urinary tract infection prevention makes the investigation of their microbial catabolism of great importance. To date, only fragmentary information is available on the microbial catabolism of A-type proanthocyanidins. A recent study utilized a pig cecum model to investigate the metabolism of procyanidin A2 and cinnamtannin B1 (an A-type trimer). They found that 80% of A-type dimers and 40% of cinnamtannin B1 were degraded within 8 h of incubation. Procyanidin A-type trimer exhibited a more complicated pattern of hydroxylated catabolites than procyanidin A2, which probably resulted from the larger and more complex structure of trimers. Both A-type procyanidins showed C-ring cleavage on the terminal unit during degradation. Further metabolism led to the generation of hydroxy- or dihydroxy-benzoic acids, phenylacetic acids, phenylpropionic acids, and phloroglucinol (Engemann, Hubner, Rzeppa, & Humpf, 2012). In contrast to human colon microbiota, no interflavan bond scission was observed in the pig cecum model.

There are questions about whether the microbial metabolites of proanthocyanidins are still bioactive. 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone had a lower antioxidant capacity than (−)-epicatechin, yet it showed a stronger antioxidant activity than L-ascorbic acid in vitro (Unno, Tamemoto, Yayabe, & Kakuda, 2003). Synthesized 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone had IC50 values of 15–73 μM for human esophageal squamous cell carcinoma cells (KYSE150), human colon adenocarcinoma cells (HT-29

<table>
<thead>
<tr>
<th>Microbial metabolites</th>
<th>Substrate</th>
<th>Procyanidin B2</th>
<th>Procyanidin A2</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structures</td>
<td>R1=OH, R2=OH</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Names</td>
<td>1-(3′,4′-Dihydroxyphenyl)-3-(2′,4′,6′-trihydroxyphenyl)propan-2-ol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeleton</td>
<td>Others</td>
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</tbody>
</table>

Data were summarized from Aura et al. (2008), Engemann et al. (2012), Griffiths (1962), Tzounis et al., 2008, Appeldoorn et al. (2009), Stoupi, Williamson, et al. (2010a).
and HCT-116), immortalized human intestinal epithelial cells (INT-407), and an immortalized rat intestinal epithelial cell line (IEC-6) (Lambert, Rice, Hong, Hou, & Yang, 2005). Seven phenolic acids were identified as metabolites in the rat serum after the administration of a diet containing freeze-dried blueberries. A mixture of these phenolic acids inhibited the production of pro-inflammatory cytokines and foam cell formation (Xie et al., 2011). Microbial degradation of proanthocyanidins results in a large number of phenolic compounds in human fecal water. 3-Phenylpropionic acid, 3-hydroxyphenylacetic acid, and 3-(4-hydroxyphenyl)-propionic acid in fecal water significantly decreased the protein level of cyclooxygenase-2 in colonic HT-29 cells (Karolson et al., 2005). These results suggested microbial metabolites of proanthocyanidins may contribute to their atheroprotection and chemoprevention effects in vivo.

A few studies investigated the activity of specific bacterial species on flavan-3-ol catabolism. Human fecal organism Eubacterium oxidoreducens was able to insert oxygen to form a new hydroxyl group in the A-ring, which facilitates A-ring opening (Stoupi, Williamson, Drynan, Barron, & Clifford, 2010b). Eggertelia lenta rK3 has the capability of cleaving the heterocyclic C-ring of both (−)-epicatechin and (+)-catechin giving rise to 1-(3,4-dihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)propan-2-ol (Kutschera, Engst, Blaut, & Braune, 2011). Flavonifractor plautii can further convert 1-(3,4-dihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)propan-2-ol to 5-(3,4-dihydroxyphenyl)-2-valerolactone and 4-hydroxy-5-(3,4-dihydroxyphenyl)valeric acid (Kutschera et al., 2011; Winter, Popoff, Grimont, & Bokkenheuer, 1991). Two probiotics, Streptococcus thermophilus and Lactobacillus casei-01 were able to metabolize A-type procyanidins from Litchi pericarp during their log phase of growth. S. thermophilus transformed procyanadins A2 to its isomer and Lactobacillus casei-01 decomposed flavan-3-ols into 3,4-dihydroxyphenylacetic acid, 4-hydroxyphenylpropionic acid, m-coumaric acid, and p-coumaric acid (Li et al., 2013). Aspergillus fumigatus was able to grow on purified B-type procyanidins from apples. Oxygenase from this fungus modified the terminal unit of procyanidin B2 to form a metabolite with a lactone moiety (Contreras-Dominguez et al., 2006).

Due to their easy accessibility, most in vitro studies used fecal microbiota as a representative of gut flora. However, this method has several drawbacks. First, the transformation of the proanthocyanidins into their metabolites varies dramatically among individual donors (Sánchez-Patán et al., 2012). This is because the colon of healthy adults harbors about 400 different cultivable species belonging to more than 190 genera (Sekirov, Russell, Antunes, & Finlay, 2010). Their composition and distribution is affected by the physiological conditions of the host (gender, ethnicity, age, stress and health status), diet habit and environmental circumstances (contact with pathogens and use of medicine). This model does not reflect variations in microbial numbers and composition across the length of the gastrointestinal tract. Most models reported pooled the fecal sample from several volunteers, which is a good way to characterize the overall trend. But this leads to the loss of information about inter-individual variation. Second, a large portion of bacteria in the human colon are non-culturable in vitro. These obstacles hampered the discovery of bacterial species related to the degradation of proanthocyanidins and the identification of new metabolites. A better understanding of human microbiota and an improvement in culturing techniques is needed to overcome these obstacles.

In vivo studies showed that microbial derived phenylvalerolactone and phenolic acids were the predominant metabolites of procyanidins in blood and urine. After humans were given cocoa procyanidins that contained dimers to decamers, urinary excretion (0–7 h after dosing) of 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone was 30 μmol in contrast to about 2 μmol for flavan-3-ol monomers (Ottaviani et al., 2012). In humans who consumed procyanidin-rich cocoa regularly, blood level of (−)-epicatechin and 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone were undetectable and 0.48 μM, respectively (Urpi-Sarda, Monagas, Khan, Llorach et al., 2009). Urinary excretion of p-coumaric acid, vanillic acid, 3-hydroxybenzoic acid, and ferulic acid increased by over 2-fold in humans after consumption of 40 g of procyanidin-rich cocoa powder (Urpi-Sarda, Monagas, Khan, Lamuela-Raventos et al., 2009). There was an increase in urinary excretion of 3′-(3′-hydroxyphenyl)propionic acid, 2′-(3′-hydroxyphenyl)acetic acid, vanillic acid (4-hydroxy-3-methoxybenzoic acid), and m-hydroxybenzoic acid after human volunteers consumed 439 mg procyanidins and 147 mg catechin monomers (Rios et al., 2003).

The major microbial metabolites of procyanidins in rat serum were 3,4-dihydroxybenzoic acid, vanillic acid, and 4-hydroxyphenylacetic acid after animals were given sorghum bran (Gu et al., 2007). About 50–80% ingested procyanidins were degraded in the gastrointestinal tract. Up to 11% of ingested procyanidins were excreted in 24 h urine in the form of phenolic acids (Gu et al., 2007). The absorption of procyanidins in the form of phenolic acids was likely underestimated because we were not able to quantify all the microbial metabolites derived from procyanidins. A recent study using 14C-labeled procyanidin B2 showed that the absolute bioavailability of dimer was about 82% using the value of total urinary excretion. After an oral dose, 58% of ingested procyanidins were excreted in 24-h urine and additional 40% of total radioactivity was excreted in feces (0–96 h after administration) (Stoupi et al., 2010). This study confirmed that a major portion of ingested procyanidins were degraded by gut microflora before absorption.

4. Absorption of proanthocyanidins may not be a prerequisite for bioactivity

Proanthocyanidins scavenge free radicals, modulate gene expression, and mediate signal transduction in cells. In this sense, absorption is a prerequisite for bioactivity. However, our knowledge on dietary fiber suggests absorption is not necessarily a prerequisite for bioactivity. Recent studies unraveled the pivotal role of gut microbiota on human health (Robles Alonso & Guarnier, 2013). Increasing evidences suggests that proanthocyanidins have the potential to confer health benefits via modulation of the gut microbiota and by exerting prebiotic-like effects. Proanthocyanidins in diets shifted the bacterial population in the rat gastrointestinal
Proanthocyanidin dimers, trimers, and tetramers were ab-

main the second focus. Metabolites derived from A-type proanthocyanidins. The ocyanidins will likely focus on identification of new and bio-

microbiota. Future research on the bioavailability of proanthocyanidins.

Lastly, absorption may not be a prerequisite for proanthocy-
in vivo

anthocyanidin dimer was 58%, counted as total radioactivity.

phenolic acids. Many microbial metabolites of proanthocyani-

noids and gut microbiota represents an exciting new trend and may shed light on the bioavailability of proanthocyanidins.

5. Conclusions

Proanthocyanidin dimers, trimers, and tetramers were ab-

sorbed in their intact form and their absorption rates were less than 10% of (−)-epicatechin. The absorbed oligomers un-

undergo lower extent of phase II metabolism in rats compared with (−)-epicatechin. Proanthocyanidins larger than tetra-

mers are not absorbed in their intact form. Depolymerization of proanthocyanidins to monomers is negligible in the gastro-

intestinal tract. As a result, oligomers and polymers did not contribute to the concentration of monomers in blood or ur-

ine. The majority of ingested proanthocyanidins were de-

graded by gut microflora into phenylvalerolactones and phenolic acids. Many microbial metabolites of proanthocyanidi-

noids have not been identified. The total absorption rate of pro-

anthocyanidin dimer was 58%, counted as total radioactivity excreted in urine. Health benefits of proanthocyanidins in vivo may attribute in part to their microbial metabolites. Lastly, absorption may not be a prerequisite for proanthocy-

anidins to exert health benefits. They may influence human health by affecting the composition and metabolism of gut microbiota. Future research on the bioavailability of proantho-
cyans will likely focus on identification of new and bio-

active microbial metabolites, especially the unique metabolites derived from A-type proanthocyanidins. The interaction of proanthocyanidins with gut microbiota will re-

main the second focus.

R E F E R E N C E S


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Please cite this article in press as: Ou, K., & Gu, L. Absorption and metabolism of proanthocyanidins, Journal of Functional Foods (2013). http://dx.doi.org/10.1016/j.jff.2013.08.004


