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Molecules of Interest

Phloridzin: Biosynthesis, distribution and physiological relevance in plants

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ABSTRACT

The phenolic compound phloridzin (phloretin 2′-*O*-glucoside, phlorizin, phlorrhizin, phlorhizin or phlorizoside) is a prominent member of the chemical class of dihydrochalcones, which are phenylpropanoids. The apple tree (*Malus* sp.) accumulates high amounts of phloridzin, whereas few other species contain this compound only in low amounts. Additionally, *Malus* sp. show a species- and tissue-specific distribution of phloridzin and its derivatives. Whereas the physiological role of phloridzin *in planta* is not fully understood, the effect on human health – especially diabetes – and membrane permeability is well documented. The biosynthesis of phloridzin was investigated only recently with recombinant enzymes and plant protein extracts and involved a NADPH-dependent dehydrogenase, chalcone synthase and UDP-glucose:phloretin 2′-*O*-glycosyltransferase.

1. Introduction

De Koninck (1835a,b) isolated and described a bitter tasting substance with antipyretic effects from the bark of the apple tree. He found that this new compound was more prevalent in root bark than in stem bark and suggested calling it phloridzin (φλοιός: bark, ρίζα: root). Phloridzin is sometimes also referred to as phlorizin, phlorrhizin, phlorhizin or phlorizoside. Phloridzin belongs to the chemical class of dihydrochalcones, phenylpropanoids with structures closely related to those of the immediate flavonoid precursors, the chalcones. It consists of a C_6–C_3–C_6 skeleton structure (two aromatic rings connected by a C_3 chain) with a β-D-glucopyranose moiety attached at position 2′ (Fig. 1). More than 700 peer-reviewed articles dealing with phloridzin or its derivatives have been published since 2000. In more than 170 years since its discovery, phloridzin and its derivatives have been widely used in human medicine and for physiological studies on biological membranes. Most studies relate to diabetes, obesity, stress hyperglycemia, antioxidative activity, membrane permeability and longevity-extending agents in foods, beverages, food additives, pharmaceuticals and cosmetics (Ehrenkranz, 2006, 2005; Gaudout et al., 2006; Rezk et al., 2002; Sukhorukov et al., 2001; Valenta et al., 2001). In particular, the effect of phloridzin on glucose uptake and diabetes has been intensively investigated and was reviewed by Ehrenkranz et al. (2005). In contrast, knowledge about the physiological relevance of phloridzin *in planta* is limited. The biosynthetic steps leading to phloridzin were recently investigated with recombinant enzymes (Gosch et al., 2010; Jugdé et al., 2008) and plant protein extracts (Gosch et al., 2009).

2. Biosynthesis of phloridzin

2.1. Precursors

Whereas the biosynthesis of chalcones and flavonoids is well understood at the enzyme and gene level, the biosynthesis of closely related dihydrochalcones such as phloridzin has only recently been elucidated. The biosynthetic pathway leading to phloridzin is shown in Fig. 2. Malonyl-CoA (**1**) is synthesized from acetyl-CoA, whereas *p*-coumaroyl-CoA (**2**) originates from phenylalanine, which is produced via the shikimate pathway. Phenylalanine ammonia-lyase catalyzes the formation of cinnamate from phenylalanine and marks the branching point between primary metabolism and cinnamate-related polyphenolic compounds. Cinnamate is further hydroxylated by the cinnamate 4-hydroxylase and activated by the hydroxycinnamate:CoA ligase, resulting in *p*-coumaroyl-CoA (4-hydroxycinnamoyl-CoA).

2.2. Formation of p-dihydrocoumaroyl-CoA from p-coumaroyl-CoA

Whereas *p*-coumaroyl-CoA is the precursor for the naringenin chalcone (**3**) and further flavonoid formation (Fig. 2),

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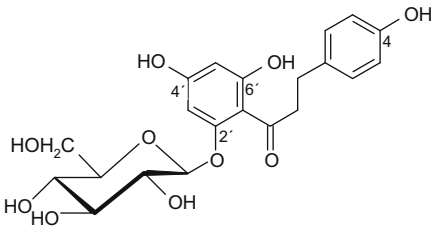

Fig. 1. The dihydrochalcone phloridzin consists of a C_6–C_3–C_6 skeleton structure with a β-D-glucopyranose moiety attached at position 2′.

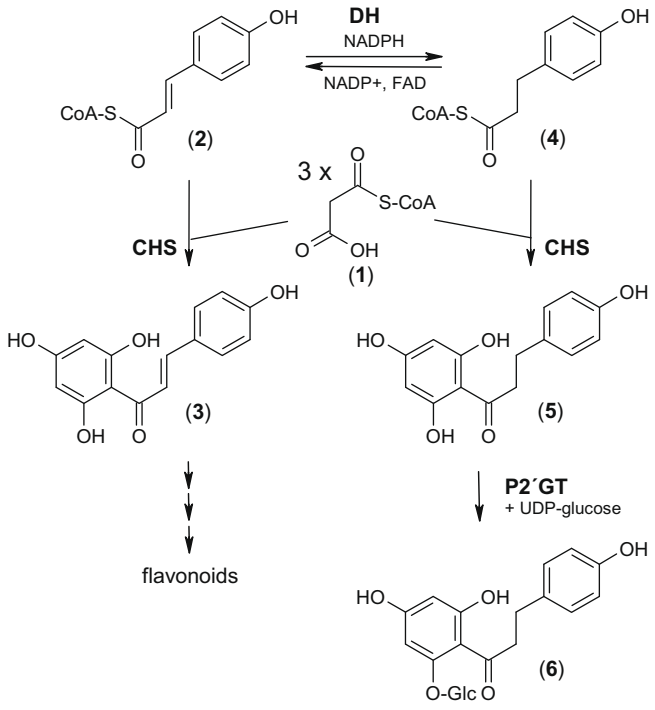

Fig. 2. Biosynthetic pathway of phloridzin and flavonoids. CHS, chalcone synthase; DH, dehydrogenase; P2′GT, UDP-glucose:phloretin 2′-*O*-glycosyltransferase.

p-dihydrocoumaroyl-CoA (4-hydroxydihydrocinnamoyl-CoA; **4**) is required for the biosynthesis of dihydrochalcones such as phloretin (**5**). Avadhani and Towers (1961) showed that young apple leaves fed with radiolabeled phenylalanine or cinnamic acid form radiolabeled phloridzin via *p*-coumaric acid, but that *p*-dihydrocoumaric acid was not detected as an intermediate. Therefore, the authors assumed that *p*-dihydrocoumaric acid was a breakdown product after fungal attack, for example, rather than a precursor during phloridzin formation. Yamazaki et al. (2001) showed with recombinant enzymes from *Psilotum nudum* that the CoA ester (*p*-dihydrocoumaroyl-CoA) can act as a precursor of phloretin. Gosch et al. (2009) showed the formation of phloretin when the CoA ester of *p*-coumaric acid, radiolabeled malonyl-CoA and NADPH were incubated with protein extracts of apple leaves. With naringenin as a substrate or without the cofactor NADPH no phloretin formation was detected. Therefore, it is assumed that *p*-dihydrocoumaroyl-CoA is formed from *p*-coumaroyl-CoA by a NADPH-dependent dehydrogenase (NADPH:*p*-coumaroyl-CoA oxidoreductase; Gosch et al., 2009). In general, the dehydrogenase catalyzing the interconversion of the CoA esters *p*-coumaroyl-CoA and *p*-dihydrocoumaroyl-CoA does not appear to be plant-specific. Watts et al. (2004) also detected the formation of naringenin besides phloretin when double transgenic *Escherichia coli* overexpressing a hydroxycinnamate:CoA ligase and a chalcone synthase were fed *p*-dihydrocoumaric acid. This implies the existence of a dehydrogenase in *E. coli*. Single transgenic *E. coli* (empty plasmid vector or overexpressing a hydroxycinnamate:CoA ligase or a chalcone synthase) revealed that this unknown dehydrogenase is active on the level of the CoA ester. Similar results were obtained by Jiang et al. (2005), who used *Saccharomyces cerevisiae* overexpressing a phenylalanine ammonia lyase, a hydroxycinnamate:CoA ligase and a chalcone synthase. After feeding either phenylalanine or tyrosine, naringenin and phloretin were detected. A similar dehydrogenase reaction was involved in the proposed degradation pathway of naringenin by *Eubacterium ramulus* with a naringenin chalcone, phloretin and *p*-dihydrocoumaric acid as intermediates (Herles et al., 2004; Schneider and Blaut, 2000). Since the dehydrogenase activity does not appear to be specific to the dihydrochalcone biosynthesis, Gosch et al. (2009) screened different enzymes, which catalyze similar dehydrogenase reaction steps and use structurally related native substrates for their ability to interconvert *p*-coumaroyl-CoA and *p*-dihydrocoumaroyl-CoA. A recombinant human medium chain acyl-CoA dehydrogenase, which actually catalyzes the FAD-dependent oxidation of octanoyl-CoA to octenoyl-CoA, could convert *p*-dihydrocoumaroyl-CoA to *p*-coumaroyl-CoA under certain conditions, but no homologous gene from apple was found. Another candidate, a recombinant enoyl-ACP (acyl carrier protein) reductase, which actually catalyzed crotonyl-ACP to butyryl-ACP, could not interconvert the CoA esters. In summary, the dehydrogenase activity involved in the formation of dihydrochalcones in apple is characterized at the enzymatic level. Some potential candidate genes can be excluded, but the identity of the dehydrogenase remains unclear.

2.3. Formation of phloretin from 4-hydroxydihydrocinnamoyl-CoA and malonyl-CoA

The high similarity of *p*-coumaroyl-CoA and *p*-dihydrocoumaroyl-CoA led to the assumption that the chalcone synthase could utilize both substrates with three molecules of malonyl-CoA to form a naringenin chalcone or phloretin, respectively. This was supported by studies of recombinant chalcone synthases from plants, which are not known to accumulate dihydrochalcones such as *Sinapis alba* (Tropf et al., 1994), *Arabidopsis thaliana* (Watts et al., 2004), *P. nudum* (Yamazaki et al., 2001) or *Pyrus communis* (Gosch et al., 2009). Studies of chalcone synthases from *M.* × *domestica* (Gosch et al., 2009) finally confirmed this hypothesis. No substrate preference was found – at least for different recombinant chalcone synthases from apple – suggesting that the formation of dihydrochalcones is catalyzed by the common chalcone synthase and not a specialized enzyme with distinct substrate specificity.

2.4. Glycosylation of phloretin to phloridzin

The attachment of a glucose moiety to phloretin at position 2′ is the final step in the formation of phloridzin (phloretin 2′-*O*-β-D-glucopyranoside, **6**). A cDNA clone from *M.* × domestica encoding a UDP-glucose:phloretin 2′-*O*-glycosyltransferase was recently isolated (Jugdé et al., 2008). Among several substrates tested, the recombinant enzyme accepted only phloretin as a substrate. Gosch et al. (2010) showed that several unspecific glycosyltransferases from *M.* × domestica with low sequence homology to one another could glycosylate phloretin to phloridzin. In addition, other phloretin glycosides (phloretin 4-*O*-glucoside and phloretin 4'-*O*-glucoside) were formed in vitro as byproducts in low amounts. In contrast to Jugdé et al. (2008), these recombinant glycosyltransferases showed a broader substrate acceptance. Therefore, more than one specific glycosyltransferase could be involved in the formation of phloridzin in apple. Interestingly, these glycosyltransferases are