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Characterization of a *Vitis vinifera* cv. Cabernet Sauvignon 3′,5′-*O*-methyltransferase showing strong preference for anthocyanins and glycosylated flavonols

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ABSTRACT

At ripening initiation in red grapevine (*Vitis vinifera*) berries, the exocarp turns color from green to red and then to purple due to the accumulation and extent of methylation of anthocyanins. The accumulation of transcripts encoding an *O*-methyltransferase was recently shown to be closely correlated with the onset of ripening and the degree of blue/purple pigmentation in grapevine berries; however, the biochemical function of this gene has remained uncharacterized. In this study, an *O*-methyltransferase cDNA that showed a distinct expression pattern when compared to closely related sequences was expressed in *Escherichia coli* and enzyme assays were carried out with a broad array of anthocyanin and other flavonoid substrates. We demonstrate that this enzyme carries out 3′, 5′-*O*-methylation of anthocyanins and flavonol compounds *in vitro*, which are known to be present in grape berries, with a preference for glycosylated substrates. The highest relative specific activity for the enzyme was found with delphinidin 3-*O*-glucoside as substrate. The enzyme is not able to methylate flavan type skeletons with chiral centers, such as either catechins or dihydroquercetin. The enzyme showed negligible specific activity for caffeoyl-CoA, compared to flavonol and anthocyanin substrates. Phylogenetic analysis of the *O*-methyltransferase suggests that it may be a member of a distinct subclass of Type 2 bivalent metal-dependent S-adenosylmethionine *O*-methyltransferases.

1. Introduction

Red grapes (*Vitis* spp.) and their fermentation products are rich sources of flavonoids that have important organoleptic and health-promoting properties. Anthocyanins impart red, blue, and purple coloration to grapes and wines, whereas flavonols in the grape exocarp (berry skin tissue) contribute to bitter taste, as well as to the stabilization of wine color through co-pigmentation with anthocyanins (Boulton, 2001). Certain anthocyanins and flavonoids were suggested to prevent the occurrence of skin cancer or act as protectants against blood and arterial diseases (Lila, 2004). Red wine anthocyanins have been shown to be absorbed by the human gastrointestinal tract into the circulatory system as intact glycosides (de Pascual-Teresa and Sanchez-Ballesta, 2008; Garcia-Alonso et al., 2009). The colorful anthocyanins are also important for the attraction of foraging animals and subsequent seed dispersal (Schaefer et al., 2008).

Grape berry growth follows a double sigmoidal curve. The onset of ripening (termed 'veraison' by viticulturists) marks the beginning of the second stage of growth when berries begin to soften and increase in size due to rapid cell enlargement. The sugar content of the berry increases rapidly and acidity decreases. During ripening initiation, the exocarp loses chlorophyll and begins to synthesize and accumulate phenolic compounds via the flavonoid pathway that are responsible for the development of characteristic colors, yellow-gold (flavonols), red (cyanidin-type anthocyanins), and blue to purple (delphinidin-type anthocyanins) (Fig. 1) (Watson, 2003). Flavonoid compounds all share the same basic skeleton, the flavan-nucleus, having two aromatic rings with six carbon atoms (rings A and B), connected by a heterocycle including three carbon atoms (ring C) (Fig. 1). Modifications of the central C-ring determine the specific structural class, such as flavanones, isoflavones, flavones, flavonols, flavanols, and anthocyanins. *Vitis* spp. berry exocarp does not contain pelargonidin-type anthocyanins, but grape cultivars accumulate diverse delphinidin- and cyanidin-type anthocyanins (Boss et al., 1996b). Flavonoids and anthocyanidins with a free hydroxyl group at the 3 position of the heterocyclic C-ring are unstable under physiological conditions and generally not found in nature (Forkmann and Heller, 1999). The enzyme, UDP-glucose:flavonoid 3-*O*-glucosyltransferase (3GT, EC 2.4.1.115), efficiently transfers a glucose moiety from

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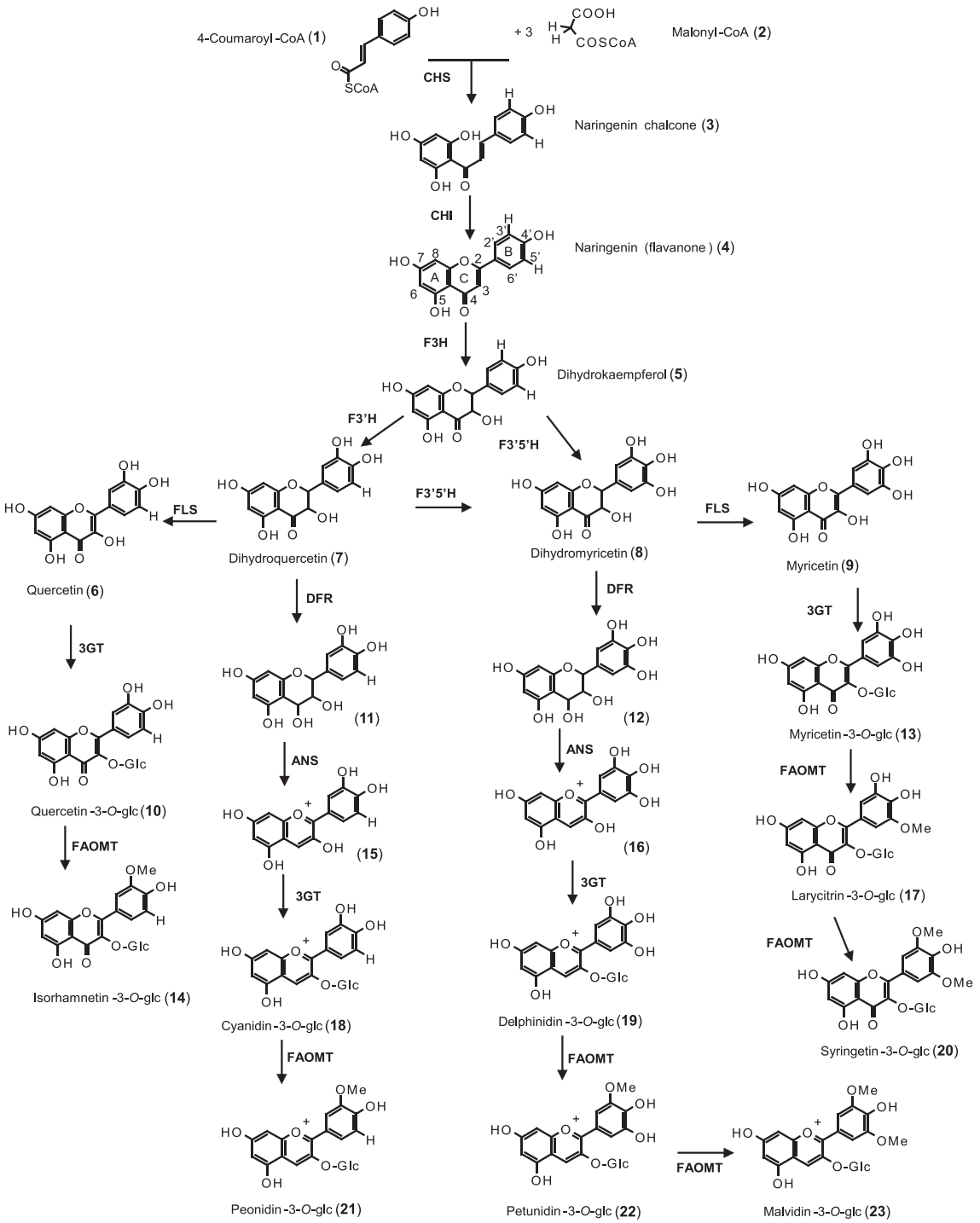

Fig. 1. The flavonoid biosynthesis pathway and structures of compounds found in *V. vinifera* berries. Branch pathways to proanthocyanidins as well as additional glycosylated and acylated anthocyanins and flavonoids are not shown. glc: glucoside.

UDP-glucose to position 3 of the C-ring, promoting stabilization. The expression of transcripts encoding 3GT coincides with the accumulation of anthocyanins in the exocarp of red grapes at ripening initiation (Boss et al., 1996a,b; Ford et al., 1998). Activity of native UDP-glucose:cyanidin 3-*O*-glucosyltransferase isolated from grape cell suspension cultures, as with the heterologously expressed enzyme, was highest with delphinidin (**16**) and cyanidin (**15**), and much lower with their respective methylated forms (Do et al., 1995; Ford et al., 1998). *O*-methylation of anthocyanins at available hydroxyl groups at the 3′ and 5′ positions of the B-ring was shown to be more efficient with cyanidin 3-*O*-glucoside (**18**) as substrate than with the respective aglycone by enzyme assays using a native *O*-methyltransferase (OMT) partially purified from *V. vinifera* cell cultures (Bailly et al., 1997). It is likely, therefore, that in *V. vinifera*, the *O*-methylation step occurs after the glycosylation step in the pathway, as shown in Fig. 1.

Expression of a putative transcript for this anthocyanin *O*-methyltransferase activity was first discovered when a caffeoyl-CoA *O*-methyltransferase (*cCoAomt*)-like gene was found to be one of nine transcripts consistently up-regulated coincident with the occurrence of red pigmentation in berry exocarp (Ageorges et al., 2006). Subsequently, transcript (Castellarin and Di Gaspero,