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Molecular evolution of flavonoid dioxygenases in the family Apiaceae

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Abstract

Plant species of the family Apiaceae are known to accumulate flavonoids mainly in the form of flavones and flavonols. Three 2-oxoglutarate-dependent dioxygenases, flavone synthase or flavanone 3 β-hydroxylase and flavonol synthase are involved in the biosynthesis of these secondary metabolites. The corresponding genes were cloned recently from parsley (*Petroselinum crispum*) leaves. Flavone synthase I appears to be confined to the Apiaceae, and the unique occurrence as well as its high sequence similarity to flavanone 3β-hydroxylase laid the basis for evolutionary studies. In order to examine the relationship of these two enzymes throughout the Apiaceae, RT-PCR based cloning and functional identification of flavone synthases I or flavanone 3β-hydroxylases were accomplished from *Ammi majus*, *Anethum graveolens*, *Apium graveolens*, *Pimpinella anisum*, *Conium maculatum* and *Daucus carota*, yielding three additional synthase and three additional hydroxylase cDNAs. Molecular and phylogenetic analyses of these sequences were compatible with the phylogeny based on morphological characteristics and suggested that flavone synthase I most likely resulted from gene duplication of flavanone 3β-hydroxylase, and functional diversification at some point during the development of the apiaceae subfamilies. Furthermore, the genomic sequences from *Petroselinum crispum* and *Daucus carota* revealed two introns in each of the synthases and a lack of introns in the hydroxylases. These results might be explained by intron losses from the hydroxylases occurring at a later stage of evolution.

Keywords: Apiaceae; Flavonoid 2-oxoglutarate dependent dioxygenases; Evolution; Flavone synthase I; Flavanone 3β-hydroxylase

1. Introduction

Among the low molecular weight polyphenols flavonoids comprise a large and widespread group of secondary metabolites, which has evolved as a consequence of the plants continuous interactions with the environment (Oksman-Caldentey and Inze, 2004), and may be traced back along the molecular relationship of relevant enzymes. Flavonoids are structurally composed of two aromatic rings (ring A and B) joined by a heterocycle (ring C). Modifications of the central C-ring differentiate the flavonoid subtypes as flavanones, flavones, isoflavones, dihydroflavonols, flavonols, flavan-3-ols and anthocyanins (Forkmann and Heller, 1999). Flavonoids serve the plants in a wide range of functions, for example as pigments in color signatures and UV-protection of tissues, or as chemical signal compounds in plant–microbe and plant–insect interactions (Schijlen et al., 2004; Harborne and Williams, 2000). Moreover, some flavonoids

Abbreviations: 2-ODD, 2-oxoglutarate-dependent dioxygenases; FHT, flavanone 3β-hydoxylase; DHF, dihydroflavonol; ANS, anthocyanidin synthase; FNS I, flavone synthase I; FLS, flavonol synthase; FNS II, flavone synthase II.

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have received considerable attention because of their beneficial effects on human health. Although flavonoids are not considered to be essential nutrients, anticarcinogenic, antiinflammatory, antihistaminic, antioxidant or antiviral activities have been reported (Hempel et al., 1999; Nielsen et al., 1999; Fejes et al., 2000; Middleton et al., 2000; Akihisa et al., 2003; Parejo et al., 2004).

The common flavonoid pathway has been well documented at the biochemical and molecular level, but little is known about the evolution of the respective enzymes. Several of these enzymes were assigned to the abundant group of 2-oxoglutarate-dependent dioxygenases (2-ODDs) which use molecular oxygen as the co-substrate and are characterized by their co-factor requirements of ferrous iron, 2-oxoglutarate and/or ascorbate (Forkmann and Heller, 1999; Springob et al., 2003). In general, these dioxygenases group into a large, functionally heterogeneous class of enzymes with widely divergent substrate specificities, ranging from flavonoids, gibberellins and alkaloids to penicillins and cephalosporins (Prescott, 1993; De Carolis and De Luca, 1994; Prescott and John, 1996; Prescott and Lloyd, 2000).

Flavanone 3β-hydoxylase (FHT) is a 2-ODD acting early in the flavonoid pathway by hydroxylating (2*S*)-flavanones stereospecifically to (2*R*,3*R*)-dihydroflavonols (DHFs) (Fig. 1). DHFs may be reduced to the corresponding flavan-*cis*-3,4-diols (leucoanthocyanidins) which are then converted further by another 2-ODD, anthocyanidin synthase (ANS), to anthocyanidins. Anthocyanidin conjugates, stabilized by glycosylation, are responsible for the coloration of many plant tissues. Alternative to the anthocyanidin branch pathway, (2*S*)-

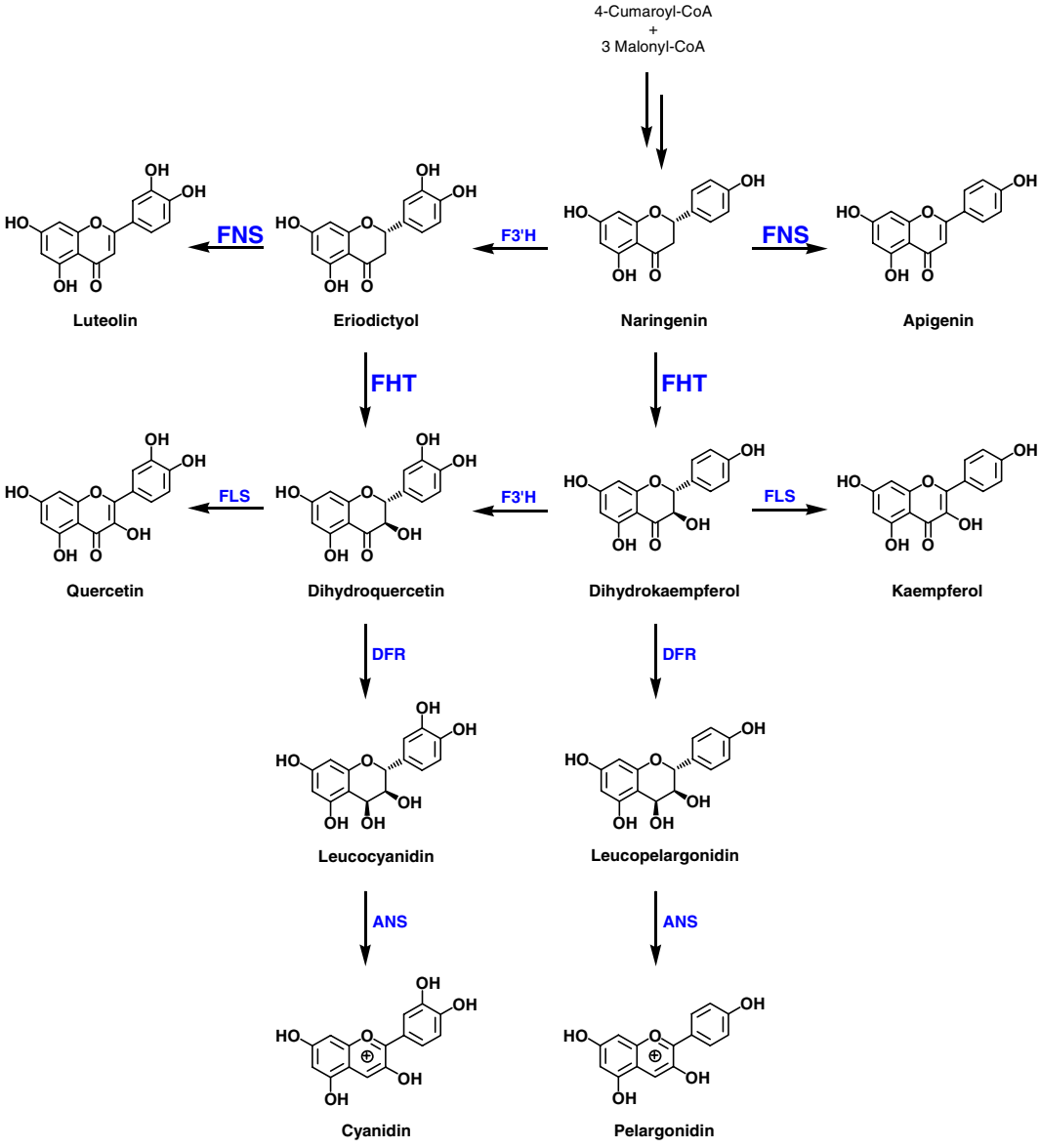

Fig. 1. Schematic flavonoid pathway. 2-ODDs: FNS I: flavone synthase I (Apiaceae), FHT: flavanone 3β-hydroxylase, FLS: flavonol synthase, ANS: anthocyanidin synthase. Others: DFR: dihydroflavonol 4-reductase, F3′H: flavonoid 3′-hydroxylase, FNS II: flavone synthase II.