ABSTRACTS (PH D THESIS)

Studies on coumarin-specific prenyltransfrase genes in plants

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Introduction

Coumarins are a large natural product group consisting of more than 1,500 derivatives as being plant secondary phenolics, and they contribute to chemical defense mechanism against a variety of environmental stresses, such as pathogens, herbivores, and abiotic stresses (ref. 1). Prenylation reactions largely diversify the chemical structures and bioactivities of coumarins, while none of prenyltransferase (PT) genes for coumarins have been identified thus far. In this study, we have performed molecular isolation, characterization, and phylogenetic analysis of PT genes characteristic to coumarins in order to elucidating prenylation reaction that is playing important roles in coumarin biosynthesis.

Geranyl diphosphate-specific coumarin PT from lemon

So far, almost all of plant-derived PT genes for aromatics encode membrane-bound enzymes accepting dimethylallyl diphosphate (DMAPP, C_5) as their specific prenyl donor. It is to be noted that they have been isolated from Fabaceae in most cases. Therefore, cDNA cloning of PT accepting longer prenyl donor, *e.g.*, geranyl diphosphate (C_{10}), was carried out using new plant taxon as its gene source.

Lemons (*Citrus limon*, Rutaceae) accumulate large amounts of geranylated coumarins in their outer pericarp, flavedo. Homology-based PCR cloning was done using cDNA pool from lemon flavedo and degenerated primers designed on conserved amino acid regions among known PT members. As a result, a strong candidate gene, *C. limon PT1* (*ClPT1*) encoding a hydrophobic polypeptide with 407 amino acids, was isolated. ClPT1 has two aspartate-rich motifs well conserved in the aromatic PT family, and i*n silico* analysis predicted that ClPT1 has plastid-sorting signal at its N-terminal region and multiple transmembrane helices. Biochemical studies using a yeast recombinant protein indicated that ClPT1 shows geranyltransferase activities for coumarin derivatives. Further enzymatic properties of this membrane-bound enzyme were preformed.

O-PT for aromatics

In general, *Citrus* species accumulate various *O*-prenylated coumarins, such as bergamottin, where prenyl moieties were attached to benzene ring via *C*-*O* bond, while no genes corresponding to the C-O bond-mediated prenylation for aromatics has been identified in plants so far. To obtain biochemical information of *O*-PT, enzymatic properties of the *O*-PT activity to yield bergamottin, bergaptol-5-*O*-geranyltransferase (B5OGT) activity, was investigated using crude enzyme prepared from lemon flavedo. B5OGT activity was detected only from membrane fraction of lemon flavedo and required divalent cations, *e.g.* Mg^{2+} and Mn^{2+}. Both properties were common to already-known aromatic PT members in plants, suggesting that *O*-PT genes in Rutaceae belong to membrane-bound PT gene family.

PT genes involved in the first reaction step in furanocoumarin formation

Furanocoumarins (FC), a tricyclic coumarin subgroup composed of liner and angular types, function as defense chemicals against biotic stresses. In FC biosynthetic pathway, umbelliferone dimehylallyltransferase (UDT), a PT catalyzing the first reaction step, determines which FC types is finally produced, i.e., dimethylallyltransferase activity at 6 or 8-position of umbelliferone (U6DT or U8DT activity) yields demethylsuberosin or osthenol leading to linear or angular FC, respectively (Fig. 1). In this study, identification of UDT genes from parsnip (*Pastinaca sativa*, Apiaceae) was performed. *In silico*

screening of EST data constructed from parsnip total RNA found two candidate cDNAs, *P. sativa PT1* and *2 (PsPT1* and *PsPT2*). *In vitro* characterization of PsPT1 and PsPT2 using *Nicotiana benthamiana* transient expression system revealed that PsPT1 and PsPT2 show U6DT and U8DT activities as their major function. Expression of both *PsPT1* and *PsPT2* was induced by methyl jasmonate, a FC elicitor, suggesting their involvement in FC biosynthesis. Moreover, when crude enzyme from parsnip was incubated with DMAPP and umbelliferone, much higher U6DT activity than U8DT activity was detected, suggesting a possibility that PsPT1 is the major UDT in parsnip.

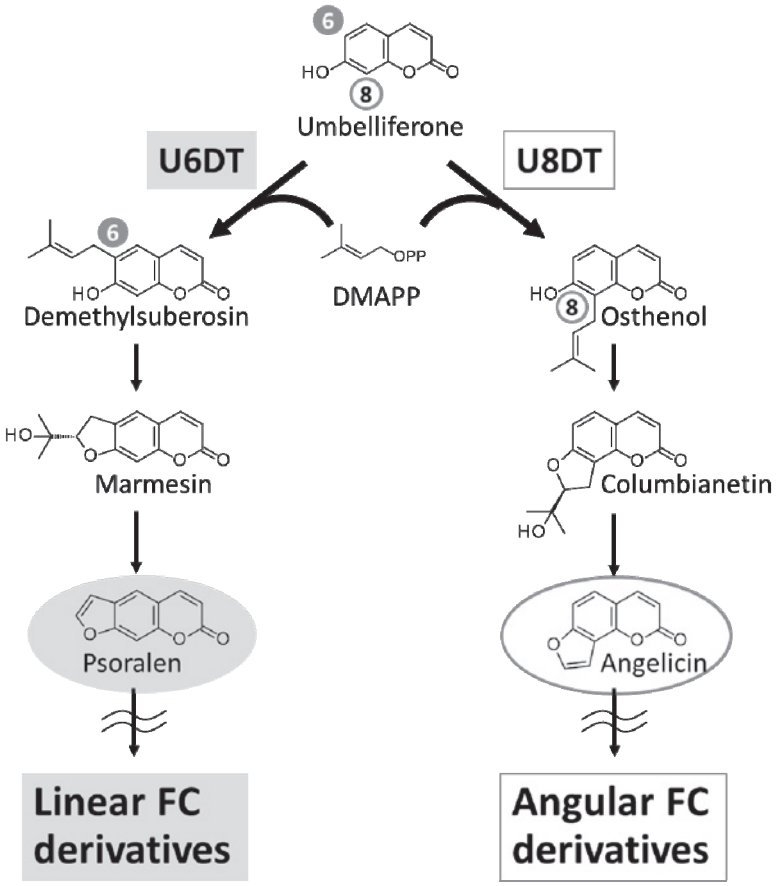

Fig. 1 Furanocoumarin biosynthetic pathway
Psoralen and angelicin were core structures of linear and angular FCs, respectively.

Reference

[1] Bourgaud, F., Hehn, A., Larbat, R., Doerper, S., Gontier, E., Kellner, S. and Matern, U., Biosynthesis of coumarins in plants: a major pathway still to be unravelled for cytochrome P450 enzymes, *Phytochemistry Reviews*, 5: 293 – 308, 2006.