Characterization of the flavonoid-specific prenyltransferase *SfN8DT-3* from *Sophora flavesecens.*

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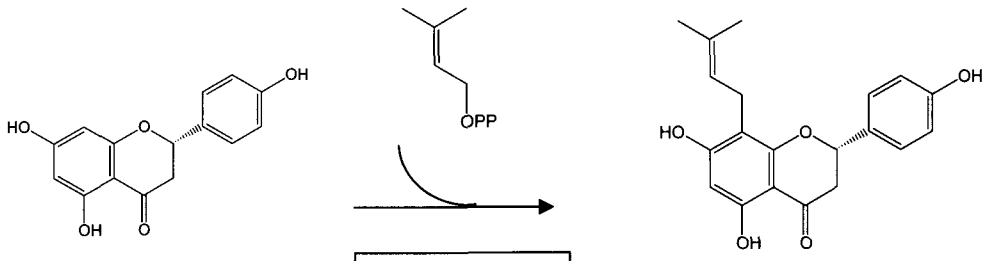
Prenylated flavonoids are natural products composed of a flavonoid core and a variety of decorations with prenyl residues of mainly dimethylallyl or geranyl groups, which are derived from isoprenoid metabolism. Many of them show diverse biological activities such as anti-bacterial, anti-tumor, anti-androgen, anti-leishmania, and anti-nitric oxide production, etc, in which prenyl residues play crucial roles for their biological activities. The prenylation reaction of flavonoid couples two major metabolic pathways, the shikimate and isoprenoid pathways, and this reaction step is often known to be a rate-limiting in their biosynthesis. We have recently previously reported the first plant gene for flavonoid-specific prenyltransferase from *Sophora flavescens*, *SfN8DT-1*. This enzyme is responsible for the prenylation of the flavanone, naringenin, at the 8-position, and is specific for dimethylallyl diphosphate (DMAPP) as its prenyl substrate [1]. This enzyme has multiple transmembrane α-helices and shares high similalry with homogentisate prenyltransferases involved in vitamin E or plastoquinone biosynthesis in their amino acid sequence (21%–55%) [2-3].

In this study, we have isolated two homologous cDNAs *SfN8DT-2* and *SfN8DT-3* from *S. flavescens* by reverse transcriptase-PCR (RT-PCR) using specifc primers designed for *SfN8DT-1* and characterized them in detail. Southern blot analysis suggested that at least four copies of *SfN8DT-1* gene were present in *S. flavescens*. *SfN8DT-3* is a paralog of *SfN8DT-1* showing 98% amino acid sequence identity with *SfN8DT-1*. *SfN8DT-3* expressed in yeast revealed clear prenyltransferase activity for naringenin in the presence of DMAPP. The Recombinant SfN8DT-3 has slightly lower affinity for substrates (naringenin, DMAPP) than that of Recombinant SfN8DT-1. Using amplified fragment length analysis, we found that *SfN8DT-3* gene expression was lower compared with *SfN8DT-1* gene. These results suggest that *SfN8DT-3* represents a paralogue gene of *SfN8DT-1* in *S. flavescens*.



(2S)- Naringenin

8-Dimethylallyl naringenin

[1] Sasaki K, et al., *Plant Physiol.*, 146, 1075-1084 (2008)

[2] Cahoon EB, et al., *Nat Biotechnol.*, 21, 1082–1087 (2003)

[3] Sadre R, et al., *FEBS Lett.*, 580, 5357–5362 (2005)