Acta Pharmaceutica Sinica B ▮▮▮▮;▮(▮):▮▮▮–▮▮▮

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Acta Pharmaceutica Sinica B

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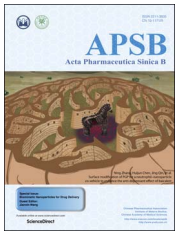

ORIGINAL ARTICLE

Biocatalytic access to diverse prenylflavonoids by combining a regiospecific *C*-prenyltransferase and a stereospecific chalcone isomerase

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Received 3 November 2017; received in revised form 8 January 2018; accepted 18 January 2018

KEY WORDS

Biocatalysis;
Prenyltransferase;
Chalcone isomerase;
Licorice;
Prenylflavonoids

Abstract Prenylflavonoids are valuable natural products that have diverse biological properties, and are usually generated biologically by multiple metabolic enzymes in nature. In this study, structurally diverse prenylflavonoids were conveniently synthesized by enzymatic catalysis by combining GuILDT, a regiospecific chalcone prenyltransferase, and GuCHI, a stereospecific chalcone isomerase that has promiscuous activity for both chalcones and prenylchalcones as substrates. Our findings provided a new approach for the synthesis of natural/unnatural bioactive prenylflavonoids, including prenylchalcones and optical prenylflavanones with chalcone origins.

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

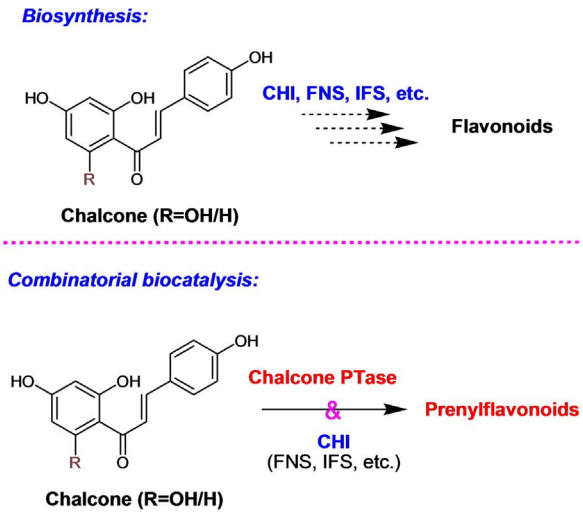
https://doi.org/10.1016/j.apsb.2018.01.009

1. Introduction

Prenylflavonoids are a diverse class of naturally occurring flavonoids that contain prenyl substituents. In higher plants, these compounds usually exhibit strong antibacterial and antifungal activities[1]. Moreover, many of them have attracted attention due to their diverse pharmacological activities, such as anti-cancer, anti-obesity, anti-inflammation activity, anti-bacterial pathogens, and the impairment of autophagosome maturation[2–8]. *C*-Prenylation of flavonoids by flavonoid prenyltransferase (PTase) represents a Friedel–Crafts alkylation of the flavonoid skeleton in the biosynthesis of natural prenylflavonoids and plays a crucial role in generating the diverse compounds due to the different prenylation positions and various types of prenyl groups as well as further tailoring modifications (*e.g.*, hydroxylation and cyclization), leading to the production of more than 1000 prenylflavonoids in plants[9]. Substitutions with prenyls of different modes on the flavonoid scaffold not only contribute significantly to the structural diversity, but also markedly enhance the bioactivity and bioavailability compared with their nonprenylated parent molecules[10].

The synthesis of prenylflavonoids by chemical methods has always had many limitations, such as regio-/stereoselectivity and variations in the number of prenyl groups attached to various flavonoid skeletons. In contrast, enzymatic catalysis is effective and superior in terms of regioselectivity and stereoselectivity in the directional synthesis of prenylflavonoids. However, only a few flavonoid PTases from plants have been identified to date, and most of them are strictly substrate-specific and regiospecific[11–17]. It has been demonstrated that most of the enzymes involved in flavonoid scaffold biosynthesis, including chalcone synthase (CHS), chalcone isomerase (CHI), flavone synthase (FNS), isoflavone synthase (IFS), and flavanone 3β-hydroxylase (F3H) have broad substrate specificity[18,19]. Of these enzymes, CHI catalyzes the stereospecific isomerization of a chalcone into the corresponding (–)-flavanone *via* intramolecular cyclization with clear substrate flexibility[20,21]. To develop an effective method to synthesize prenylflavonoids, we focused on cascade reactions by combining chalcone PTase and CHI to produce prenylflavonoids with diverse structures (Scheme 1).

Licorice from the roots and rhizomes of various species of *Glycyrrhiza* (Leguminosae) is one of the most frequently used traditional medicines in China and in other countries[22,23]. More than 200 prenylated flavonoids have been identified in licorice, including prenylchalcones, prenylflavanones, prenylflavones, and prenylisoflavonoids[24,25]. Therefore, *Glycyrrhiza* is considered an ideal source for the discovery of biosynthetic enzymes/genes for the biosynthesis of prenylflavonoids. In this article, a new chalcone-specific PTase, GuILDT, and a new stereo- and regio-specific type II CHI, GuCHI, were characterized from *Glycyrrhiza uralensis* and employed for cascade reactions to produce diverse bioactive prenylflavonoids. Their enzymatic synthesis applications as well as roles they played in the biosynthesis of structurally different prenylflavonoids were also discussed.



Scheme 1 Biocatalytic access to diverse prenylflavonoids by combining various biosynthetic enzymes.

2. Materials and methods

2.1. General material and reagents

Plasmid pESC was purchased from Stratagene. Synthetic dextrose dropout medium lacking histone (SD-His) were used to select *Sacharomyces cerevisiae* transformants containing the assembled plasmids. Complex medium (YPDA) consists of 2% peptone, 1% yeast extract, and 2% glucose supplemented with 0.01% adenine hemisulfate. *S. cerevisiae* YPH499 was used as the host for DNA assembly and heterologous expression. Plasmid pET-28a was purchased from Invitrogen. The prenyl donors dimethylallyl diphosphate (DMAPP), geranyl diphosphate (GPP), farnesyl diphosphate (FPP), geranylgeranyl diphosphate (GGPP), and phytyl diphosphate (PPP) were chemically synthesized as describe previously[26]. Isoliquiritigenin (**1**), 2′-hydroxychalcone (**5**), and 2-hydroxychalcone (**6**) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Narigenin chalcone (4,2′,4′,6′-tetrahydroxychalcone, **2**), 2′,4′-dihydroxychalcone (**3**), 2,4,2′,4′-tetrahydroxychalcone (**4**), were chemically synthesized according to the literatures[27].

2.2. Plant material and culture conditions

G. uralensis callus cultures were maintained on Murashige and Skoog basal medium containing 30 g/L sucrose, 6 g/L agar, 0.2 mg/L α-naphthaleneacetic acid (NAA), 0.5 mg/L 6-benzylaminopurine (6-BA), and 0.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), pH 6.2[28]. The suspension of cultured cells were initiated by inoculating two-week-old callus cultures into 150 mL of Murashige and Skoog liquid medium, incubated on a rotary shaker at 120 rpm in the darkness at 25 °C, and subcultured every 15 days.

2.3. Analytical methods

^{1}H and ^{13}C NMR spectra were recorded on VNOVA SYSTEM-600 spectrometers using CD_3COCD_3 or CD_3OD as solvent. Optical rotations were determined using a Perkin-Elmer Model 341 LC polarimeter (Perkin-Elmer). Enzymatic products were analyzed with a 1200 HPLC system (Agilent) coupled with an LCQ Fleet ion trap mass spectrometer (Thermo Scientific). HPLC analyses were conducted with an RP-18 end-capped Purospher STAR LP column (250 mm × 4.6 mm, 5 µm, Merck Millipore) with a flow rate of 1 mL/min. UV detection was set at 290 or 380 nm, and the column was operated at 30 °C. The solvent system consisted of a linear gradient (45%–95%, *v*/*v*) of methanol in water

Please cite this article as: Li Jianhua, et al. Biocatalytic access to diverse prenylflavonoids by combining a regiospecific *C*-prenyltransferase and a stereospecific chalcone isomerase. *Acta Pharmaceutica Sinica B* (2018), https://doi.org/10.1016/j.apsb.2018.01.009