Acta Pharmaceutica Sinica B 2017;7(5):603–609

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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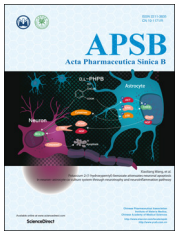

ORIGINAL ARTICLE

Molecular cloning and functional identification of sterol C24-methyltransferase gene from *Tripterygium wilfordii*

Hongyu Guan[a,b,c,†], Yujun Zhao[b,†], Ping Su[b], Yuru Tong[b], Yujia Liu[a], Tianyuan Hu[a], Yifeng Zhang[a], Xianan Zhang[a], Jia Li[a], Xiaoyi Wu[a], Luqi Huang[b,], Wei Gao[a,*]**

[a]*School of Traditional Chinese Medicine, Capital Medical University, Beijing 100069, China*
[b]*State Key Laboratory Breeding Base of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China*
[c]*Beijing University of Chinese Medicine Third Affiliated Hospital, Beijing 100029, China*

Received 26 May 2016; received in revised from 27 June 2016; accepted 29 June 2016

KEY WORDS

Cloning;
Cycloartenol C24-methyl transferase;
Enzymatic assay;
Inducible expression;
Tissue expression

Abstract Sterol C24-methyltransferase (SMT) plays multiple important roles in plant growth and development. SMT1, which belongs to the family of transferases and transforms cycloartenol into 24-methylene cycloartenol, is involved in the biosynthesis of 24-methyl sterols. Here, we report the cloning and characterization of a cDNA encoding a sterol C24-methyltransferase from *Tripterygium wilfordii* (*TwSMT1*). *TwSMT1* (GenBank access number KU885950) is a 1530 bp cDNA with a 1041 bp open reading frame predicted to encode a 346-amino acid, 38.62 kDa protein. The polypeptide encoded by the *SMT1* cDNA was expressed and purified as a recombinant protein from *Escherichia coli* (*E. coli*) and showed SMT activity. The expression of *TwSMT1* was highly up-regulated in *T. wilfordii* cell suspension cultures treated with methyl jasmonate (MeJA). Tissue expression pattern analysis showed higher expression in the phellem layer compared to the other four organs (leaf, stem, xylem and phloem), which is about ten times that of the lowest expression in leaf. The results are meaningful for the study of sterol

*Corresponding author. Tel.: +86 10 83911671; fax: +86 10 83911627.
**Corresponding author. Tel.: +86 10 64014411; fax: +86 10 64013996.
E-mail addresses: huangluqi01@126.com (Luqi Huang), weigao@ccmu.edu.cn (Wei Gao).
†These authors made equal contribution to this work.

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

http://dx.doi.org/10.1016/j.apsb.2017.07.001

biosynthesis of *T. wilfordii* and will further lay the foundations for the research in regulating both the content of other main compounds and growth and development of *T. wilfordii*.

1. Introduction

Tripterygium wilfordii Hook. F. is a traditional Chinese medicinal plant that has analgesic and anti-microbial properties, and thus it has been widely used to treat inflammatory diseases[1]. Moreover, recent research showed that *T. wilfordii* could treat immune and tumour diseases[2–4].

Isoprenoid compounds are main active ingredients of *T. wilfordii*. Several important enzymes have been cloned and identified for their biosynthetic pathways[5,6]. The isoprenoid compounds in *T. wilfordii* include sterols, chlorophyll, gibberellin, and a variety of terpenes[7]. Among these, sterols are hydrocarbon derivatives that consist of a four-membered cyclopentanoperhydrophenanthrene ring. Plant sterols are essential components of eukaryotic membranes. They help to maintain membrane integrity and permeability[8], participate in mammalian, yeast and plant cell endocytosis and production processes[9], and serve as precursors in the brassinosteroid hormone biosynthesis[10]. In addition, phytosterols can act as signalling molecules in plants , participating in the regulation of various physiological activities, such as photosynthesis, reproduction and immunization[11]. Sterol C24-methyltransferase (SMTs) have been found to play a key role in the synthesis of steroids with its methyltransferase property[12]. The analysis of different amino acid sequences in all the cDNAs suggested that *SMTs* can be separated into two gene families, *SMT1* and *SMT2*[13]. It has been reported that the two compounds play important roles in plant growth and development. The metabolic pathway chart is shown in Fig. 1. It has been indicated that the methylation reactions of cycloartenol and 24-methylene lophenol are catalysed by SMT1 and SMT2, respectively[14]. The two gene families are involved in the biosynthesis of 24-methyl and 24-ethyl sterols, respectively. Thus, cloning of the plant *SMT* genes and characterization of the gene products would provide an alternative approach to addressing some of the important questions regarding *SMTs*, such as the C-24 methylation mechanism and developmental regulation of the enzyme.

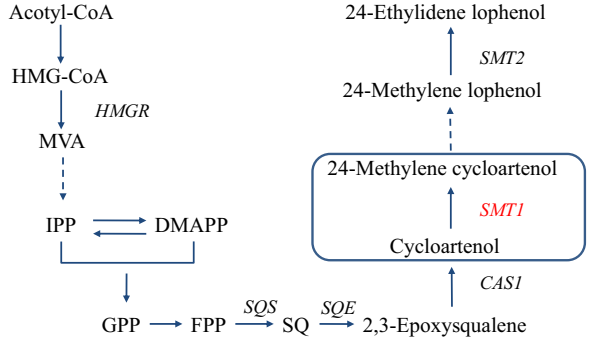

Figure 1 The biosynthetic pathway of phytosterol involving sterol C24-methyltransferase 1 (*SMT1*) gene and sterol C24-methyltransferase 2 (*SMT2*) gene. 3-Hydroxy-3-methylglutary CoA (HMG-CoA); 3-hydroxy-3-methyl glutaryl coenzyme A reductase (*HMGR*); mevalonate pathway (MVA); isopenteny pyrophosphate (IPP); dimethylally pyrophosphate (DMAPP); gerqnyl pyphosphate (GPP); famesyl pyrophosphate (FPP); squalene synthase (*SQS*); squalene (SQ); squalene epoxidase (*SQE*); cycloartenol synthase 1 (*CAS1*).

Molecular cloning of *SMTs* was recently achieved in a number of higher plant species, including *Astragalus bisulcatus*[15], *Arabidopsis thaliana*[16], *Oryza sativa*[17], *Nicotiana tobacum*[18], *Brassica oleracea*[19], and *Camellia sinensis*[20]. Until now, no *SMT* gene from *T. wilfordii* has been cloned. In this paper, we report the isolation and identification of a cDNA encoding SMT1 from *T. wilfordii* for the first time. The polypeptide encoded by the *T. wilfordii* cDNA was expressed in *E. coli* and shown to be an active SMT enzyme. The real-time quantitative PCR analysis of *TwSMT1* expression was found to be promoted upon the methyl jasmonate (MeJA) elicitor treatment.

2. Materials and methods

2.1. Plant materials

Cell suspensions of *T. wilfordii* in the study were cultured in Murashige and Skoog (MS) medium (pH 5.8) containing 2,4-dichlorophenoxyacetic acid (2,4-D, 0.5 mg/L), cytokinin (KT, 0.1 mg/L), indole-3-butytricacid (IBA, 0.5 mg/L), and sucrose (30 g/L), shaking at 120 rpm (Eppendorf, 5810 R, Germany) 25 °C in dark culture and subculture suspension cells (2 g) in the same medium (25 mL) every 20 days. The plants of *T. wilfordii* in the tissue expression analysis were obtained from Fujian province and have grown for seven years.

2.2. Cloning of TwSMT1 from T. wilfordii

Total RNA was extracted from *T. wilfordii* suspension cells stored at –70 °C using the CTAB-LiCl method[21]. The extract was purified using DNase I (Biolabs, Beijing, China) and an RNA cleaning kit (TIANGEN, Beijing, China) to remove contaminating genomic DNAs.

The purified product was reverse transcribed into first-stand 5′-RACE-Ready cDNA and 3′-RACE-Ready cDNA with the SMART RACE cDNA Amplification Kit (Takara Bio Group, Japan). According to mRNA fragments obtained from the transcription data, specific primers (3′-RACE Primer: 5′-TGGATGTAGGATGTGGAATCGGTGGA-3′; 5′-RACE Primer: 5′-TTAGGGCCTCAAGGCATTGTCTGGTC-3′) were designed to amplify 5′ and 3′ cDNA, respectively, followed by ligation into the *pEASY*-T3 vector (TransGen Biotech, Beijing, China) and transfer into *E. coli* Trans5*α* competent cells (TransGen Biotech,