KSABC
The Korean Society for Applied Biological Chemistry

NOTE

Open Access

Biosynthesis of fraxetin from three different substrates using engineered *Escherichia coli*

Seung Hoon An, Gyu-Sik Choi and Joong-Hoon Ahn*

Abstract

Fraxetin, which is a simple coumarin, is a phytochemical present in medicinal plants, such as *Fraxinus rhynchophylla*, and *Cortex Fraxini*. In plants, it serves as a controller of iron homeostasis. The health-enhancing activities of fraxetin, such as anticancer, neuroprotective and antibacterial activities, are known. Scopoletin 8-hydroxylase (S8H) is a key enzyme involved in the synthesis of fraxetin from scopoletin. Scopoletin can be synthesized either from esculetin by *O*-methylation or from ferulic acid by feruloyl CoA 6′-hydroxylase (F6′H) and 4-coumaric acid CoA ligase (4CL). To enable fraxetin synthesis, the fraxetin biosynthesis pathway was introduced into *Escherichia coli*. Three distinct routes, from ferulic acid, esculetin, and scopoletin, were designed for the synthesis of fraxetin. In the first approach, *E. coli* strain harboring S8H was used and found to synthesize 84.8 μM fraxetin from 100 μM scopoletin. Two *E. coli* strains were used for the other two approaches because these approaches required at least two enzymatic reactions. Through this approach, 41.4 μM fraxetin was synthesized from 100 μM esculetin, while 33.3 μM fraxetin was synthesized from 100 μM ferulic acid.

Keywords: Coumarin, Fraxetin, Scopoletin 8-hydroxylase

Introduction

Coumarins (benzo-alpha-pyrones) were first isolated from the tonka bean (*Dipteryx odorata*) in 1820. Since then, their presence has been detected in various parts of different plants, including the fruit (e.g., in Bael fruit or *Aegle marmelos*), seed (e.g., in tonka beans or *Calophyllum inophyllum*), root (e.g., in *Ferulago campestris*), and leaf (e.g., *Murraya paniculata*) [1]. All coumarins have a hydroxy or methoxy group at position 7. Scopoletin, esculetin, umbelliferone, fraxetin, as well as their respective glycosides, are termed simple coumarins; they are widespread in higher plants [2]. These coumarins play a pivotal role in protecting plants against pathogens [3]; furthermore, a simple coumarin, such as fraxetin, was found to modulate vital physiological processes such as iron homeostasis [4]. As naturally occurring phytochemicals, coumarins possess health-enhancing properties, including anticancer [1], neuroprotective [5], and antibacterial properties [6].

Coumarins were synthesized from hydroxycinnamic acids, such as *p*-coumaric acid, caffeic acid, and ferulic acid, in plants; *p*-coumaric acid, caffeic acid, and ferulic acid resulted in the synthesis of umbelliferone, esculetin, and scopoletin, respectively. The key enzyme for coumarin biosynthesis was *p*-coumaroyl CoA 2′-hydroxylase (C2′H) or feruloyl CoA 6′-hydroxylase (F6′H); the corresponding genes were cloned in *Arabidopsis thaliana* [7], *Ruta graveolens* [8], *Ipomoea batatas* [9], *Manihot esculenta* [10], *Angelica decursiva* [11], and *Peucedanum praeruptorum* [12]. This enzyme is a 2-oxglutarate-dependent dioxygenase; the hydroxylation of hydroxycinnamoyl-CoA resulted in the formation of a pyrone ring.

Fraxetin belongs to the family of simple coumarins and is synthesized from scopoletin by the hydroxylation of its carbon at position 8. Fraxetin is involved in iron metabolism in plants [4, 13]. Similar to other phytochemicals, fraxetin was found to exert beneficial effects in humans. These included antitumor [10, 14], neuroprotective [15],

*Correspondence: jhahn@konkuk.ac.kr
Department of Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 05029, Republic of Korea

Springer Open

An *et al. Appl Biol Chem*   (2020) 63:55

Page 2 of 6

antihyperglycemic [16], and anti-inflammatory [17] effects.

Since the metabolic pathway responsible for the synthesis of simple coumarins is well established, these compounds have been synthesized in *E. coli*. Scopoletin, esculetin, umbelliferone, skimming (umbelliferone 7-*O*-glucoside), and herniarin (7-*O*-methyl umbelliferone) were synthesized in *E. coli* [18, 19]. For the fraxetin synthesis process in *E. coli*, two routes were postulated (Fig. 1). The first route involved the synthesis of esculetin from glucose, followed by the conversion of esculetin into fraxetin by 7-*O*-methylation and 8-hydroxylation. The second route started with the synthesis of scopoletin from ferulic acid, followed by 8-hydroxylation. In the present study, one coumarin, namely fraxetin, was synthesized using *E. coli* via these two routes.

Materials and methods
Plasmid construction
Reverse transcription polymerase chain reaction (RT-PCR) was used to clone cDNA of scopoletin 8-hydroxylase (*S8H*) from *Arabidopsis thaliana* (*AtS8H*; GenBank: DQ446658.1). Two primers 5′-aagaattcaATGGGTATCAATTTCGAGGA-3′ and 5′-aagcggccgcTCACTCGGCACGTG-3′ were used (restriction sites for *Eco*RI and *Not*I have been underlined). Additionally, the *S8H* homologue from *Oryza sativa* (*OsS8H*; GenBank: XM_026024461) was cloned by RT-PCR using two primers: 5′-aagaattcaATGCCGTCCGGCTACGAC-3′ and 5′-aagcggccgcCTAATCTAGACTAGCGGCGG-3′ (restriction sites for *Eco*RI and *Not*I have been underlined). *AtS8H* was digested using the *Eco*RI and *Not*I sites and subcloned into pGEX 5X-3 (pG-AtS8H),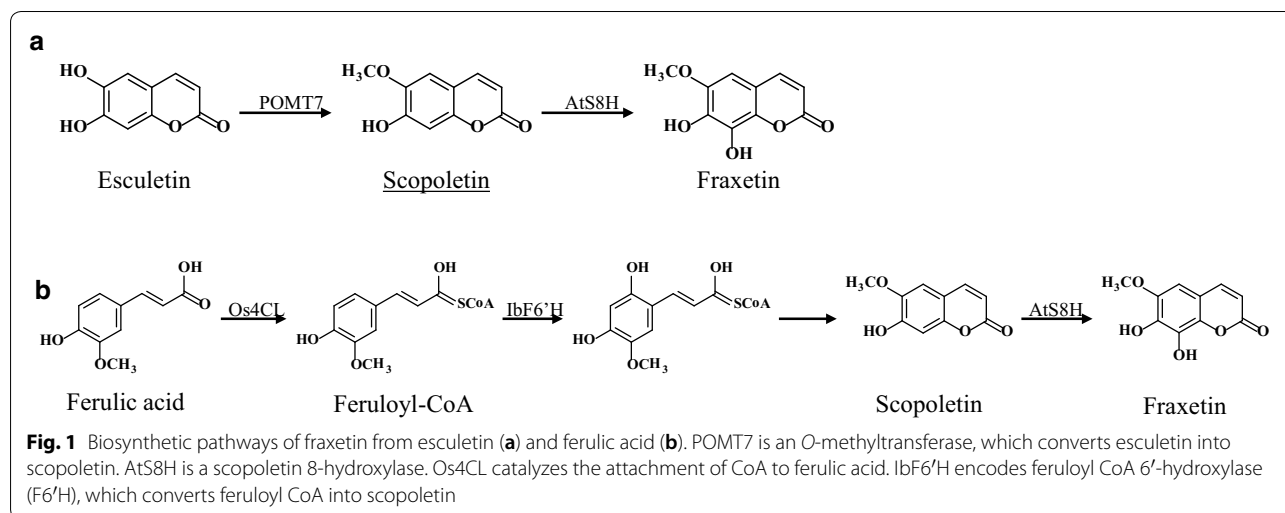 pET-duet1 (pE-AtS8H), pRSF-duet1 (pR-AtS8H), and pCDF-duet1 (pC-AtS8H). *OsS8H* was subcloned into *Eco*RI/*Not*I sites of pGEX 5X-3 (pG-OsS8H).

F6′H2 from *Ipomoea batatas* (*IbF6′H2*; GenBank : AB636154) and *4CL* (*Os4CL*; 4-coumarate: CoA ligase) from *O. sativa* had been previously cloned using RT-PCR [18]. *F6′H2* was first cloned into pET-duet1 (*Eco*RI/*Not*I) using PCR and, then, *Os4CL* was subcloned into pET-duet1 containing *F6′H2 to* generate pE-IbF6′H2-Os4CL (*Nde*I/*Xho*I). Subsequently, *Os4CL* was re-amplified with a forward primer, adding a *Not*I site and ribosomal-binding site (RBS), and a reverse primer, containing a *Xho*I site. Thereafter, *Os4CL* was subcloned into the *Not*I/*Xho*I sites of pET-duet1 containing *IbF6′H2* to generate pE-IbF6′H-Os4CL controlled by a single promoter (operon). The IbF6′H-Os4CL operon was subcloned into pGEX 5X-3 (*Eco*RI/*Xho*I).

POMT7 (flavone 7-*O*-methyltransferase) [20] and *POMT9* from *Populus deltoids* [21] and *ROMT9* (flavonoid 3′-*O*-methyltransferase) from *O. sativa* [22] have also been cloned previously. These genes were subcloned into pGEX 5X-3 vector.

Production and analysis of metabolites
For the synthesis of fraxetin from scopoletin, an overnight culture of an *E. coli* transformant containing pG-OsS8H, pG-AtS8H, pC-AtS8H, pE-AtS8H, or pR-AtS8H was inoculated into fresh LB medium containing 50 μg/mL ampicillin and grown at 37 °C until the OD_{600} reached 0.8; following this, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the medium at a final concentration of 0.1 mM or 1 mM and incubated at 18 °C for 16 h. Cells were harvested and the cell concentration was adjusted to an OD_{600} of 3.0. The cells were resuspended in M9 medium containing 2% glucose, ampicillin (50 μg/mL), and either 0.1 mM or 1 mM IPTG

Fig. 1 Biosynthetic pathways of fraxetin from esculetin (**a**) and ferulic acid (**b**). POMT7 is an *O*-methyltransferase, which converts esculetin into scopoletin. AtS8H is a scopoletin 8-hydroxylase. Os4CL catalyzes the attachment of CoA to ferulic acid. IbF6′H encodes feruloyl CoA 6′-hydroxylase (F6′H), which converts feruloyl CoA into scopoletin

An *et al. Appl Biol Chem*   *(2020) 63:55*

Page 3 of 6

in a test tube. A total concentration of 100 μM of the substrate (esculetin, isoscopoletin, scopoletin, or scoparone) was added, and the resulting culture was incubated at 30 °C for 24 h. An *E. coli* transformant containing the pG-AtS8H construct was employed to determine the substrate (scopoletin) concentration. The cell concentration was adjusted to an OD_{600} of 3.0. The substrate was added to the appropriate M9 medium at 0.1, 0.2, 0.3, or 0.5 mM. The reaction culture was incubated at 30 °C for 24 h.

The *E. coli* transformant harboring *ROMT9* was used to methylate esculetin to scopoletin, isoscopoletin, and scopoletin. Three reaction products were purified using thin layer chromatography (silica gel 60 F254, Millipore). A mixture of benzene and ethyl acetate (3:1) was used as a solvent. The *E. coli* transformant harboring *POMT9* was used to synthesize scopoletin from esculetin. The methylation reaction using *E. coli* was carried out as described by Kim et al. [20].

Analysis of the reaction products was carried out using Thermo Ultimate 3000 HPLC [23]. Mass spectrometry and proton nuclear magnetic resonance (NMR) were performed as previously described [24, 25]. The ^{1}H NMR of fraxetin in acetone-d_6 (in ppm) is δ 3.87 (3H, s, 6-OCH$_3$),

6.15 (1H, d, $J = 9.3$ Hz, H-3), 6.76 (1H, s, H-5), 7.91 (1H, d, $J = 9.3$ Hz, H-4) [26].

Results and discussion

Biotransformation of scopoletin into fraxetin using *E. coli* harboring scopoletin 8-hydroxylase

Fraxetin is 8-hydroxy scopoletin. *S8H* from *A. thaliana* (*AtS8H*) and its homologue from rice (*OsS8H*) were cloned as a glutathione *S*-transferase fusion protein and expressed in *E. coli*. Scopoletin was tested, along with other structurally-related coumarins, such as esculetin, isoscopoletin, and scoparone. These four compounds have esculetin derivatives. Three methylated esculetins (isoscopoletin, scopoletin, and scoparone) were synthesized using *E. coli* harboring ROMT9, purified, and used as substrates.

E. coli harboring *AtS8H* or *OsS8H* was tested for the conversion of esculetin, isoscopoletin, scopoletin, and scoparone by the administration of each compound. *E. coli* harboring *OsS8H* did not convert any coumarins used. However, for *E. coli* harboring *AtS8H*, scopoletin and isoscopoletin were converted into novel compounds that had retention times different from those of the corresponding substrates (Fig. 2). Other substrates
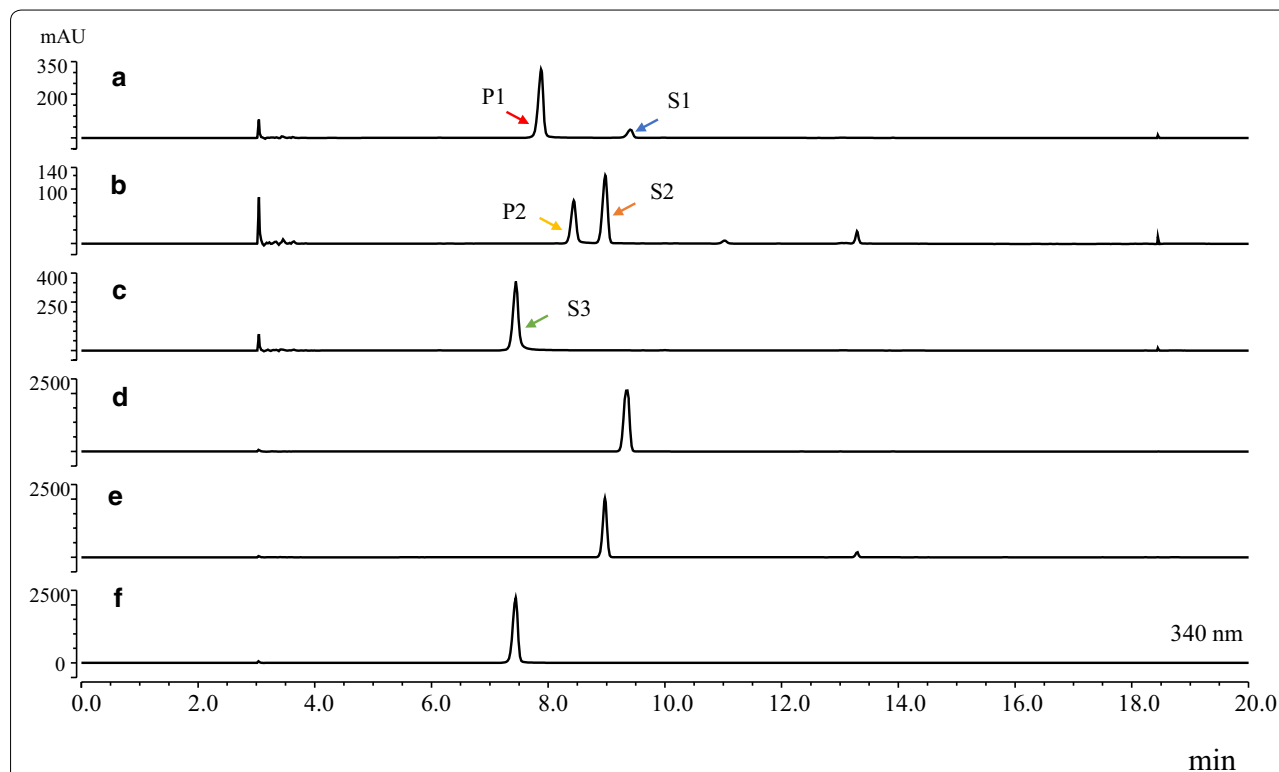

Fig. 2 HPLC analysis of the reaction in *E. coli* harboring AtS8H. *E. coli* harboring AtS8H was administered scopoletin (**a**), isoscopoletin (**b**), and esculetin (**c**); the reaction product was analyzed. **d**–**f** denote standard scopoletin, isoscopoletin, and esculetin, respectively. P1, reaction product from scopoletin; P2, reaction product from isoscopoletin; S1, scopletin; S2, isoscopoletin; S3, esculetin

An *et al. Appl Biol Chem*   (2020) 63:55

Page 4 of 6

(esculetin and scoparone) did not generate any new product. The molecular mass of the products from scopoletin and isoscopoletin was 207.937 Da, which is the molecular mass obtained if hydroxylation occurs. S8H utilized one methylated esculetins (scopoletin and isoscopoletin) as a substrate and did not utilize dimethylated (scoparone) or unmethylated esculetin. Scopoletin was a better substrate than isoscopoletin; 84.8% of scopoletin was converted, as opposed to the conversion of only 55% isoscopoletin. To determine the structure of the biotransformation product from scopoletin, the reaction product was purified, and its structure was analyzed using proton NMR. The reaction product was determined to be fraxetin (see Materials and Methods). *E. coli* harboring different constructs (pG-AtS8H, pE-AtS8H, pR-AtS8H, or pC-AtS8H) synthesized the approximately same amount fraxetin from scopoletin.

To optimize the initial concentration of scopoletin and the final yield of fraxetin, *E. coli* harboring *AtS8H* was prepared at an OD_{600} of 3.0 after induction of *AtS8H*. Subsequently, four different concentrations of scopoletin (100, 200, 300, and 500 μM) were added. The highest rate of conversion of scopoletin into fraxetin was seen at 100 μM scopoletin; 84.8 μM fraxetin was synthesized (84.8% conversion rate). However, fraxetin production was highest at 200 μM scopoletin; approximately 139.5 μM fraxetin was synthesized (69.8% conversion rate). Above 200 μM scopoletin, the production level of fraxetin registered a decline. The optimum initial cell concentrations were also determined. Five initial cell concentrations ($OD_{600} = 1.0$, 2.0, 3.0, 4.0, and 5.0) were tested and 200 μM scopoletin was administered. As the initial cell concentration increased, the conversion of

scopoletin also registered a concomitant increase. At an OD_{600} of 5.0, approximately 152.0 μM of scopoletin was converted into fraxetin.

Synthesis of fraxetin from esculetin and ferulic acid

Fraxetin may also be synthesized from esculetin. Two enzymatic reactions are required; the first is the conversion of esculetin into scopoletin by an *O*-methyltransferase (OMT), and the second is the synthesis of fraxetin from scopoletin by S8H. For the synthesis of scopoletin from esculetin, three OMT genes (*POMT7*, *POMT9*, and *ROMT9*) were evaluated. *E. coli* harboring *POMT7* synthesized 56.8 μM scopoletin from 100 μM esculetin (Fig. 3a). However, *E. coli* harboring *ROMT9* produced three methylated esculetins (isoscopoletin, scopoletin, and scoparone), with isoscopoletin as a major product. The ratio of isoscopoletin, scopoletin, and scoparone was 83: 13: 3. POMT9 generated almost the same amounts of isoscopoletin (38.1 μM) and scopoletin (37.4 μM) from 100 μM esculetin. Therefore, *E. coli* harboring *POMT7* was utilized to synthesize scopoletin from esculetin.

A two-step reaction was conducted using two *E. coli* transformants to augment the final yield of fraxetin. The first reaction was carried out using *E. coli* harboring *POMT7*. Approximately 56.9 μM scopoletin was synthesized from 100 μM esculetin (Fig. 3a). Further incubation did not result in the conversion of more esculetin. Thereafter, the culture filtrate from the first reaction was combined with *E. coli* harboring *AtS8H*. Approximately 41.4 μM fraxetin was synthesized from 56.9 μM scopoletin (Fig. 3b), indicating that there was approximately 72.7% conversion from the synthesized fraxetin.
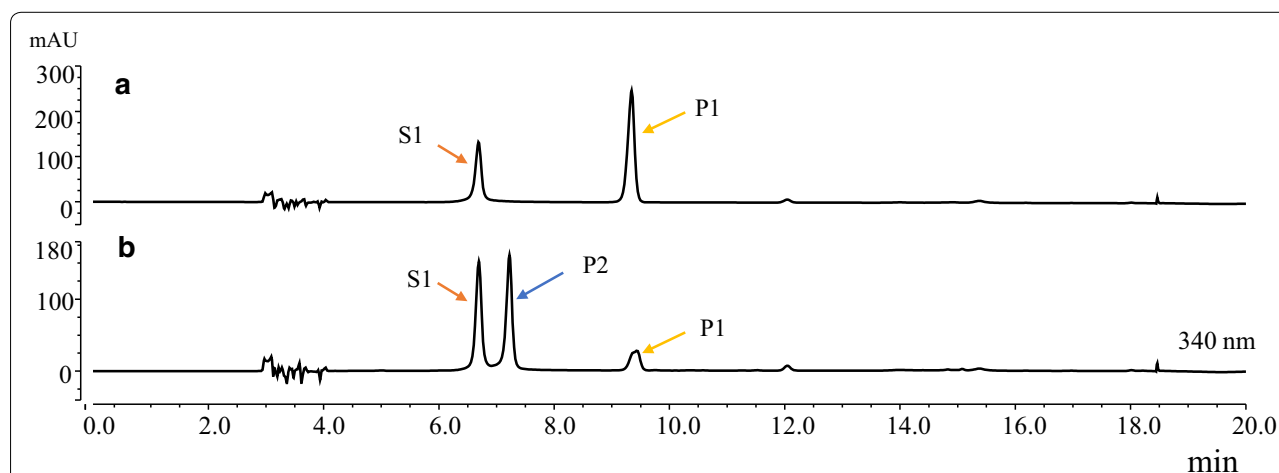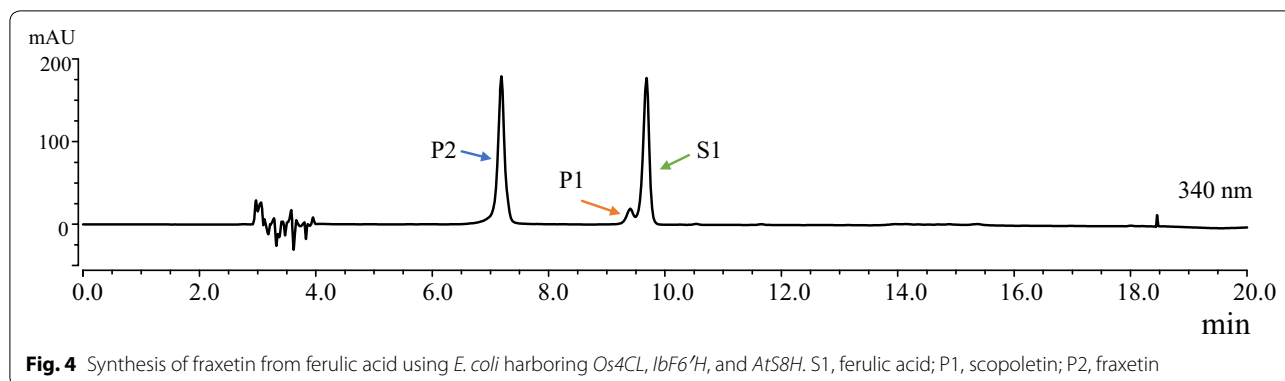

Fig. 3 Synthesis of fraxetin from esculetin using two *E. coli* transformants. **a** Conversion of esculetin into scopoletin using *E. coli* harboring *POMT7*. *E. coli* harboring *POMT7* was administered esculetin (S1), following which the reaction product was analyzed. P1 denotes the reaction product from scopoletin. **b** Synthesis of fraxetin from scopoletin using *E. coli* harboring *AtS8H*. The culture filtrate from *E. coli* harboring *POMT7* was administered to the *E. coli* harboring *AtS8H*, and the reaction product was analyzed

An *et al. Appl Biol Chem*   (2020) 63:55

Page 5 of 6

Fig. 4 Synthesis of fraxetin from ferulic acid using *E. coli* harboring *Os4CL*, *IbF6′H*, and *AtS8H*. S1, ferulic acid; P1, scopoletin; P2, fraxetin

Fraxetin was successfully synthesized from esculetin by a two-step reaction. The final yield of fraxetin synthesized from esculetin was lower than that from scopoletin; moreover, the conversion rate of scopoletin into fraxetin in the two-step reaction was lower than that seen for the direct conversion. This could possibly be attributed to the metabolite(s) in the first step inhibiting the second reaction. It was attempted herein to synthesize fraxetin from esculetin using an *E. coli* transformant harboring both *POMT7* and *AtS8H*. Only 3.4 μM fraxetin and 17.2 μM scopoletin were synthesized from 100 μM esculetin.

Next, fraxetin was synthesized from ferulic acid. Three enzymatic reactions are required for this. Previously, scopoletin was successfully synthesized from ferulic acid using *E. coli* harboring *IbF6′H2* and *Os4CL*. It was reasoned that introducing *AtS8H* into *E. coli* harboring *IbF6′H2* and *Os4CL* could result in the synthesis of fraxetin from ferulic acid. Three genes (*IbF6′H*, *Os4CL*, and *AtS8H*) were introduced into *E. coli*, and the resulting transformant was administered ferulic acid. The *E. coli* transformant synthesized fraxetin from ferulic acid. To optimize fraxetin synthesis, several initial ferulic acid concentrations (100, 200, 300, and 500 μM) were tested. The synthesis of fraxetin was optimal at 100 μM of initial ferulic acid, and approximately 33.3 μM fraxetin was synthesized (Fig. 4). Unreacted ferulic acid and scopoletin were accumulated at the higher concentrations of ferulic acid.

Fraxetin was synthesized from three different substrates (scopoletin, esculetin, and ferulic acid). As shown in Fig. 1, more enzymes are required when fraxetin is synthesized from ferulic acid or esculetin than when it is synthesized from scopoletin. Consequently, the final yield of fraxetin was higher (84.8 μM) when it was synthesized from scopoletin (100 μM). Its yield was decreased when synthesis was carried out from esculetin (41.4 μM) or ferulic acid (33.3 μM).

An attempt was made to synthesize fraxetin from esculetin or ferulic acid. One *E. coli* transformant harboring both *POMT7* and *AtS8H* synthesized a lower amount of fraxetin from esculetin than the other two *E. coli* transformants, each of which conducted one reaction. However, fraxetin was successfully synthesized from ferulic acid using one *E. coli* transformant harboring three genes (*Os4CL*, *IbF6′H*, and *AtS8H*). Esculetin may compete with scopoletin for AtS8H. In the *E. coli* transformant harboring both *POMT7* and *AtS8H*, esculetin served as a substrate for POMT7 and an inhibitor of AtS8H. Therefore, following the synthesis of scopoletin by POMT7, AtS8H could not utilize scopoletin because it was inhibited by esculetin. Conversely, when two independent *E. coli* transformants were used, more scopoletin synthesized by the first *E. coli* transformant harboring *POMT7* was present in the medium and was converted into fraxetin by the second *E. coli* transformant harboring *AtS8H*. When fraxetin was synthesized from ferulic acid, only scopoletin was synthesized; therefore, it was possible to synthesize fraxetin using *E. coli* harboring *Os4CL*, *IbF6′H*, and *AtS8H*.

An *et al. Appl Biol Chem* (2020) 63:55

Page 6 of 6

References

1. Thakur A, Singla R, Jaitak V (2015) Coumarins as anticancer agents: a review on synthetic strategies, mechanism of action and SAR studies. Eur J Med Chem 101:476–495
2. Bourgaud F, Hehn A, Larbat R, Doerper S, Gontier E, Kellner S, Matern U (2006) Biosynthesis of coumarins in plants: a major pathway still to be unraveled for cytochrome P450 enzymes. Phytochem Rev 5:293–308
3. Stringlis IA, de Jonge R, Pieterse CMJ (2019) The age of coumarins in plant–microbe interactions. Plant Cell Physiol 60:1405–1419
4. Tsai HH, Rodríguez-Celma J, Lan P, Wu YC, Vélez-Bermúdez IC, Schmidt W (2018) Scopoletin 8-hydroxylase-mediated fraxetin production is crucial for iron mobilization. Plant Physiol 177:194–207
5. Kang SY, Kim YC (2007) Neuroprotective coumarins from the root of *Angelica gigas*: structure-activity relationships. Arch Pharm Res 30:1368–1373
6. Kayser O, Kolodziej H (1999) Antibacterial activity of simple coumarins: structural requirements for biological activity. Z Naturforsch, C: J Biosci 54:169–174
7. Kai K, Mizutani M, Kawamura N, Yamamoto R, Tamai M, Yamaguchi H, Sakata K, Schimizu B-I (2008) Scopoletin is biosynthesized via ortho-hydroxylation of feruloyl CoA by a 2-oxoglutarate-dependent dioxygenase in *Arabidopsis thaliana*. Plant J 55:989–999
8. Vialart G, Hehn A, Olry A, Ito K, Krieger C, Larbat R, Paris C, Bun-Ichi S, Sugimoto Y, Mizutani M, Bourgaud F (2012) A 2-oxoglutarate dependent dioxygenase from *Ruta graveolens* L. exhibits *p*-coumaroyl CoA 2′ hydroxylase activity (C2′H): a missing step in the synthesis of umbelliferone. in plants. Plant J 70:460–470
9. Matsumoto S, Mizutani M, Sakata K, Shimizu B (2012) Molecular cloning and functional analysis of the ortho-hydroxylases of *p*-coumaroyl coenzyme A/feruloyl coenzyme A involved in formation of umbelliferone and scopoletin in sweet potato, *Ipomoea batatas* (L.) Lam. Phytochemistry 74:49–57
10. Liu S, Zainuddin IM, Vanderschuren H, Doughty J, Beeching JR (2017) RNAi inhibition of feruloyl CoA 6′-hydroxylase reduces scopoletin biosynthesis and post-harvest physiological deterioration in cassava (*Manihot esculenta* Crantz) storage roots. Plant Mol Biol 94:185–195
11. Zhao Y, Jian X, Wu J, Huang W, Huang C, Luo J, Kong L (2019) Elucidation of the biosynthesis pathway and heterologous construction of a sustainable route for producing umbelliferone. J Biol Eng 13:44
12. Yao R, Zhao Y, Liu T, Huang C, Xu S, Sui Z, Luo J, Kong L (2017) Identification and functional characterization of a *p*-coumaroyl CoA 2′-hydroxylase involved in the biosynthesis of coumarin skeleton from *Peucedanum praeruptorum* Dunn. Plant Mol Biol 95:199–213
13. Siwinska J, Siatkowska K, Olry A, Grosjean J, Hehn A, Bourgaud F, Meharg AA, Carey M, Lojkowska E, Ihnatowicz A (2018) Scopoletin 8-hydroxylase: a novel enzyme involved in coumarin biosynthesis and iron-deficiency responses in Arabidopsis. J Exp Bot 69:1735–1748
14. Kimura Y, Sumiyoshi M (2015) Antitumor and antimetastatic actions of dihydroxycoumarins (esculetin or fraxetin) through the inhibition of M2 macrophage differentiation in tumor-associated macrophages and/or G1 arrest in tumor cells. Eur J Pharm 746:115–125
15. Molina-Jiménez MF, Sánchez-Reus MI, Andres D, Cascales M, Benedi J (2004) Neuroprotective effect of fraxetin and myricetin against rotenone-induced apoptosis in neuroblastoma cells. Brain Res 1009:9–16
16. Murali R, Srinivasan S, Ashokkumar N (2013) Antihyperglycemic effect of fraxetin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats. Biochimie 95:1848–1854
17. Kundu J, Chae IG, Chun K-S (2016) Fraxetin induces heme oxygenase-1 expression by activation of Akt/Nrf2 or AMP-activated protein kinase α/Nrf2 pathway in HaCaT cells. J Cancer Prev 21:135–143
18. Yang S-M, Shim GY, Kim B-G, Ahn J-H (2015) Biological synthesis of coumarins in *Escherichia coli*. Microb Cell Fact 14:65
19. Chu LL, Pandey RP, Lim HN, Jung HJ, Thuan NH, Kim T-S, Sohng JK (2017) Synthesis of umbelliferone derivatives in *Escherichia coli* and their biological activities. J Biol Eng 11:15
20. Kim BG, Lee YJ, Lee S, Lim Y, Cheong Y, Ahn J-H (2008) Altered regioselectivity of a poplar *O*-methyltransferase, POMT-7. J Biotech 138:107–111
21. Kim BG, Lee Y, Hur HG, Lim Y, Ahn J-H (2006) Production of Three *O*-Methylated Esculetins with *E. coli* Expressing *O*-Methyltransferase from Poplar. Biosci Biotech Biochem 70:1269–1272
22. Kim BG, Lee Y, Hur H-G, Lim Y, Ahn J-H (2006) Flavonoid 3′-*O*-methyltransferase from rice: cDNA cloning, characterization and functional expression. Phytochemistry 67:387–394
23. Lee SJ, Sim GY, Kang H, Yeo WS, Kim B-G, Ahn J-H (2018) Synthesis of avenanthramides using engineered *Escherichia coli*. Microb Cell Fact 17:46
24. Song MK, Cho AR, Sim GY, Ahn J-H (2019) Synthesis of diverse hydroxycinnamoyl phenylethanoid esters using *Escherichia coli*. J Agric Food Chem 67:2028–2035
25. Yoon J-A, Kim B-G, Lee WJ, Lim Y, Chong Y, Ahn J-H (2012) Production of a novel quercetin glycoside through metabolic engineering of *Escherichia coli*. Appl Env Microbiol. 78:4256–4262
26. Yu M, Sun A, Zhnag Y, Liu R (2014) Purification of coumarin compounds from *Cortex fraxinus* by adsorption chromatography. J Chromatogr Sci 52:1033–1037

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.