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Biocatalytic Synthesis of Calycosin-7-O-*β*-D-Glucoside with Uridine Diphosphate–Glucose Regeneration System

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Abstract: Calycosin-7-O-*β*-D-glucoside (Cy7G) is one of the principal components of *Radix astragali*. This isoflavonoid glucoside is regarded as an indicator to assess the quality of *R. astragali* and exhibits diverse pharmacological activities. In this study, uridine diphosphate-dependent glucosyltransferase (UGT) UGT88E18 was isolated from *Glycine max* and expressed in *Escherichia coli*. Recombinant UGT88E18 could selectively and effectively glucosylate the C7 hydroxyl group of calycosin to synthesize Cy7G. a one-pot reaction by coupling UGT88E18 to sucrose synthase (SuSy) from *G. max* was developed. The UGT88E18–SuSy cascade reaction could recycle the costly uridine diphosphate glucose (UDPG) from cheap sucrose and catalytic amounts of uridine diphosphate (UDP). The important factors for UGT88E18–SuSy cascade reaction, including UGT88E18/SuSy ratios, different temperatures, and pH values, different concentrations of dimethyl sulfoxide (DMSO), UDP, sucrose, and calycosin, were optimized. We produced 10.5 g L^{-1} Cy7G in the optimal reaction conditions by the stepwise addition of calycosin. The molar conversion of calycosin was 97.5%, with a space–time yield of 747 mg L^{-1} h^{-1} and a UDPG recycle of 78 times. The present study provides a new avenue for the efficient and cost-effective semisynthesis of Cy7G and other valuable isoflavonoid glucosides by UGT–SuSy cascade reaction.

Keywords: calycosin; calycosin-7-O-β-D-glucoside; glucosyltransferase; sucrose synthase; UDP-glucose recycle; UGT–SuSy cascade reaction

1. Introduction

Flavonoids are a group of structurally diverse, plant-derived polyphenols widely present in fruits, vegetables, and beverages [1,2]. These compounds play an important role in human diet related to health [3]. More than 10,000 flavonoids have been isolated from the plant kingdom, which can be categorized into flavonols, flavones, isoflavonoids, flavanones, chalcone, catechins, and anthocyanins according to the variability in their chemical structures [4,5]. Glucosylation mediated by uridine diphosphate-dependent glucosyltransferase (UGT) is one of the most common modifications of flavonoids, which presents immense structural diversity after this process [6]. The attachment of glucosyl moiety to flavonoids not only increases their solubility and stability, but also improves their bioavailability and pharmacokinetic properties [7,8].

Calycosin-7-O-β-D-glucoside (Cy7G) is an isoflavonoid glucoside (Figure 1). This compound is one of the principal components of *Radix astragali*—a famous medicinal and edible herbal plant recorded in European, Japanese, and Chinese pharmacopoeias [9]. The contents of Cy7G are generally regarded as an indicator to assess the quality of *R. astragali* and *R. astragali*-based drugs or foods [9,10]. Cy7G exhibits various health-beneficial bioactivities, including antioxidant, anti-inflammatory, antiapoptotic, and bone-regeneration-enhancing activities [11,12]. Commercially available Cy7G is mainly extracted from plants; however, the natural scarcity and high cost of production are major obstacles that limit its applications in pharmaceutical and healthcare industries [13].

Compounds	R1	R2
Calycosin (**1**)	-H	-OH
Cy7G (**1a**)	-Glc	-OH
Formononetin (**2**)	H	H
Ononin (**2a**)	-Glc	H
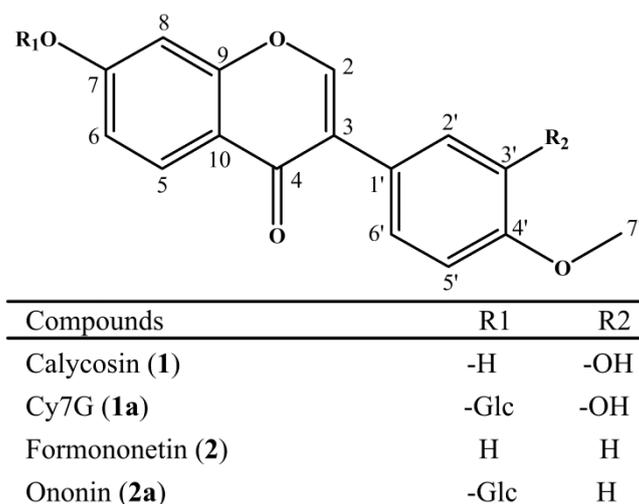
Figure 1. Chemical structures of calycosin, formononetin, and glucosylated products synthesized by UGT88E18.

Chemical glucosylation is notoriously complicated because of various disadvantages such as low efficiency, poor stereospecificity, a series of protection and deprotection steps, and environmental pollution [14]. In this respect, enzymatic glucosylation using regio- and stereoselective UGTs can alleviate these disadvantages [15,16]. Extensive studies indicate that sucrose synthase (SuSy) is a versatile biocatalyst that can synthesize costly uridine diphosphate glucose (UDPG) from abundant and cheap sucrose and catalytic amounts of uridine diphosphate (UDP) [17,18]. Furthermore, SuSy can be coupled with a UGT in a one-pot reaction that could recycle UDPG from UDP and sucrose, thereby providing a cost-effective approach for the glucosylation of natural products [19,20].

A considerable number of UGTs involved in the glucosylation of flavonoids have been isolated and functionally characterized from plants [21]. These UGTs typically tolerate a broad range of aglycones with high regiospecifity in vitro [22,23]. In the present study, the regioselective and effective glucosylation of calycosin was achieved using UGT88E18 isolated from *Glycine max*. In addition, a UGT88E18–SuSy cascade reaction was developed to synthesize Cy7G using cheap sucrose as the expedient glucosyl donor.

2. Results and Discussion

2.1. Regioselective Glucosylation of Calycosin and Formononetin by UGT88E18

UGT88E18 from *G. max* was specific for isoflavones, including genistein and daidzein [23]. Thus, N-terminal His$_6$-tagged UGT88E18 was heterologously expressed in *Escherichia coli* BL21 (DE3), and purified by one-step nickel chelate affinity chromatography. SDS-PAGE analysis showed a clear band at around 72.2 kDa that corresponded to the calculated molecular weight of recombinant UGT88E18 (Figure S1). The purified UGT88E18 was applied to the in vitro reaction with calycosin (Compound **1**) or formononetin (Compound **2**, another important isoflavonoid present in *R. astragali*)

as the acceptor and UDPG as the glucosyl donor, respectively (Figure 1). High-performance liquid chromatography (HPLC) analysis of the reactants revealed that over 97% of calycosin and formononetin could be transformed into their corresponding glucosides (Figure 2A), suggesting that UGT88E18 could effectively glucosylate calycosin and formononetin. These results were in agreement with a previous study that reported that UGT88E18 is an isoflavone UGT [23]. Only one new product with higher polarity was produced using calycosin or formononetin as the aglycone. The retention time (RT) of new Products **1a** and **2a** was 9.8 and 13.1 min, respectively. HPLC electrospray-ionization mass-spectrometry (HPLC-ESI-MS) analysis of Products **1a** ([2M+H]$^+$ m/z$^+$ ~893.2509]$^+$, [M+H]$^+$ m/z$^+$ ~447.1304, and [M-Glc+H]$^+$ m/z$^+$ ~285.0747) and **2a** ([2M+H]$^+$ m/z$^+$ ~861.2614]$^+$, [M+H]$^+$ m/z$^+$ ~431.1365, and [M-Glc+H]$^+$ m/z$^+$ ~269.0808) indicated the addition of one glucosyl moiety from calycosin ($C_{16}H_{12}O_5$, [M+H]$^+$ m/z$^+$ ~285.0743) and formononetin ($C_{16}H_{12}O_4$, [M+H]$^+$ m/z$^+$ ~269.0809), respectively (Figure 2B).
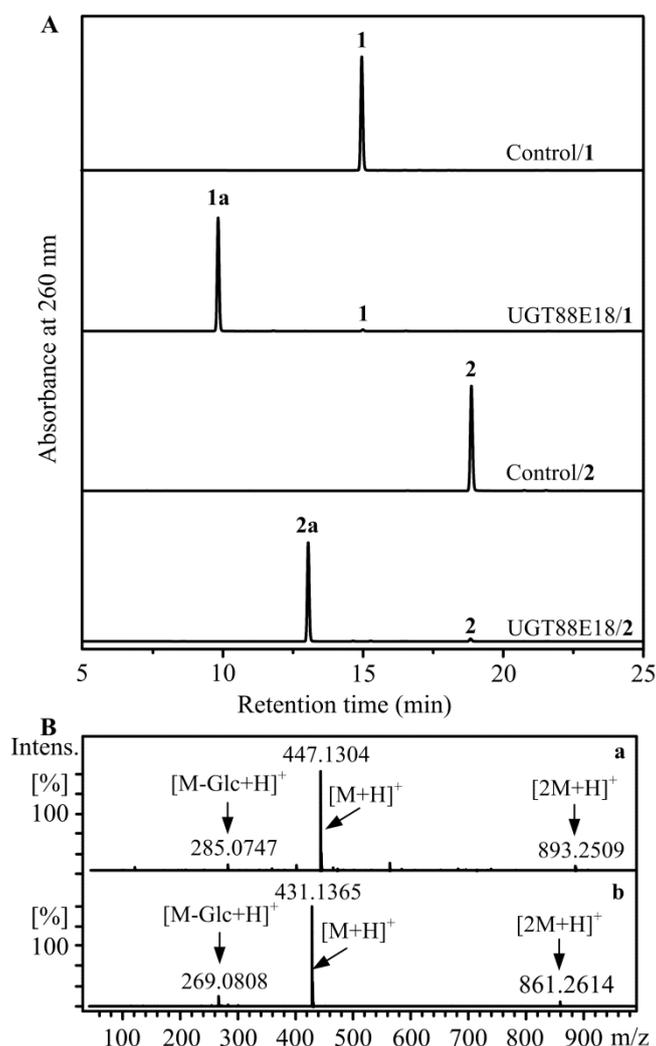
Figure 2. HPLC electrospray-ionization mass-spectrometry (HPLC-ESI-MS) analysis of glucosylated products of calycosin and formononetin. (**A**) HPLC chromatograms of calycosin, formononetin, and glucosylated products synthesized by UGT88E18. (**B**) MS spectra for Products (**a**) **1** and (**b**) **2**.

Products **1a** and **2a** were purified by preparative HPLC system, and their chemical structures were determined by 1D NMR (^{1}H NMR and ^{13}C NMR) and 2D NMR spectra (homonuclear correlation spectroscopy (COSY), heteronuclear multiple-bond correlation spectroscopy (HMBC), and heteronuclear singular quantum correlation (HSQC))) (Figures S2–S11). For Product **1a** (Table S1),

glucosyl proton signals (δ_H 3.1–5.2 ppm) in the ^{1}H NMR spectra and carbon signal (δ_C 60–101 ppm) in the ^{13}C NMR spectra suggested the presence of a glucosyl moiety [24]. The HMBC correlations of the glucosyl moiety anomeric signal H1' (δ_H 5.11 ppm, d, J = 7.3 Hz) with C7 (δ_C 161.32 ppm) indicated the attachment of a glucosyl moiety to the C7 hydroxyl group of calycosin. The ^{1}H- and ^{13}C-NMR spectra of Product **2a** were highly similar to those of Product **1a** [24]. The additional signals of glucosyl proton in the δ_H 3.1–5.2 ppm region and anomeric proton H1' at δ_H 5.12 ppm (d, *J*=7.4 Hz) in the ^{1}H NMR spectra indicated the presence of a glucosyl moiety. In ^{13}C-NMR analysis, the observation of the anomeric carbon signal C1' (δ_C 100.47 ppm) and five other new carbon signals (δc 61.12, 70.11, 73.61, 76.96, and 77.69 ppm) further justified the presence of a glucosyl moiety. The long-range correlations between glucosyl anomeric signal H1' (δ_H 5.12 ppm, d, *J* = 7.4 Hz) and C7 (δ_H 161.93 ppm) suggested that the glucosyl moiety was attached to the C7 hydroxyl group of formononetin. In addition, the large anomeric proton-coupling constants (*J* = 7.3–7.4 Hz) of Products **1** and **2** suggested the formation of the *β*-anomers, which agreed with the inverting mechanism for UGTs [25]. The present results revealed that UGT88E18 could selectively glucosylate the C7 hydroxyl group of calycosin and formononetin to synthesize Cy7G and formononetin-7-O-*β*-D-glucoside (ononin), respectively.

2.2. Kinetic Analysis of UGT88E18 Toward Formononetin and Calycosin

The K_m values of UGT88E18 toward calycosin and formononetin were 18.67 and 30.64 µM, respectively (Table 1 and Figure S12). These results suggested that UGT88E18 had a higher substrate preference for calycosin than for formononetin. In addition, these K_m values were comparable to those of other UGTs involved in glycosylation of isoflavonoids [22,26]. The *k*cat values of UGT88E18 toward calycosin and formononetin were 7.67 and 5.39 s^{-1}, respectively. The turnover rates of UGT88E18 toward formononetin and calycosin were considerably high compared with other UGTs involved in flavonoid biosynthesis [1,22]. Thus, the catalytic efficiency of UGT88E18 toward calycosin and formononetin was 4.11×10^5 and 1.76×10^5 M^{-1} s^{-1}, respectively. Kinetic analysis of UGT88E18 suggested that it was a powerful catalyst for the in vitro glucosylation of calycosin and formononetin.

Table 1. Kinetic parameters of UGT88E18 toward calycosin and formononetin.

Substrate	K_m (µM)	*k*cat (s^{-1})	*k*cat/ K_m (s^{-1} M^{-1})
Calycosin	18.67 ± 1.36	7.67 ± 0.13	4.11×10^5
Formononetin	30.64 ± 2.30	5.39 ± 0.11	1.76×10^5

2.3. Optimization of UGT88E18–SuSy Cascade Reaction for Cy7G Synthesis

The UGT88E18-catalyzed synthesis of Cy7G requires the costly UDPG as the glucosyl donor, which is one of the major bottlenecks for in vitro enzymatic reactions [27]. Thus, SuSy from *G. max* was also heterologously expressed in *E. coli* and purified to homogeneity (Figure S1). A one-pot reaction was developed by coupling UGT88E18 to SuSy to regenerate costly UDPG from UDP and cheap sucrose. As expected, calycosin could be transformed into Cy7G by a UGT88E18–SuSy cascade reaction, similar to in vitro reactions using UDPG as the glucosyl donor (Figure S13), suggesting that the UGT88E18–SuSy cascade reaction was applicable to semisynthesize Cy7G from calycosin.

The effects of different ratios of UGT88E18 and SuSy on Cy7G production were primarily determined (Table 2). Only 1.30 mM Cy7G was produced in the reaction mixtures containing 50 mU mL^{-1} UGT88E18. The content of Cy7G doubled when the amount of UGT88E18 was raised from 50 to 100 mU mL^{-1}. The concentration of Cy7G continued to increase from 3.35 to 3.78 mM when the amount of UGT88E18 was increased from 150 to 200 mU mL^{-1}. Thus, 200 mU mL^{-1} UGT88E18 was selected for the following UGT88E18–SuSy cascade reactions. The titer of Cy7G increased by 106% when the amount of SuSy was increased from 50 to 150 mU mL^{-1}, suggesting that glucosylation catalyzed by UGT88E18 was the rate-limiting step in the UGT88E18–SuSy cascade reaction system. Approximately 95% of calycosin (3.80 mM) could be transformed into Cy7G in the presence of 150

and 200 mU mL^{-1} SuSy. Thus, 200 mU mL^{-1} UGT88E18 and 150 mU mL^{-1} SuSy were selected as the optimal ratios for the subsequent optimization assays.

Table 2. Effect of different enzyme ratios on product yields of calycosin-7-O-β-D-glucoside (Cy7G).

Entry	UGT88E18 (mU mL^{-1})	SuSy (mU mL^{-1})	Cy7G (mM)
1	50	150	1.30
2	100	150	2.56
3	150	150	3.35
4	200	150	3.78
5	200	50	1.82
6	200	100	3.31
7	200	150	3.75
8	200	200	3.80

Different temperature and pH values and different concentrations of dimethyl sulfoxide (DMSO), UDP, sucrose, and calycosin were further optimized (Figure 3). Cy7G production in UGT88E18–SuSy cascade reaction was investigated in different temperature ranges (25–45 °C). Only 1.25 mM Cy7G was produced at 25 °C. The concentration of Cy7G increased to 3.28 mM at 30°C. Over 93% calycosin could be transformed into Cy7G (3.72–3.84 mM) at 35 and 40 °C. However, the concentration of Cy7G decreased to 3.09 mM at 45°C. Considering a lower temperature could be more conducive to maintaining the thermostability of plant-derived UGT88E18 and SuSy, 35 °C was selected as the optimal temperature. The pH values were in the range of 6.5–8.0 and were also optimized. The UGT88E18–SuSy cascade reaction performed well in the selected pH values. The highest Cy7G titers (~3.75 mM) were obtained at pH 7.0 and 7.5. Thus, pH 7.5 in the Tris-HCl buffer was selected in the following experiments. The poor solubility of aglycones could inhibit the in vitro UGT–SuSy cascade reactions. DMSO is a versatile organic solvent to promote the dissolution of hydrophilic aglycones [28]. The Cy7G titer increased from 2.60 to 4.70 mM when the DMSO concentration increased to 10% (v/v). The UGT–SuSy cascade system could tolerate 10%–15% DMSO when the conversion of calycosin reached over 98%. However, Cy7G production (2.32 mM) decreased rapidly when the concentration of DMSO increased to 20%. Considering the concentration of DMSO would be further increased in the subsequent stepwise addition of calycosin (stocked in DMSO), 10% DMSO (v/v) was selected as the optimal condition. UDP is a costly cofactor in the UGT–SuSy cascade system. UDP is involved in the formation of UDPG in the SuSy-catalyzed hydrolysis of sucrose. In addition, as a byproduct of the UGT-catalyzed reaction, a high concentration of UDP could inhibit UGT activity [29]. Only 1.30 mM Cy7G was formed in the presence of 0.1 mM UDP. The production of Cy7G increased to 3.74 mM when the concentration of UDP exceeded 0.3 mM. Thus, 0.3 mM UDP was adequate for the UGT88E18–SuSy cascade reaction. Cy7G titers increased with increasing concentration of sucrose. Maximal Cy7G production reached 3.74 when the concentration of sucrose increased to 400–500 mM. Then, 2–3 mM calycosin could be completely transformed into Cy7G by the UGT88E18–SuSy cascade reaction. The maximal titer of Cy7G reached 3.75 mM in the presence of 4 mM calycosin. Collectively, 35 °C, pH 7.5, 10% DMSO (v/v), 0.3 mM UDP, 400 mM sucrose, and 4 mM calycosin were selected as the optimal parameters for the subsequent fed-batch reaction.
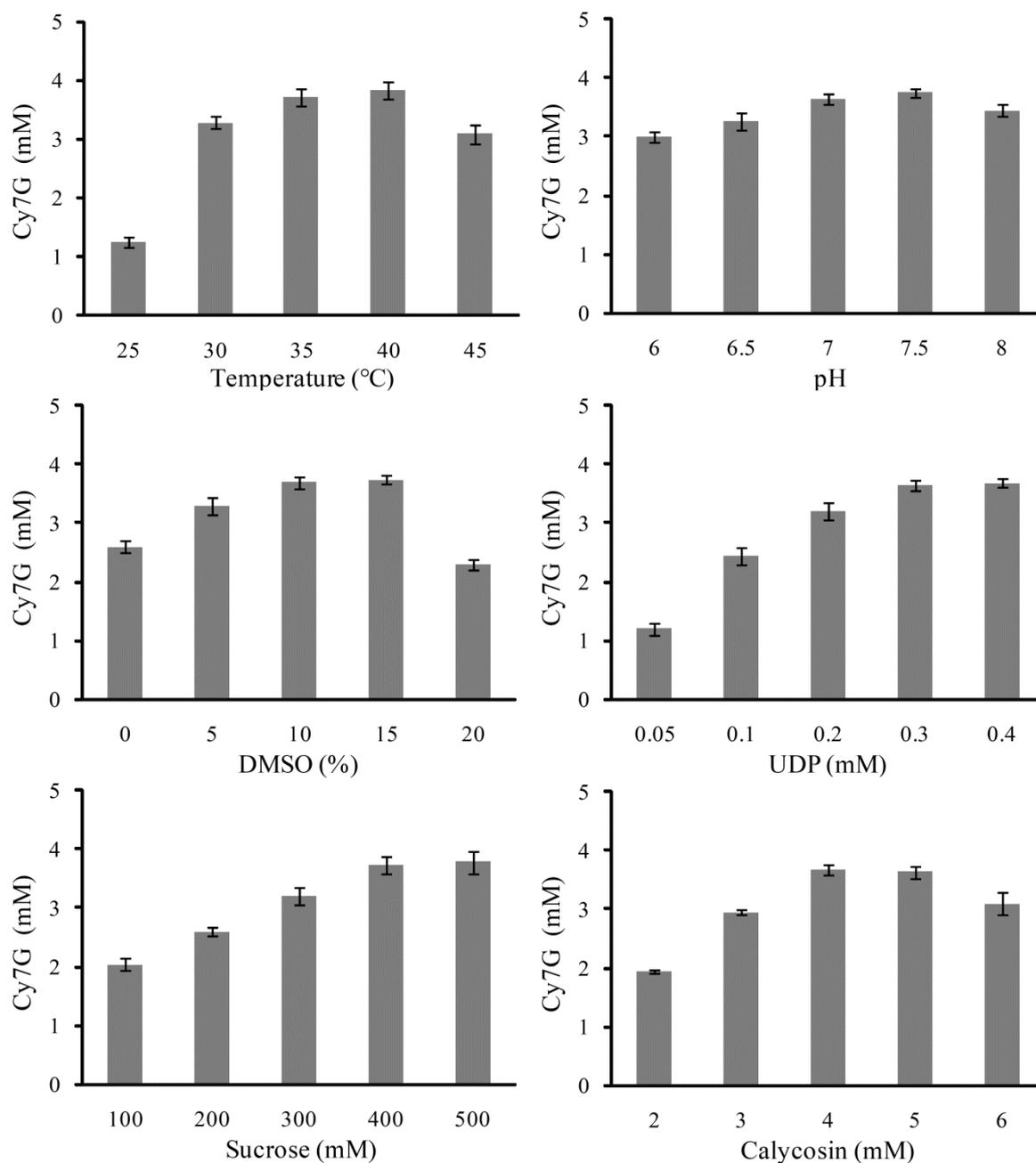
Figure 3. Optimization of reaction conditions for UGT88E18–SuSy cascade reactions.

2.4. Fed-Batch Synthesis of Cy7G by UGT88E18–SuSy Cascade Reaction

UGT88E18–SuSy cascade reaction by the periodical addition of calycosin was conducted to increase the final titer of Cy7G and to avoid the inhibition of a high concentration of calycosin to the reaction system. As shown in Figure 4, 7.80 mM Cy7G was produced in the first hour with a space–time yield (STY) of 3487 mg L^{-1} h^{-1}. Specific productivity gradually decreased with the stepwise addition of calycosin. STYs were 849 mg L^{-1} h^{-1} over a reaction time of 1–5 h, 581 mg L^{-1} h^{-1} over a reaction time of 5–8 h, and 305 mg L^{-1} h^{-1} over a reaction time of 8–14 h. The main cause of the decrease in STY might be the product inhibition by a high concentration of fructose and Cy7G in the system [30–33]. Eventually, 10.46 g L^{-1} Cy7G was produced with the conversion rate of calycosin being 97.5%. The overall STY of Cy7G was 747 mg L^{-1} h^{-1}, with a maximal number of UDPG regeneration cycles of 78 times (= 23.4/0.3). These results provide a new approach for the efficient and cost-effective semisynthesis of Cy7G and other valuable isoflavonoid glucosides by UGT–SuSy cascade reaction.
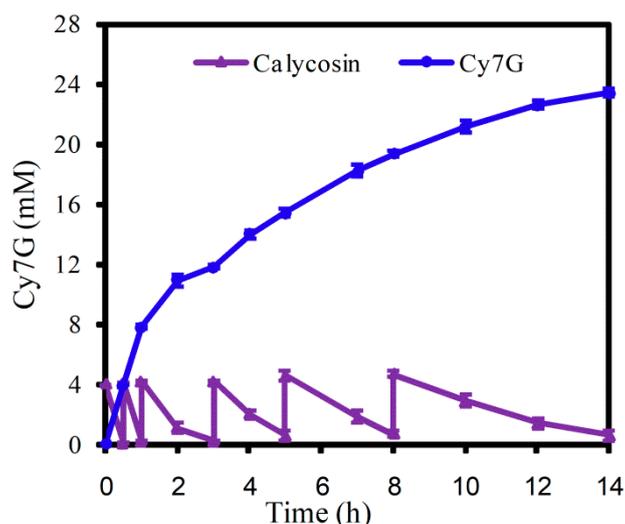
Figure 4. Fed-batch synthesis of Cy7G by UGT88E18–SuSy cascade reaction. Stepwise addition of 100 μL of fresh calycosin, stocked in dimethyl sulfoxide (DMSO, 400 mM), to reactants at 0.5, 1, 3, 5, and 8 h.

3. Materials and Methods

3.1. Chemicals and Reagents

Calycosin was provided by Chengdu Biopurify Phytochemicals Ltd. (Chengdu, China). Formononetin, UDP, and DMSO were purchased from J&K Scientific Ltd. (Beijing, China). UDPG, deuterium dimethyl sulfoxide (DMSO-d_6), and chromatography-grade acetonitrile were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents were of the highest chemical grade.

3.2. Expression and Purification of Recombinant Proteins

UGT88E18 (GenBank accession number, AB904893) and SuSy (GenBank accession number, NP_001237525.1) from *G. max* were codon-optimized and synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). UGT88E18 and SuSy were subcloned into a pET32a vector to construct recombinant vectors pET32-UGT88E18 and pET32-SuSy, respectively. Expression vectors pET32-UGT88E18 and pET32-SuSy were transformed into *E. coli* BL21 (DE3) cells for heterologous expression, respectively. The recombinant *E. coli* cells were cultured in a Luria–Bertani medium containing 100 mg L^{-1} ampicillin at 37 °C and 200 rpm. After optical density (OD$_{600}$) reached 0.6–0.8, the recombinant *E. coli* cells were induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside for 20 h at 16 °C and 200 rpm. Subsequently, induced cells were harvested by centrifugation at 5000 g for 10 min at 4 °C and resuspended in lysis buffer (50 mM Tris-HCl, 25 mM imidazole, and 150 mM NaCl, pH 7.5). After disruption by high-press homogenization, the resultant cell debris was removed by centrifugation at 17,000 g for 60 min at 4 °C. The supernatant was applied to an AKTA Purifier system equipped with a nickel nitrilotriacetic acid agarose affinity column (GE Healthcare, Piscataway, NJ, USA). The purity of the purified proteins was analyzed by 4%–12% SurePAGE Bis-Tris gels (GenScript, Nanjing, China). Protein concentration was determined by bicinchoninic acid assay using bovine serum albumin as a reference according to the manufacturer's instructions (Solarbio, Beijing, China). Purified UGT88E18 and SuSy were kept in 50 mM Tis-HCl (pH 7.5) at −80 °C.

3.3. Enzyme Activity

The reaction mixtures (3 μg UGT88E18, 1 mM calycosin or formononetin, 5 mM UDPG, 10% DMSO (v/v), 50 mM Tris-HCl buffer, pH 7.5) were carried out in a total reaction volume of 500 μL.

The reactions were performed at 35 °C for 1 h, and were stopped by adding twofold volumes of methanol. After centrifugation at 13,000 g for 10 min, the reactants were filtered with a 0.22 μm nylon filter prior to analysis by a Shimadzu LC-20AD HPLC system coupled with a photodiode array detector (Shimadzu Corporation, Kyoto, Japan). The reverse-phase InertSustain C18 column (4.6 × 250 mm, 5 μm; GL Science, Kyoto, Japan) was eluted with double-distilled water and chromatography-grade acetonitrile using a gradient of 15%–85% chromatography-grade acetonitrile in 0–25 min. HPLC-ESI-MS was performed in the same positive-ion mode as in our previous study [24]. One unit of enzyme activity was defined as the amount of UGT88E18 that generated 1 μmole of Cy7G per min under the assay conditions. Enzyme activity of SuSy was measured by the bicinchoninic acid method as described previously [31].

3.4. Kinetic Parameters of UGT88E18

Kinetic analysis of UGT88E18 was carried out in 300 μL volumes containing 50 mM Tris-HCl (pH 7.5), 5 mM of UDPG, 10% DMSO (v/v), and 10–400 μM calycosin or formononetin. The reaction mixtures were precultured at 35 °C for 5 min and initiated by adding 0.5 μg UGT88E18. Subsequently, reactants were incubated at 35 °C for 10 min and quenched by an equal volume of methanol. Finally, the aliquots were centrifuged at 13,000 g for 10 min, filtered with a 0.22 μm nylon filter, and analyzed by HPLC, as described above. Data were obtained from three parallel experiments. Kinetic parameters were determined by nonlinear regression analysis using GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA).

3.5. Optimization of UGT88E18–SuSy Cascade Reaction

UGT88E18–SuSy cascade reactions (4 mM calycosin, 0.5 mM UDP, 10% DMSO (v/v), 500 mM sucrose, 50 mM Tris-HCl (pH 7.5), 200 mU mL^{-1} UGT88E18, and 150 mU mL^{-1} SuSy) were performed at 35 °C for 0.5 h. The optimal ratio of UGT88E18 and SuSy was determined by adding different ratios of UGT88E18 and SuSy (50–200 mU mL^{-1}). Subsequently, different temperature values (25, 30, 35, 40, and 45 °C) and pH values (NaH$_2$PO$_4$–Na$_2$HPO$_4$ buffer, 6.0, 6.5, and 7.0; Tris-HCl buffer, 7.5 and 8.0) were further determined. Finally, the effects of different concentrations of DMSO (0–20%, v/v), UDP (0.05–0.4 mM), sucrose (100–500 mM), and calycosin (2–6 mM) on the UGT88E18–SuSy cascade reaction were optimized.

3.6. Fed-Batch Synthesis of Cy7G

UGT88E18–SuSy cascade reaction (10 mL) was conducted in optimal conditions (50 mM Tris-HCl (pH 7.5), 4 mM calycosin, 0.3 mM UDP, 10% DMSO (v/v), 400 mM sucrose, 200 mU mL^{-1} UGT88E18, and 150 mU mL^{-1} SuSy) at 35 °C and 150 rpm. Samples (100 μL) were collected at different time intervals (0.5, 1, 2, 3, 4, 5, 7, 8, 10, 12, and 14 h). Samples were quenched and diluted using 2.9 mL of methanol. After filtration with 0.22 μm nylon filters, the reaction mixtures were analyzed by HPLC, as described above. In addition, 100 μL of fresh calycosin stocked in DMSO (400 mM) was stepwise added to the reaction mixtures at 0.5, 1, 3, 5, and 8 h.

3.7. Purification and Structural Elucidation of the Glucosylated Products

A scaled-up UGT88E18–SuSy cascade reaction (30 mL) was performed with calycosin or formononetin as the substrate, respectively. After quenching using twofold volumes of methanol, the reaction mixtures were concentrated by reduced-pressure distillation and resuspended in 10 mL volumes of methanol. The synthesized compounds were purified using a preparative HPLC system coupled with a reverse-phase Ultimate C18 column (21.2 × 250 mm, 5 μm particles, Welch, Shanghai, China). The purified glucosylated products were dissolved in DMSO–d_6, respectively. The chemical structures of the purified products were determined by 1D NMR (^{1}H NMR and ^{13}C NMR) and 2D NMR spectra (COSY, HMBC and HSQC) using a 400 MHz NMR spectrometer (Bruker, Karlsruhe,

Germany). The chemical-shift values were quoted in parts per million (ppm). All raw data were analyzed using the MestReNova 9 program.

4. Conclusions

In summary, UGT88E18 from *G. max* was demonstrated to selectively and effectively glucosylate the C7 hydroxyl group of calycosin to synthesize Cy7G. Our study revealed that the UGT88E18–SuSy cascade reaction is a powerful approach for the biocatalytic synthesis of Cy7G. UGT88E18–SuSy cascade reaction provides a new avenue for the cost-effective and scaled-up production of Cy7G and other isoflavonoids.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4344/10/2/258/s1. Table S1. ^{1}H- and ^{13}C-NMR spectra for Products **1** and **2**. Figure S1. SDS-PAGE analysis of purified UGT88E18 and SuSy. Figure S2. ^{1}H NMR of Product **1**. Figure S3. ^{13}C NMR of Product **1**. Figure S4. HMBC spectra of Product **1**. Figure S5. HSQC spectra of Product **1**. Figure S6. COSY spectra of Product **1**. Figure S7. ^{1}H NMR of Product **2**. Figure S8. ^{13}C NMR of Product **2**. Figure S9. HMBC spectra of Product **2**. Figure S10. HSQC spectra of Product **2**. Figure S11. COSY spectra of Product **2**. Figure S12. Kinetic analysis of UGT88E18 toward formononetin and calycosin. Figure S13. Fed-batch synthesis of Cy7G by UGT88E18–SuSy cascade reaction.

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