Abstract: Sulfuretin glucosides are important sources of innovative drugs. However, few glucosides of sulfuretin have been observed in nature. Therefore, it is urgent to diversify sulfuretin glucosides. Herein, glycosyltransferase (GT)-catalyzed glycodiversification of sulfuretin was achieved. Specifically, a flavonoid GT designated as OcUGT1 was used as a biocatalyst for the glucosylation of sulfuretin with UDP-Glc. The OcUGT1-assisted glucosylation of sulfuretin yielded ten glycosylated products, including three monoglucosides, five diglucosides and two triglucosides. The three monoglucosides were thus identified to be sulfuretin 3′-, 4′- and 6-glucoside according to HR-ESI-TOFMS data and their coelution with respective standards. A major diglucoside was assigned as sulfuretin 4′,6-diglucoside by HR-ESI-TOFMS in conjunction with NMR analysis. The exact structure of the other four diglucosides was not well characterized due to their trace amount. However, they were reasonably inferred as sulfuretin 3′,6-diglucoside, sulfuretin 3′,4′-diglucoside and two disaccharide glucosides. In addition, the structural identification of the remaining two triglucosides was not performed because of their small amount. However, one of the triglucosides was deduced to be sulfuretin 3′,4′,6-triglucoside based on the catalytic behavior of OcUGT1. Of the ten sulfuretin glucosides, at least six were new compounds. This is the first time to obtain monoglucosides, diglucosides and triglucosides of sulfuretin simultaneously by a single glycosyltransferase.

Keywords: glycosyltransferase; glycodiverfication; sulfuretin; OcUGT1

1. Introduction

Glycodiversification is a collective strategy of natural product glycosylation, in which varied activated sugars are attached to natural-product acceptors by enzymatic or chemical means, thereby providing diverse carbohydrate structures and functions [1,2]. The resultant glycosylated bioactive compounds have been shown to exert various biological and pharmacological activities with improved physicochemical characters, such as solubility and stability [3,4]. Many glycosides are thus developed to clinical drugs, e.g., rutin [5–7], puerarin [8] and scutellarin [9]. Hence, glycodiversification of natural products is deemed an effective strategy to broaden the scope of new compounds [2].

Owing to the structural complexity of many glycosylated compounds, glycodiversification of natural products by chemical synthesis may be a formidable task [2]. Conversely, enzymatic glycodiversification is becoming a main strategy for diversifying glycosylated natural products due to the great strides made in the generation of glycosyltransferase with catalytic promiscuity [10–13].

Sulfuretin (1, also designated as sulphuretin, Figure 1 and Figure S1), a naturally occurring aurone [14–16], is found to display a remarkable spectrum of biological activities such as therapeutic...
activity against acquired lymphedema [14], anti-Parkinson’s disease activity [15], antioxidant action [16], therapeutic benefits in bone disease and regeneration [17,18] and neuroprotective effect [19], suggesting sulfuretin is a promising molecule for drug development. Accordingly, the interest for the discovery or synthesis of sulfuretin derivatives is increasing. Many sulfuretin derivatives featuring varied functional groups were thus observed to display a wide range of biological activities [20–23]. Of these derivatives, glycosides of sulfuretin, e.g., sulfuretin 6-glucoside (sulfurein) [24–26], sulfuretin 3′-glucoside [25] and palasitrin (sulfuretin 3′,6-diglucoside) [27], have been determined to exhibit diverse activities such as antioxidant activity [26,28] and influenza A neuraminidase inhibitory activity [25], suggesting sulfuretin glycosides are a potent source of drug discovery. Thus far, however, few sulfuretin glycosides have been obtained through direct extraction or enzymatic synthesis [29,30], which limited their druggability study. Therefore, it is urgent to diversify sulfuretin glycosides for drug screening.

Figure 1. OcUGT1-catalyzed glucosylation of sulfuretin (1) resulted in the generation of ten glucosides (1a, 1b, 1c, 1g and six unidentified compounds).

OcUGT1 (Ornithogalum caudatum UDP-glycosyltransferase), isolated from O. caudatum previously [13], is a flavonoid glycosyltransferase (GT) with catalyzing promiscuity. OcUGT1 can glucosylate diverse sugar acceptors including flavonoids. Moreover, OcUGT1 has been observed to function on multiple sites of flavonoids, yielding a number of flavonoid glycosides [13]. Both indicate OcUGT1 is an ideal tool for glycodiversification of small molecules. OcUGT1 was used as a biocatalyst...
for the glucosylation of sulfuretin with UDP-D-glucose (UDP-Glc). OcUGT1-assisted glucosylation of sulfuretin resulted in the formation of ten glucosides including three monoglucosides, five diglucosides and two triglucosides. Of these ten newly formed glycosides, at least six glucosides were new compounds (Figure 1). Thus, the use of single glycosyltransferases capable of forming multiple glycosides is an effective way to achieve glycosidic diversification, and can significantly increase the probability of drug discovery.

2. Results and Discussion

2.1. Protein Expression and Purification

After induction by IPTG, total proteins of Escherichia coli strain BL21(DE3) [pET28a-OcUGT1 + pKJE7] were subject to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis [13]. As shown in Figure 2A, an intense band with 53 kDa was detected in the sample. No corresponding band was present in the control strain, suggesting a soluble OcUGT1 was expressed in E. coli (Figure 2). The expressed OcUGT1 was thus purified to near homogeneity and its concentration was determined for glucosylation reaction.

2.2. OcUGT1-Catalyzed Glycosylation towards Sulfuretin

After incubated at 50 °C for 2 h, the reaction mixture containing purified OcUGT1, sulfuretin and UDP-Glc was analyzed by reverse phase high performance liquid chromatography (RP-HPLC). As shown in Figure 3, ten new peaks 1a–j were present in the reaction mixture (Figure 3), while there were no new peaks in the control reaction harboring no purified OcUGT1 (Figure 3) suggesting the ten peaks might be glucosylated metabolites of sulfuretin.
The evidence suggests that all three metabolites were monoglucosylated sulfuretins. Coelutions of these metabolites with their standards assigned 

\( \delta \) proton signals at 

2.4. Structural Identification of Sulfuretin Diglucosides

Compounds 1d, 1e, 1f, 1g and 1h have the same molecular formula \( \text{C}_{27}\text{H}_{30}\text{O}_{15}\text{Na} \) with \([M + \text{Na}]^+\) ion peaks at \( m/z \) 617.1479, 617.1484, 617.1492, 617.1481 and 617.1500, respectively, suggesting their diglucosylation of sulfuretin (Figure S3). Compound 1g is the major product of these sulfuretin diglucosides. To further determine the structure of 1g, it was collected using RP-HPLC and subjected to nuclear magnetic resonance (NMR) analyses. The \(^1\)H-NMR spectrum (Figure S4 and Table 1) showed signals for the following protons: an olefinic proton at \( \delta = 6.77 \) (1H, s, H-10); and two sets of ABX type aromatic protons at \( \delta = 7.71 \) (1H, d, \( J = 8.5 \) Hz, H-4), 6.77 (1H, s, H-10); and two sets of ABX type aromatic protons at \( \delta = 7.71 \) (1H, d, \( J = 8.5 \) Hz, H-4), 6.92 (1H, dd, \( J = 2.0, 8.5 \) Hz, H-7), and 7.49 (1H, d, \( J = 2.1 \) Hz, H-2'), 7.21 (1H, d, \( J = 8.6 \) Hz, H-5'), 7.41 (1H, dd, \( J = 2.1, 8.6 \) Hz, H-6'). The \(^{13}\)C-NMR and spectroscopic data (Figure S4 and Table 1) indicated 27 carbon resonances, including two glucose moiety carbons, one carbonyl carbon, five aromatic oxygenated carbons, and nine aromatic carbons. The above data revealed that compound 1g has a typical sulfuretin skeleton. In the HMBC (Figure 4 and Figure S5) spectrum of compound 1g, long-range correlations between H-1'' and C-6 (\( \delta = 164.9 \)), H-1''' and C-4' (\( \delta = 146.7 \)), demonstrated that the glucosyl group was located at C-6 and C-4', respectively. The \( \beta \)-configuration of sugars were concluded from the anomeric proton signals at \( \delta = 5.18 \) (1H, d, 7.3 Hz, H-1''), and 4.84 (1H, d, 7.3 Hz, H-1'''') in the \(^1\)H-NMR spectrum. Based on these observations, the metabolite 1g was elucidated as sulfuretin 4',6-diglucoside (Figure 4, Figure S1, S4 and S5 and Table 1). The four other diglucosides were not well characterized due to...
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their trace amount. According to the catalytic behavior of OcUGT1 towards luteolin [13], the four diglucosides should include sulfuretin 3′,6-diglucoside and sulfuretin 3′,4′-diglucoside. Previous study indicated that OcUGT1 was able to attack the hydroxyl group of sugar moiety in monoglucosides, thereby forming disaccharide glycosides [30]. It is therefore reasonable to infer the remaining two diglucosides were disaccharide glucosides of sulfuretin. Thus, OcUGT1-assisted glucosylation of sulfuretin resulted in five diglucosides, namely sulfuretin 4′,6-diglucoside (1g) (Figure S1), sulfuretin 3′,6-diglucoside, sulfuretin 3′,4′-diglucoside and two disaccharide glucosides (Figure 1). Of the five diglucosides, the two diglucoside sulfuretin 4′,6-diglucoside (1g) and sulfuretin 3′,4′-diglucoside, as well as two disaccharide glucosides of sulfuretin, were new compounds.

| Position | 13C | 1H          |
|----------|-----|-------------|
| 2        | 146.2, C |            |
| 3        | 181.7, C |            |
| 4        | 125.4, CH | 7.71, d (8.5) |
| 5        | 113.7, CH | 6.92, dd (8.5, 2.0) |
| 6        | 164.9, C |            |
| 7        | 99.4, CH | 7.21, d (2.0) |
| 8        | 167.4, C |            |
| 9        | 115.1, C |            |
| 10       | 111.9, CH | 6.77, s    |
| 1′       | 126.3, C |            |
| 2′       | 118.2, CH | 7.49, d (2.1) |
| 3′       | 147.2, C |            |
| 4′       | 146.7, C |            |
| 5′       | 116.0, CH | 7.21, d (8.6) |
| 6′       | 124.0, CH | 7.41, dd (8.6,2.1) |
| Glc      |           |            |
| 1′′      | 101.4, CH | 5.18, d (7.3) |
| 2′′      | 73.3, CH |            |
| 3′′      | 76.4, CH |            |
| 4′′      | 69.9, CH | 3.0–3.8, m (overlapped) |
| 5′′      | 77.3, CH |            |
| 6′′      | 60.8, CH₂ |            |
| Glc      |           |            |
| 1′′′     | 99.7, CH | 4.84, d (7.3) |
| 2′′′     | 73.1, CH |            |
| 3′′′     | 75.8, CH |            |
| 4′′′     | 69.6, CH | 3.0–3.8, m (overlapped) |
| 5′′′     | 77.1, CH |            |
| 6′′′     | 60.7, CH₂ |            |

2.5. Structural Identification of Sulfuretin Triglucosides

The HR-ESI-MS of 1i and 1j displayed molecular ion [M + Na]⁺ peaks at m/z 779.2011 and 779.2031, respectively, both corresponding to the molecular formula of C₃₃H₄₀O₂₀Na, which indicated that both compounds were triglucosides of sulfuretin (Figure S6). The structures of the two triglucosides were not well characterized due to their trace amount. According to the catalytic behavior of OcUGT1 [13], one of the triglucosides was sulfuretin 3′,4′,6-triglucoside. The other triglucoside could not be deduced from the HR-ESI-MS data. To the best of our knowledge, the two triglucosides were also new compounds.
Overall, OcUGT1-catalyzed glucosylation of sulfuretin led to the generation of ten glucosides including six new compounds. The data revealed that enzyme-mediated glucosylation is an effective way to diversify glucosides. Previously, glycosyltransferases capable of accepting glycosides for further glycosylation have been reported [3]. However, there are few glycosyltransferases that catalyze the formation of monoglycosides, diglucosides and triglycosides of a single substrate simultaneously. In this study, OcUGT1 has been demonstrated to catalyze sulfuretin to form corresponding monoglycosides, disaccharides and triglycosides simultaneously, indicating that OcUGT1 has a very wide substrate specificity. These results, together with previous reports [13,31,32], indicate that OcUGT1 has potential applications as a biocatalyst in glycodiversification of natural products.

3. Materials and Methods

3.1. Chemicals

Sulfuretin (CAS No.:120-05-8) was purchased from BioBioPha (Kunming, Yunnan, China) (Figure S1). UDP-Glc was obtained from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). The other chemicals were either reagents or analytical grade when available.

3.2. Protein Expression and Purification

Heterologous expression and purification of OcUGT1 was performed as described previously [13]. As introduced by Yuan et al., an expression plasmid pET28a-OcUGT1 and a chaperone plasmid pKJE7 (Takara, Dalian, China) were co-transformed into E. coli strain BL21 (DE3) for soluble expression. Total protein extracts from isopropyl-β-D-thiogalactoside (IPTG)-induced bacterial cells were separated by SDS-PAGE. The expressed recombinant protein with His-Tag were purified by affinity chromatography. The concentration of the purified protein was determined based on the procedure introduced by Yin et al. [33]. The resultant purified OcUGT1 was applied as the biocatalyst for the glycosylation towards sulfuretin (1) (Figure S1).
3.3. Glycosylation Assay

The reaction mixture and reaction conditions of OcUGT1-catalyzed glycosylation assay was the same as that of our previous reports [13]. In brief, a total of 100 µL phosphate buffer (10 mM, pH 8.0) harboring 10 mg purified OcUGT1, 1 mM sulfuretin and 1 mM UDP-Glc were incubated at 50 °C for 2 h. The glycosylation reaction was monitored by RP-HPLC. The HPLC conditions were the same as previously described by Yuan et al. [13].

3.4. Structural Identification

HR-ESI-MS spectra were recorded on A Triple TOF™ 5600 system (AB SCIEX, CA, USA) with a DuoSpray ionization source operating in the positive ESI mode.

NMR spectroscopic data were obtained as previously described [29,34–36]. Chemical shifts (δ) and coupling constants (J) were provided in ppm and hertz (Hz), respectively.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4344/8/10/416/s1, Figure S1: the general position numeration of sulfuretin (1) and sulfuretin 4′,6-diglucoside (1g), Figure S2: The mass spectra of 1a (A), 1b (B) and 1c (C) acquired by ESI-HRMS, Figure S3: The mass spectra of 1d (A), 1e (B) 1f (C), 1g (D) and 1h (E) acquired by ESI-HRMS, Figure S4: 1H-NMR spectrum (600 MHz, DMSO-d6) (A) and 13C-NMR spectrum of 1g (150 MHz, DMSO-d6) (B), Figure S5: HMBC spectrum of 1g, Figure S6: The mass spectra of 1i (A) and 1j (B) acquired by ESI-HRMS.

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Abbreviations

GT glycosyltransferase
HR-ESI-MS high-resolution electrospray ionization mass spectrometry
IPTG isopropyl-β-D-thiogalactoside
NMR nuclear magnetic resonance
OcUGT1 Ornithogalum caudatum UDP-glycosyltransferase
RP-HPLC reverse phase high performance liquid chromatography
SDS-PAGE sodium dodecyl sulfate polyacrylamide gel electrophoresis
UDP-Glc UDP-D-glucose

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