



Glycosylation of Ganoderic Acid F by *Bacillus* Glycosyltransferase

Te-Sheng Chang

Department of Biological Sciences and Technology, National University of Tainan, Tainan 70005, Taiwan; mozyme2001@gmail.com; Tel./Fax: +886-6-2602137

Abstract: *Ganoderma lucidum* is a medicinal fungus and has been used for improvements of health or prevention of certain diseases in Asia for thousands of years. Despite numerous kinds of triterpenoids having been identified from *G. lucidum*, few natural *Ganoderma* triterpenoids exist in the form of glycosides (saponins). To expand the diversity of *Ganoderma* triterpenoids and find rare *Ganoderma* saponins, ganoderic acid F (GAF), a *Ganoderma* triterpenoid, was biotransformed by a glycosyltransferase (BsGT110) from *Bacillus subtilis* ATCC (American type culture collection) 6633. The results showed that BsGT110 catalyzed biotransformation of GAF to produce a metabolite, which was confirmed as a GAF glucoside by mass–mass spectroscopy. The GAF glucoside showed 89-fold higher aqueous solubility than that of GAF. The present study highlights the utility of BsGT110 in the production of novel *Ganoderma* triterpenoid saponins, and the newly identified and highly soluble GAF glucoside can be studied for its bioactivity in the future.

Keywords: biotransformation; *Ganoderma lucidum*; glycosyltransferase; saponin; triterpenoid



Citation: Chang, T.-S. Glycosylation of Ganoderic Acid F by *Bacillus* Glycosyltransferase. *Agrochemicals* **2022**, *1*, 17–21. <https://doi.org/10.3390/agrochemicals1010003>

Academic Editor: Christos G. Athanassiou

Received: 3 August 2022

Accepted: 16 September 2022

Published: 22 September 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Ganoderma lucidum (in Chinese, “Lingzhi”) has been used as a nutrition supplement by Chinese for more than 2000 years. This fungus is used in the prevention of some diseases, including immunomodulatory and antitumor activities [1]. Many bioactive constituents have been isolated from *G. lucidum*, such as polysaccharides and triterpenoids. To date, more than 300 different triterpenoids have been isolated and identified from *Ganoderma* spp. [2]. Although many bioactive triterpenoids have been isolated from *G. lucidum*, few studies have focused on the biotransformation of *Ganoderma* triterpenoids. Among various biotransformations, glycosylation (the attachment of a bulky sugar group to triterpenoids) can improve physical and chemical stability and aqueous solubility and reduce the cytotoxicity of natural triterpenoids. Increasing aqueous solubility and decreasing cytotoxicity could expand the applications of the triterpenoids. However, *Ganoderma* contains more than 374 triterpenoids, while only three triterpenoid glycosides exist [1,2].

Glycosylation is a common modification reaction in the biosynthesis of natural compounds. Generally, glycosylation is catalyzed by glycosyltransferases (GTs, EC 2.4.x.y), which transfer sugar moieties from the activated donor molecules, such as uridine-diphosphate glucose (UDP-G), to specific acceptor molecules [3–5]. A previous study used a recombinant BsGT110 from *Bacillus subtilis* ATCC (American type culture collection) 6633 to catalyze glycosylation of a soybean isoflavone, 8-hydroxydaidzein (8-OHDe) [6]. The results showed that the aqueous solubility and stability of the isoflavone glucosides (8-OHDe-7-O- β -glucoside and 8-OHDe-8-O- β -glucoside) were greatly improved. Moreover, BsGT110 was recently demonstrated to glycosylate both ganoderic acid A (GAA) and ganoderic acid G (GAG), two *Ganoderma* triterpenoids, to produce two novel *Ganoderma* saponins, GAA-26-O- β -glucoside [7] and GAG-26-O- β -glucoside [8], respectively. In fact, BsGT110 was the first identified GT catalyzing the glycosylation of a triterpenoid at the C-26 position. The study revealed that BsGT110 might have glycosylation activity toward other *Ganoderma* triterpenoids.

To expand the diversity of *Ganoderma* triterpenoids and find new *Ganoderma* saponins, BsGT110 was used to glycosylate ganoderic acid F (GAF), a *Ganoderma* triterpenoid, in

the present study. One metabolite was produced and the structure and the solubility of the product were determined.

2. Results and Discussion

2.1. *BsGT110* Biotransforming GAF

The previous study showed that *BsGT110* could glycosylate the C-26 carboxyl groups of GAA [7] and GAG [8]. Thus, to produce rare *Ganoderma* saponins, the enzyme was evaluated for its glycosylation activity toward another *Ganoderma* triterpenoid, GAF (Figure 1). GAF contains only one functional group for glycosylation, the C-26 carboxyl group, which is identical with those of GAA and GAG. The results showed that one metabolite, compound (1), appeared in the HPLC analysis after the biotransformation (Figure 2). The result revealed that *BsGT110* could catalyze biotransformation of GAF.

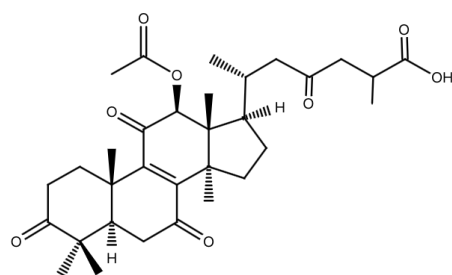


Figure 1. Chemical structure of ganoderic acid F (GAF).

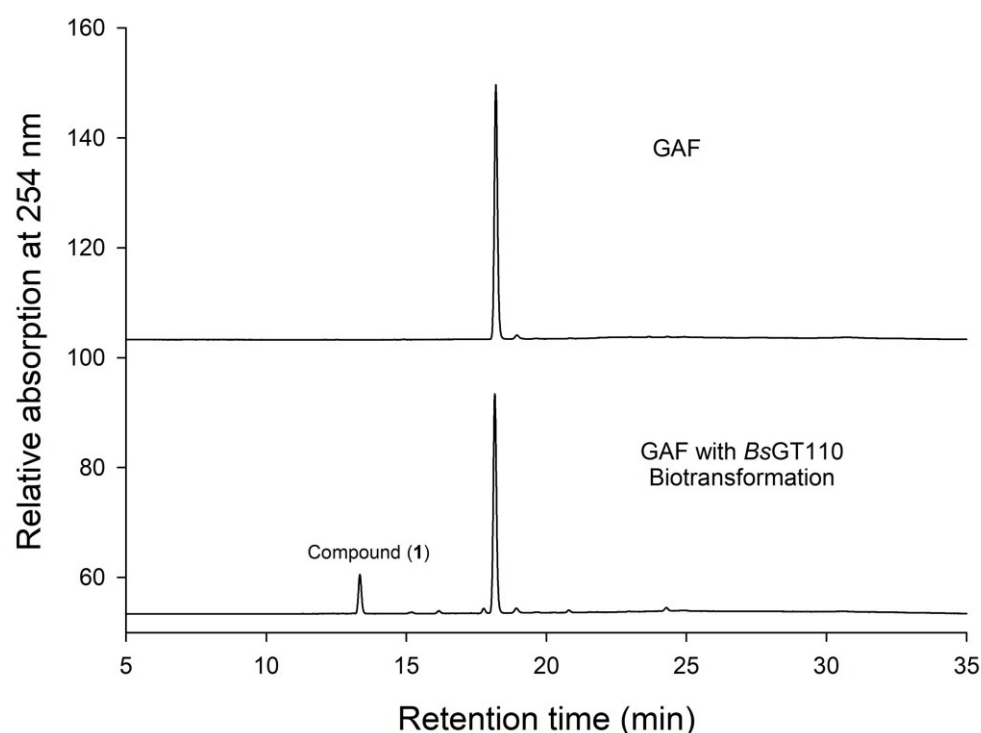


Figure 2. High-Performance Liquid Chromatography (HPLC) of the biotransformation reaction of GAF with *BsGT110*. Twenty-five micrograms of purified *BsGT110* were incubated with 10 mM UDP-G and 1 mg/mL of GAF in the presence of 50 mM phosphate buffer (PB) pH 6.0 and 10 mM of $MgCl_2$. After 24 h incubation, the mixture was analyzed with HPLC. The UPLC conditions are described in Materials and Methods.

2.2. GAF Glucoside Produced

To determine whether the biotransformation product was the glycoside derivative of GAF, the biotransformation was scaled up to 10 mL. About 1.7 mg of the product in the 10 mL reaction was purified with preparative HPLC. The chemical structure of the purified compound (**1**) was resolved with mass and mass–mass spectral analysis. The mass analysis of the compound showed an $[M-H]^-$ ion peak at m/z : 731.5 in the electrospray ionization mass (ESI-MS) spectrum corresponding to the m/z signal of molecular weight 732 of GAF-glucoside ($570+180-18$) at the negative mode (Figure S1a). In addition, two significant peaks appeared from mass–mass spectral analysis at m/z 551.5 and 179.0, which represented the signals of molecular weight 552 of GAF with loss of a H_2O ($570-18$) and molecular weight 180 of glucose from the breakdown of compound (**1**) (Figure S1b). Accordingly, compound (**1**) was assigned as a GAF monoglucoside. GAF is commercially available in tiny amounts, and only 1.7 mg of the GAF monoglucoside was isolated from the biotransformation. Therefore, the chemical structure of the GAF monoglucoside was not resolved by nucleic magnetic resonance spectral analysis in advance. However, GAF contains only one functional group for glycosylation, the C-26 carboxyl group, which is identical with those of GAA and GAG. Moreover, our previous study showed that *BsGT110* could glycosylate the C-26 carboxyl groups of GAA and GAG. Taken all these results together, it was concluded that *BsGT110* glycosylated GAF to a new *Ganoderma* triterpenoid saponin, GAF glucoside, whose chemical structure is predicted as GAF-26-O-glucoside. Figure 3 illustrates the assumed biotransformation of GAF by *BsGT110* to produce GAF-26-O-glucoside.

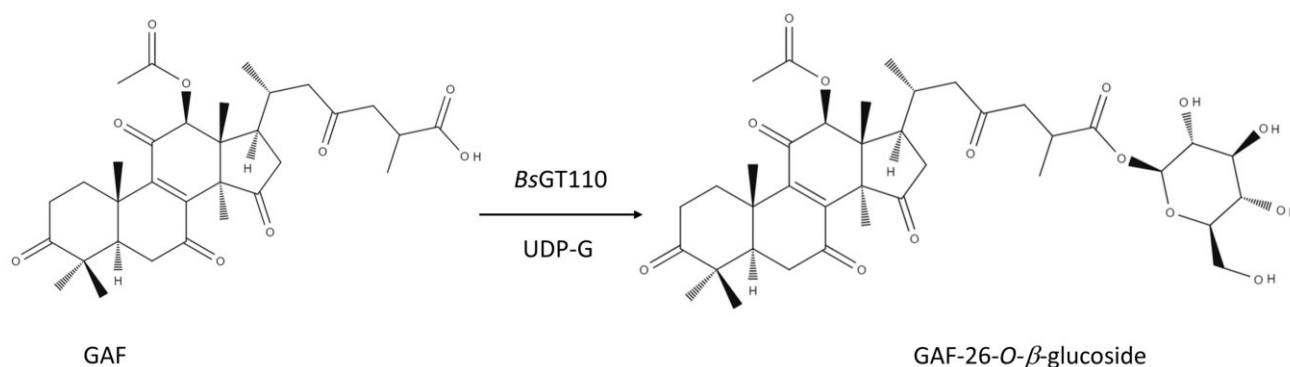


Figure 3. The assumed biotransformation process of GAF to GAF-26-O-glucoside by *BsGT110*. UDP-G: uridine-diphosphate glucose.

2.3. GAF Glucoside Possessing High Aqueous Solubility

Previous studies have proven that the aqueous solubility of flavonoids can be improved through glycosylation [9], which would advance the oral bioavailability of the original molecules [10,11]. Thus, the aqueous solubility of GAF and the GAF glucoside were determined with the methods we used in a previous study [6]. The results showed that the aqueous solubility of the GAF glucoside was 89.2-fold higher than that of GAF (Table 1). Due to its high aqueous solubility, the produced novel *Ganoderma* saponin could have broader applications in biotechnology. Thus, it is worthy to study the bioactivities of the newly identified GAF glucoside in the future.

Table 1. Aqueous solubility of GAF and its glucoside.

Triterpenoid	Aqueous Solubility (mg/L) ¹	Fold ²
GAF	11.7 ± 1.4	1.0
GAF glucoside	1044.2 ± 61.4	89.2

¹ The mean ($n = 3$) is shown, and the standard deviations are represented by error bars. ² The fold of aqueous solubility of GAF glucoside is expressed as relative to that of GAF, normalized to 1.

3. Materials and Methods

3.1. Enzymes and Chemicals

Recombinant *BsGT110* enzyme was prepared from the previous study [6]. UDP-G was obtained from Cayman Chemical (Ann Arbor, MI, U.S.A.). A total of 20 mg of GAF was purchased from Baoji Herbest Bio-Tech (Xi-An, Shaanxi, China).

3.2. In Vitro Biotransformation Assay

The in vitro biotransformation was conducted with the purified *BsGT110*. The reaction (0.1 mL) containing 25 µg of the tested enzyme, 1 mg/mL of GAF, 10 mM of UDP-G, 10 mM of MgCl₂, and 50 mM of phosphate buffer (PB) at pH 6.0 was carried out at 40 °C for 24 h. After the reaction, the mixture was stopped by adding an equal volume of methanol and analyzed with high-performance liquid chromatography (HPLC).

3.3. HPLC Analysis

HPLC was performed with the Agilent 1100 series HPLC system (Santa Clara, CA, USA) equipped with a gradient pump (Waters 600, Waters, Milford, MA, USA). The stationary phase was a C18 column (5 µm, 4.6 i.d. × 250 mm; Sharpsil H-C18, Sharpsil, Bei-jing, China), and the mobile phase was 1% acetic acid in water (A) and methanol (B). The elution condition was a linear gradient from 0 min with 50% B to 20 min with 95% B, an isocratic elution from 20 min to 25 min with 95% B, a linear gradient from 25 min with 95% B to 28 min with 50% B, and an isocratic elution from 28 min to 34 min with 50% B. All eluants were eluted at a flow rate of 1 mL/min. The sample volume was 10 µL. The detection condition was set at 254 nm.

3.4. Isolation and Identification of the Biotransformation Product

To purify compound (1), the biotransformation reaction as described in Section 3.2 was scaled up to 10 mL. After reaction, compound (1) was purified by a preparative YoungLin HPLC system. The elution corresponding to the peak of compound (1) in the HPLC analysis was collected, condensed under a vacuum, and then dehydrated by freeze drying. Finally, 1.7 mg of compound (1) was obtained. The structure of the compound was confirmed with mass spectral analyses.

3.5. Determination of Solubility

The aqueous solubility of GAF and its glucoside were examined by the previous method [6]. An excess of GAF (1 mg/200 µL) and the purified glucoside (0.5 mg/20 µL) were mixed with double-deionized water by vortexing for 10 min at 25 °C, followed by incubation for 1 h in an ultrasonic bath (Branson, Danbury, CT, USA) to maximize the solubility of the compound. The mixture was then centrifuged and the supernatant was filtrated with a nylon membrane for HPLC analysis.

4. Conclusions

A new *Ganoderma* triterpenoid saponin, GAF glucoside, was produced and identified from the biotransformation of GAF by *BsGT110*, which was proven to be a promiscuous biocatalyst for production of novel *Ganoderma* saponins. The newly identified GAF glucoside possessed 89-fold higher aqueous solubility than that of GAF and might have unique bioactivities that could benefit the medicinal industry.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agrochemicals1010003/s1>. Figure S1. The mass (a) and mass–mass (b) analyses of compound (1) at the negative mode.

Funding: This research was funded by the Ministry of Science and Technology of Taiwan under grant number MOST 111-2918-I-024-001.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Wu, J.W.; Zhao, W.; Zhong, J.J. Biotechnological production and application of ganoderic acids. *Appl. Microbiol. Biotechnol.* **2010**, *87*, 457–466.
2. Xia, Q.; Zhang, H.; Sun, X.; Zhao, H.; Wu, L.; Zhu, D.; Yang, G.; Shao, Y.; Zhang, X.; Mao, X.; et al. A comprehensive review of the structure elucidation and biological activity of triterpenoids from *Ganoderma* spp. *Molecules* **2014**, *19*, 17478–17535. [[CrossRef](#)]
3. Tiwari, P.; Sangwan, R.S.; Sangwan, N.S. Plant secondary metabolism linked glycosyltransferases: An update on expanding knowledge and scopes. *Biotechnol. Adv.* **2016**, *34*, 716–739. [[CrossRef](#)] [[PubMed](#)]
4. Kim, B.G.; Yang, S.M.; Kim, S.Y.; Cha, M.N.; Ahn, J.H. Biosynthesis and production of glycosylated flavonoids in *Escherichia coli*: Current state and perspectives. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 2979–2988. [[CrossRef](#)] [[PubMed](#)]
5. Hofer, B. Recent developments in the enzymatic O-glycosylation of flavonoids. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 4269–4281. [[CrossRef](#)] [[PubMed](#)]
6. Chiang, C.M.; Wang, T.Y.; Yang, S.Y.; Wu, J.Y.; Chang, T.S. Production of new isoflavone glucosides from glycosylation of 8-hydroxydaidzein by glycosyltransferase from *Bacillus subtilis* ATCC 6633. *Catalysts* **2018**, *8*, 387. [[CrossRef](#)]
7. Chang, T.S.; Chiang, C.M.; Kao, Y.H.; Wu, J.Y.; Wu, Y.W.; Wang, T.Y. A new triterpenoid glucoside from a novel acidic glycosylation of ganoderic acid A via recombinant glycosyltransferase of *Bacillus subtilis*. *Molecules* **2019**, *24*, 3457. [[CrossRef](#)] [[PubMed](#)]
8. Wu, J.Y.; Ding, H.Y.; Wang, T.Y.; Zhang, Y.R.; Chang, T.S. Glycosylation of ganoderic acid G by *Bacillus* glycosyltransferases. *Int. J. Mol. Sci.* **2021**, *22*, 9744. [[CrossRef](#)] [[PubMed](#)]
9. Huang, G.; Lv, M.; Hu, J.; Huang, K.; Xu, H. Glycosylation and activities of natural products. *Mini Rev. Med. Chem.* **2016**, *16*, 1013–1016. [[CrossRef](#)] [[PubMed](#)]
10. Zhao, J.; Yang, J.; Xie, Y. Improvement strategies for the oral bioavailability of poorly water-soluble flavonoids: An overview. *Int. J. Pharm.* **2019**, *570*, 118642. [[CrossRef](#)] [[PubMed](#)]
11. Fu, J.; Wu, Z.; Zhang, L. Clinical applications of the naturally occurring or synthetic glycosylated low molecular weight. *Prog. Mol. Biol. Transl. Sci.* **2019**, *163*, 487–522. [[PubMed](#)]