jmb

Gram-Scale Production of Ginsenoside F1 Using a Recombinant Bacterial β-Glucosidase

Dong-Shan An[1#], Chang-Hao Cui[2], Muhammad Zubair Siddiqi[3,4], Hong Shan Yu[5], Feng-Xie Jin[5], Song-Gun Kim[1,6*], and Wan-Taek Im[3,4*]

[1]Biological Resource Center/Korean Collection for Type Cultures (KCTC), Korea Research Institute of Bioscience and Biotechnology, Jeongeup 56212, Republic of Korea

[2]KAIST Institute for Biocentury, Korea Advanced Institute of Science and Technology, Daejeon 34141, Republic of Korea

[3]Department of Biotechnology, Hankyong National University, Anseong 17579, Republic of Korea

[4]Center for Genetic Information, Graduate School of Bio and Information Technology, Hankyong National University, Anseong 17579, Republic of Korea

[5]College of Biotechnology, Dalian Polytechnic University, Dalian 116034, P.R. China

[6]University of Science and Technology (UST), Daejeon 34113, Republic of Korea

Naturally occurring ginsenoside F1 (20-*O*-β-D-glucopyranosyl-20(S)-protopanaxatriol) is rare. Here, we produced gram-scale quantities of ginsenoside F1 from a crude protopanaxatriol saponin mixture comprised mainly of Re and Rg1 through enzyme-mediated biotransformation using recombinant β-glucosidase (BgpA) cloned from a soil bacterium, *Terrabacter ginsenosidimutans* Gsoil 3082[T]. In a systematic step-by-step process, the concentrations of substrate, enzyme, and NaCl were determined for maximal production of F1. At an optimized NaCl concentration of 200 mM, the protopanaxatriol saponin mixture (25 mg/ml) was incubated with recombinant BgpA (20 mg/ml) for 3 days in a 2.4 L reaction. Following octadecylsilyl silica gel column chromatography, 9.6 g of F1 was obtained from 60 g of substrate mixture at 95% purity, as assessed by chromatography. These results represent the first report of gram-scale F1 production via recombinant enzyme-mediated biotransformation.

Keywords: Protopanaxatriol, bioconversion, gram-scale, glycoside hydrolase family 3, rare ginsenoside

Introduction

Ginseng, the common term for the root of the ginseng plant, has been used as a medicinal plant in Oriental medicine for thousands of years. Even today, ginseng is a widely consumed botanical dietary supplement in Western as well as Oriental cultures. Ginsenoside is the major active component of ginseng [1–3], and to date, more than 180 different types of ginsenoside have been identified [4].

However, the majority of ginsenosides in dried ginseng, as well as in Korean red ginseng, which is prepared by a sequential steaming and drying process, are composed of only six compounds: protopanaxadiol-type Rb1, Rb2, Rc, and Rd; and protopanaxatriol (PPT)-type Re and Rg [5, 6]. These compounds constitute 3–5% of ginseng on a weight/weight basis [7, 8].

In 2004, Hasegawa [9] was the first to report the biotransformation activity of intestinal bacteria in converting

structurally large ginsenoside into a deglycosylated smaller form that is absorbed into circulation. As the absorption and efficacy of deglycosylated ginsenoside appears to be higher than that of the major component of untreated ginsenoside [10, 11], there has been considerable focus on techniques for producing deglycosylated ginsenosides in vitro. Conversion of a rare ginsenoside that is not a natural component of ginseng by physicochemical methods such as acid hydrolysis and steaming is quite difficult. Acid or alkaline hydrolysis of the major ginsenosides produces the Rg3 racemic mixture [12]. In heat processed ginseng, only Rg3 and Rh2 appear as the major components, but the conversion to Rg3 and Rh2 is not complete [13]. Methods of enzymatic hydrolysis to produce the smaller deglycosylated forms of ginsenoside have been in active development in recent years, as this method could produce rare ginsenosides of interest using a single enzyme or a combination of enzymes [14–16]. The production of protopanaxadiol-type ginsenosides (gypenoside (Gyp) XVII, Gyp LXXV, and compound K) using a recombinant glycoside hydrolase family 3 (GH3) enzyme was reported [17], as was the production of C-K from Rd using a thermostable archaeal or bacterial enzyme [18].

Ginsenoside F1 (20-*O*-β-D-glucopyranosyl-20(S)-protopanaxatriol; CAS No. 53963-43-2), a rare PPT-type ginsenoside, has only one glucopyranosyl moiety on C20 of the aglycone. F1 has been shown to have a protective effect against ultraviolet-(UV)B-induced apoptosis in human HaCaT keratinocytes, and modulates cellular responses of skin melanoma cells [18, 19]. It can be produced by hydrolysis of the disaccharide of the α-L-rhamnopyranosyl-β-D-glucopyranosyl moiety of C6 of ginsenoside Re, or by hydrolysis of the glucose moiety of C6 of Rg1. In 2003, Ko *et al.* [20] used glycosidases to enzymatically produce 100 mg of F1, Rh1, Rg2, and Rg1 from 0.5 g of a PPT-type mixture, and several groups have reported the production of F1 from Rg1 using β-glucosidases isolated from fungi [21–23]. However, large-scale production of F1 has yet to be reported. This is largely due to the lack of an available recombinant enzyme that can deglycosylate PPT-type ginsenosides [13]. Large-scale production of F1 using a wild-type enzyme has yet to be established because the production of wild-type enzyme from a microorganism is limited.

In the current study, the rare PPT-type ginsenoside F1 was produced by biotransformation using a recombinant β-glucosidase (GH3) that was previously reported to possess deglycosylation activity towards protopanaxadiol-type ginsenosides. We produced 9.6 g of F1 with 95% purity based on high-performance liquid chromatography (HPLC)

analysis from 60 g of a ginsenoside mixture. This is the first report of the production of gram-scale quantities of F1 using a recombinant glycoside hydrolase.

Materials and Methods

Chemicals, Bacterial Strains, and Plasmids

The standard ginsenosides Re, Rg1, Rg2, Rh1, F1, and protopanaxatriol aglycon (PPTA) were purchased from Nanjing Zelang Medical Technological Co. Ltd (China). *p*-Nitrophenyl-β-D-glucopyranoside (pNPGlc) was obtained from Sigma (USA). Diaion HP-20 resin was obtained from Mitsubishi Co. Ltd (Japan). The recombinant β-glucosidase was cloned from *Terrabacter ginsenosidimutans* Gsoil 3082^T (= KCTC 19421^T), which was isolated from a ginseng field in Korea [16]. The cDNA for BgpA was cloned into pGEX-4T-1 (GE Healthcare, USA) to generate pGEX-bgpA, in which glutathione *S*-transferase was fused to the N-terminus of BgpA. Recombinant protein was expressed in *E. coli* C41 (DE3) (Lucigen Corporation, USA). All other chemicals used in this study were at least of analytical reagent grade, as noted individually in the Methods sections.

Preparation of BgpA

E. coli was cultivated in Luria-Bertani (LB) medium supplemented with ampicillin (50 μg/ml) for the production of recombinant enzyme. Cultures were incubated at 37°C until they reached an OD_{600} of 0.6, at which point the temperature was decreased to 25°C. The pH of the medium was adjusted to 7.5 with a solution of 50 mM sodium phosphate. Protein expression was induced by the addition of isopropyl-β-D-thiogalactopyranoside at a final concentration of 0.1 mM. Bacterial cells were incubated for an additional 12 h at 25°C, and then harvested by centrifugation at 5,000 ×*g* for 20 min at 4°C. Cells were suspended in 50 mM phosphate buffer (pH 7.5) and disrupted by sonication (Vibra-cell; Sonics & Materials, USA). The resulting lysates were subjected to centrifugation at 20,000 ×*g* for 30 min at 4°C, and the supernatants were recovered as the crude enzyme preparation. The crude enzyme was diluted to the desired concentration with 50 mM phosphate buffer (pH 7.5) and used in the biotransformation reactions.

Preparation of a Crude Mixture of the PPT-Type Ginsenosides Re and Rg1

A mixture of dried Korean (*Panax ginseng* C.A. Meyer) and American ginseng (*Panax quinquefolius*) root powder (4:1 (w/w)) was used as the source for ginsenoside extraction. Ginseng root powder (10 kg) was twice extracted with 100 L of 70% ethanol. The extract was filtered through filter paper and then dried using a rotary evaporator. The resultant dried powder was dissolved in water and loaded onto a glass column (400 mm × 100 mm (L × diameter) packed with Diaion HP-20 resin (Mitsubishi, Japan). HP-20 absorbed beads were washed with 8 column volumes of

water to remove free sugar molecules and unwanted hydrophilic compounds, and then the PPT-type ginsenosides were eluted with 4 column volumes of 40% ethanol. The ethanol extracts were evaporated in vacuo, and the dried residue was used as the ginsenoside substrate mixture for F1 production by BgpA. HPLC analysis indicated that the mixture was composed of 32.4% Rg1, 31.9% Re, 6.6% Rb1, 6.0% Rc, and 6.2% Rd based on mol ratio. This crude ginsenoside mixture (hereafter referred to as PPTx) was used to optimize the conditions for the biotransformation reaction. PPTx was soluble up to 100 mg/ml in 50 mM phosphate buffer.

Substrate Specificity of BgpA for PPT-Type Ginsenosides

To test the biotransformation activity of BgpA, 40 mg/ml of crude enzyme was incubated with an equal volume of the PPT-type ginsenosides Re, Rg1, Rg2, F1, or Rh1 at a final concentration of 0.1% (w/v) in 50 mM sodium phosphate buffer (pH 7.5) at 37°C. Samples were collected after 24 h of incubation and analyzed by thin-layer chromatography (TLC) after pretreatment (see below).

Optimization of Substrate and NaCl Concentrations

To determine the optimal concentration of PPTx for the biotransformation reaction, crude BgpA (40 mg/ml) was mixed with an equal volume of PPTx at concentrations of 5, 10, 20, 25, 30, or 60 mg/ml. Samples were withdrawn at regular intervals and analyzed by HPLC. To increase the solubility of PPTx and further optimize F1 production, various concentrations of NaCl were tested. The formation of F1 from PPTx in reactions containing 100, 200, 500, or 1,000 mM NaCl was tested at a fixed enzyme concentration of 20 mg/ml.

Scale-Up of F1 Production by BgpA-Mediated Hydroylsis

The scaled-up biotransformation reaction was carried out in a 5 L stirred-tank reactor (KoBioTech Co., Korea) in a 2.4 L working volume at 50 rpm for 3 days at 37°C. The reaction was carried out under optimized conditions of 25 mg/ml PPTx (substrate), 20 mg/ml recombinant BgpA, and 200 mM NaCl in 50 mM phosphate buffer (pH 7.5). Samples were collected at regular intervals and analyzed by HPLC to determine the extent of biotransformation of PPTx to F1.

Purification of F1

The 2.4 L large-scale reaction mixture was cooled at 4°C and then subjected to centrifugation at 4,000 g for 15 min. The metabolites in the supernatant and pellet were isolated separately. The composition of ginsenoside in the supernatant was assessed by HPLC, after the extraction of ginsenosides with an equal volume of water-saturated butanol. The precipitate was twice dissolved in 1.5 L of 70% ethanol and then filtered through filter paper (Advantec, Japan). The ethanol extracts were pooled and evaporated in vacuo. The resulting powder was dissolved in 50% methanol and then loaded onto a reverse-phase silica gel column (Biotage 180 × 70 φ mm) packed with ODS resin (ZEOprep 60 C$_{18}$

40–63 μm; ZEOCHEM, Switzerland). F1 was eluted in a methanol gradient up to 100%.

Analytic Methods

To analyze the ginsenoside composition of the substrate and the product of the biotransformation reaction, the reaction mixture was extracted with an equal volume of water-saturated n-butanol, and the resulting n-butanol fraction was evaporated to dryness. The residual material was dissolved in CH_3OH and examined by TLC and/or HPLC.

TLC analysis. TLC was performed using 60F$_{254}$ silica gel plates (Merck, Germany) with $CHCl_3$-CH_3OH-H_2O (65:35:10 (v/v/v) lower phase) as the solvent. The TLC plates were sprayed with 10% (v/v) H_2SO_4, followed by heating at 110°C for 5 min to visualize the spots. The spots were identified by comparison to standard ginsenoside.

HPLC analysis. HPLC analysis of the ginsenosides was performed using a HPLC system (Younglin Co. Ltd, Korea) equipped with a quaternary pump, automatic injector, single wavelength UV detector (model 730D), and Younglin's AutoChro 3000 software for peak identification and integration. The separation was carried out on a Prodigy ODS(2) C$_{18}$ column (5 μm, 150 × 4.6 mm i.d.) (Phenomenex, USA) with a guard column (Eclipse XDB C$_{18}$, 5 μm, 12.5 × 4.6 mm i.d.). The mobile phase was A (acetonitrile) and B (water). Gradient elution started with 17% solvent A and 83% solvent B, followed by 17% to 25% A, from 12 to 20 min; 25% to 32% A, from 20 to 30 min; 32% to 55% A, from 30 to 35 min; 55% to 60% A, from 35 to 40 min; 60% to 80% A, from 40 to 45 min; 80% to 100% A, from 45 to 50 min; 100% A, from 50 to 54 min; 100% to 17% A, from 54.0 to 54.1 min; and 17% A, from 54.1 to 65 min. The flow rate was 1.0 ml/min and the injection volume was 25 μl. Detection was performed by monitoring the absorbance at 203 nm (A$_{203}$).

Results and Discussion

Biotransformation of PPT-Type Ginsenosides

Maximal protein expression was achieved at a culture temperature of 20°C, 1% glucose (w/v), and pH 7.5, adjusted with 50 mM sodium phosphate buffer. Under these conditions, nearly all of the expressed BgpA was in a soluble form (data not shown). To identify the pattern of hydrolysis of PPT-type ginsenosides by BgpA, crude enzyme was incubated with ginsenoside Re, Rg1, Rg2, Rh1, or F1, and the hydrolysis products were analyzed by TLC (Fig. 1). BgpA hydrolyzed ginsenosides Re and Rg1 to F1. F1 was further hydrolyzed to PPTA if the concentration of BgpA was increased or the reaction period was extended. Notably, ginsenosides Rg2 and Rh1 were hydrolyzed directly to PPTA. These results indicated that BgpA hydrolyzed the glucose moieties at both the C6 and C20 sites of the PPT-
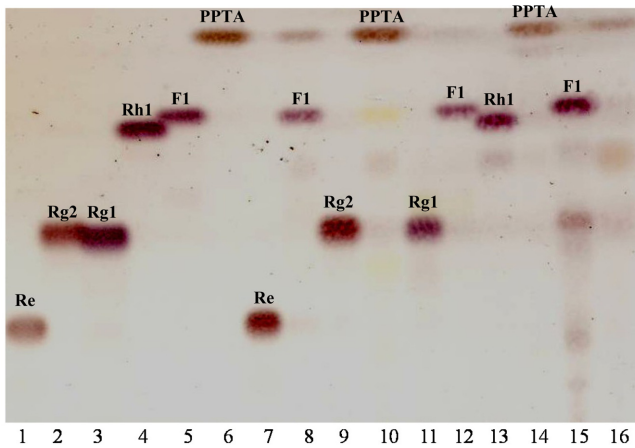
Fig. 1. BgpA-mediated biotransformation of ginsenosides Re, Rg2, Rg1, Rh1, and F1.

Lanes 1, 2, 3, 4, 5, and 6: Re, Rg2, Rg1, Rh1, F1, and PPTA standards; lanes 7, 9, 11, 13, and 15: ginsenosides Re, Rg2, Rg1, Rh1, and F1, respectively, without enzyme treatment; lanes 8, 10, 12, 14, and 16: ginsenosides Re, Rg2, Rg1, and F1, respectively, after incubation with BgpA for 24 h.

type ginsenosides, which was distinct from the mode of action of BgpA toward PPD-type ginsenosides (Fig. 2).

In a previous report, BgpA preferentially hydrolyzed the glucose attached to C3 of the aglycone of the PPD-type ginsenoside Rb1, such that Rb1 was first converted to GypVII, then converted to GypLXXV, and finally converted to C-K through hydrolysis of the C20 glucose moiety [17]. With PPT-type ginsenosides, BgpA hydrolyzed the inner glucose moiety of C6, which was protected by the outer rhamnose moiety, whereas with PPD-type ginsenosides, BgpA hydrolyzed the outer glucose and then the inner glucose of C3 in a step-wise manner [17]. Thus, BgpA hydrolyzes the C3 glucose moieties of PPD-type ginsenosides in an exotype mode of action (*i.e.,* sequential hydrolysis of an outer glucopyranosyl moiety followed by an inner glucopyranosyl moiety), whereas it has an endotype mode of action toward the C3 glucose moieties of PPT-type ginsenosides (*i.e.,* hydrolysis of the disaccharide of the rhamnose-glucopyranose moiety).

In the case of the PPT-type ginsenosides, BgpA also hydrolyzed the monosaccharide of the glucose moiety (Rg1) or the disaccharide of the β-D-glucose (2-1)-α-L-rhamnose moiety (Re) attached to the hydroxyl group at C6 to produce F1. It is noteworthy that BgpA hydrolyzed the disaccharide of Re, as opposed to carrying out sequential hydrolysis, because BgpA does not possess rhamnosidase activity [17].
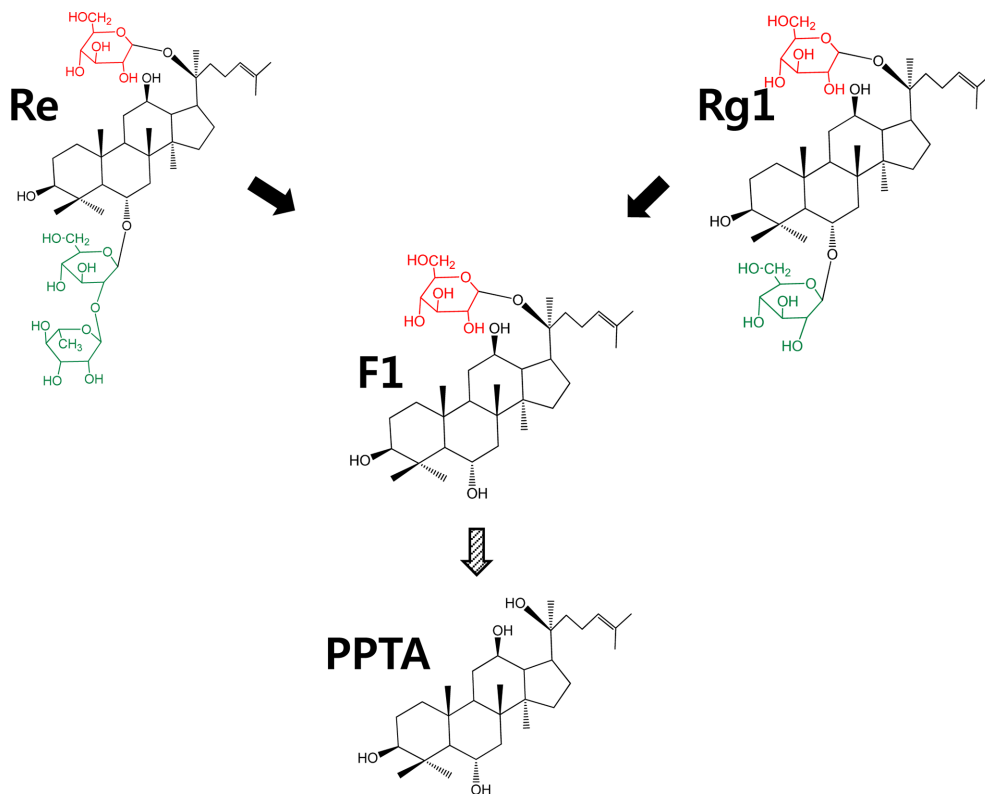

Fig. 2. Proposed biotransformation pathway for the production of ginsenoside F1 by BgpA-mediated hydrolysis.

BgpA belongs to family 3 of the glycoside hydrolases. At present, the data are not sufficient to generalize the specificity of the glycoside hydrolases based on classification, with the exception of α-L-arabinofuranosidase of the GH51 family, which hydrolyzes only the arabinofuranoside moiety of ginsenoside Rc and its derivatives [24].

Preparation of the PPTx Substrate

Because of its hydrophobic nature, pure ginsenoside Re was highly insoluble in 50 mM phosphate buffer (pH 7.5); thus, the concentration of pure Re hydrolyzed by BgpA was very low (less than 0.2 mg/ml). On the other hand, ginsenoside Rg1 was quite soluble in 50 mM phosphate buffer (pH 7.5) and was readily hydrolyzed to F1 by BgpA at high concentrations (more than 100 mg/ml). More interestingly, the solubility of Re could be increased when Re was mixed with Rg1; thus, the activity of BgpA towards ginsenoside Re could be increased.

Using ethanol extraction, 2,894 g of crude ginseng extract was obtained from 10 kg of ginseng powder. The crude ginseng extract was then divided into three equal parts, one of which was loaded onto a glass HP-20 column. Washing with water followed by elution in 40% EtOH yielded 62 g of PPTx.

Optimization of the Biotransformation Reaction of Ginsenosides Re and Rg1 to F1

Selection of PPTx concentration. The production of F1 was tested at various concentrations of PPTx to determine the optimal concentration of substrate for biotransformation. When the concentration of crude BgpA was fixed at 20 mg/ml, the optimal PPTx concentration was determined to be 25 mg/ml (Fig. 3). From day 2, production of PPTA rather than F1 increased at a low concentration of substrate, such as 5 or 10 mg/ml, whereas the conversion ratio was decreased at a high concentration of substrate (30 or 60 mg/ml) (data not shown).

Effect of NaCl on biotransformation activity. To determine the NaCl concentration for proper solubility of PPTx and enzyme activity, the production of F1 in the presence of increasing concentrations of NaCl (100, 200, 500, and 1,000 mM) was tested at an enzyme concentration of 20 mg/ml (Fig. 4). Changes in substrate (Re and Rg1) and product (F1 and PPTA) over time were determined by HPLC. Rg1 was not detected after 1 day of incubation, which indicated that Rg1 was readily transformed to F1. There was a general trend of increased F1 production with increasing concentration of NaCl. A concentration of 200 mM NaCl was chosen because it yielded the highest
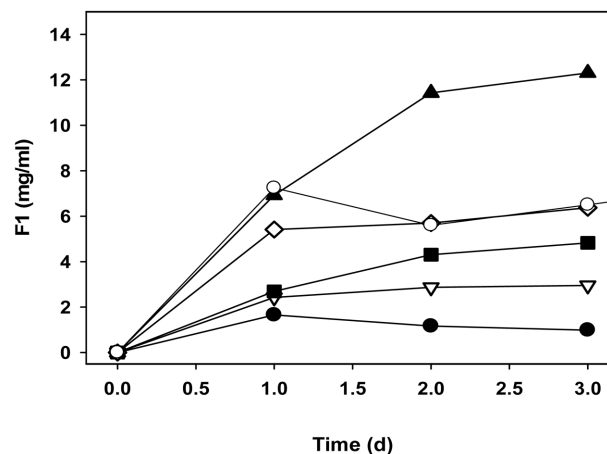

Fig. 3. Effect of substrate (PPTx) concentration on the production of ginsenoside F1 by BgpA.
The crude BgpA enzyme preparation (40 mg/ml) was mixed with an equal volume of PPTx at the indicated concentrations: 5 (●), 10 (▽), 20 (■), 25 (◇), 30 (○), and 60 (▲) mg/ml. The F1 concentration in the reaction mixture was determined by HPLC analysis.

concentration of F1 on day 3, and there was no further increase in F1 production with 500 or 1,000 mM NaCl.

Scale-Up of F1 Production by Biotransformation

A scaled-up reaction of 2.4 L was carried out under the selected conditions of 25 mg/ml substrate, 200 mM NaCl, and 20 mg/ml enzyme. One of the main substrates in the
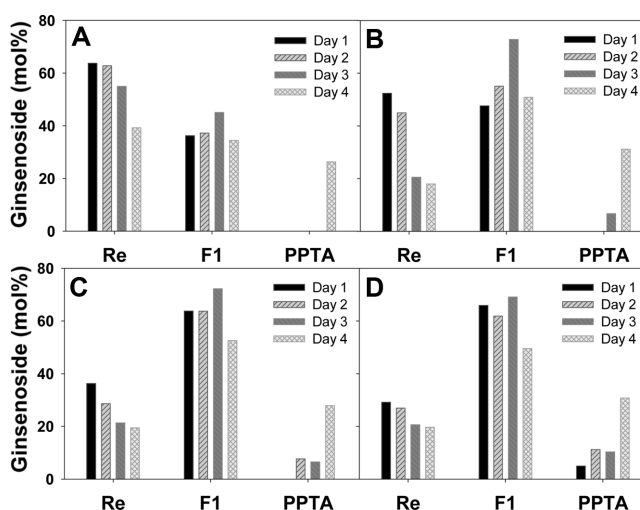

Fig. 4. Effect of NaCl concentration on the production of ginsenoside F1 from PPTx by BgpA.
(**A**) 0 mM NaCl; (**B**) 200 mM NaCl; (**C**) 500 mM NaCl; and (**D**) 1,000 mM NaCl. The concentration of the ginsengosides Re, F1, and PPTA was determined by HPLC on the dates shown.
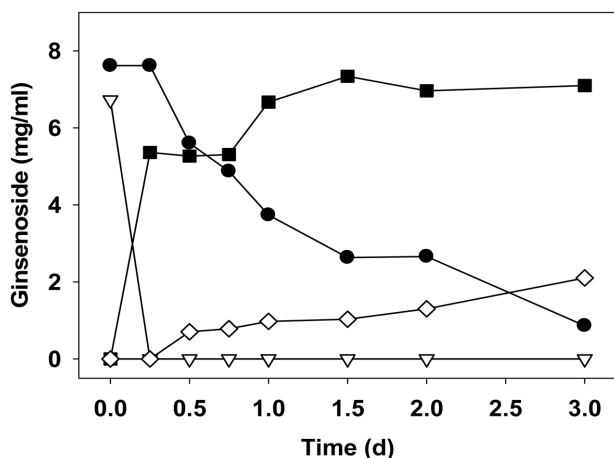
Fig. 5. Scale-up of the biotransformation of PPTx to F1.

The reaction consisted of a 2.4 L volume containing 25 mg/ml of PPTx and 20 mg/ml of BgpA. The amounts of the ginsenosides Re (●), Rg1 (▽), F1 (■), and PPTA (◇) were determined by HPLC.

Fig. 6. HPLC chromatograms of (**A**) PPTx (substrate); (**B**) ethanol extracts from the scaled-up biotransformation reaction; and (**C**) ODS eluent from the scaled-up reaction.

PPTx mixture, Rg1, disappeared from the reaction mixture at 6 h, whereas there was a more gradual disappearance and transformation of Re to F1 (Fig. 5). The reaction was stopped on day 3 and the supernatant was harvested, as further incubation resulted in an increase in PPTA, which is the end-product of hydrolysis by BgpA.

Purification of F1

Enzyme, salt, and free sugars were removed from the reaction by first cooling the mixture at 4°C followed by centrifugation at 4,000 g for 15 min. This resulted in the precipitation of F1 into a solid form. The supernatant was discarded as it contained very little ginsenoside F1. F1 was purified and recovered by dissolving the precipitate in 2 × 1.5 L of 70% ethanol. The resulting 3 L solution was evaporated to yield 27.0 g of solid ginsenoside F1, which was contaminated with salts and free sugars. After purification on an OSD column, 9.6 g of F1 was recovered (Table 1). The compositions of the substrate, reactants, and ODS column eluent were analyzed by HPLC. The purity of the final product was determined to be 95.3%, based on the area of the chromatogram (Fig. 6). Among 60 g of PPTx, the total molar amount of Re and Rg1 that could be biotransformed
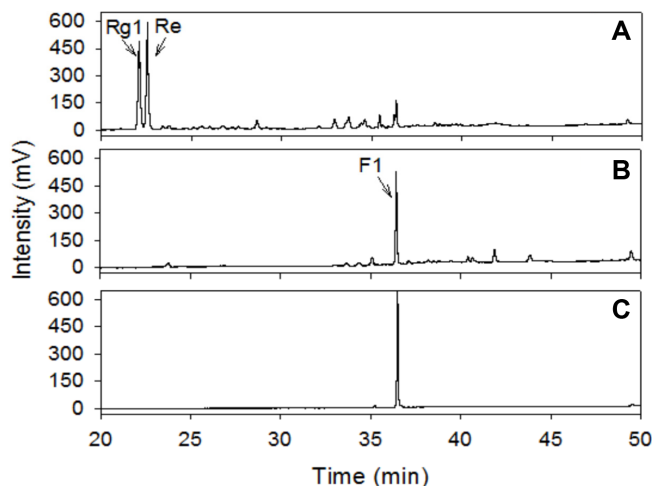
into F1 using BgpA was 30.7 mmol, which corresponds to 26.9 g of 60 g. The residue (33.1 g) consisted of other types of ginsenosides and unknown impurities. The molar amount of the produced 70% ethanol extract (27.0 g) and high-purity ginsenoside F1 (9.6 g) were 22.9 and 14.3 mmol, respectively. This indicates that the recovery ratio through the biotransformation process using ginsenosides Re and Rg1 of PPTx to F1 reached 46.6% during the entire bioprocess engineering.

Large-scale production of F1 should facilitate further basic research on the potential medicinal and cosmetic benefits of the ginsenosides, including the inhibition of UVB-induced skin ageing [19] and their potential anticancer activity [6].

Gram-scale production of the rare protopanaxatriol F1 was accomplished by enzyme-mediated biotransformation of a protopanaxatriol mixture under optimized conditions of substrate and NaCl concentration. Purified recombinant BgpA converted both Re and Rg1 to F1 by hydrolysis of sugar moieties on C6 of the aglycone. The biotransformation of ginsenoside using recombinant enzymes could be applied to the large-scale production of other ginsenosides. This is the first report of a gram-scale production of F1.

Table 1. Analysis of F1 production and purification following large-scale biotransformation of PPTx by BgpA.

Step	Weight (g)	Chromatographic purity of F1	Recovery (%)
Starting substrate: PPTx	60	-	-
70% ethanol extract	27.0	54.1	74.5
Purified F1 after ODS chromatography	9.6	95.3	46.6

Acknowledgments

References

1. Bae M, Jang S, Lim JW, Kang J, Bak EJ, Cha JH, *et al*. 2014. Protective effect of Korean Red Ginseng extract against *Helicobacter pylori*-induced gastric inflammation in Mongolian gerbils. *J. Ginseng Res*. **38:** 8-15.

2. Lee CH, Kim JH. 2014. A review on the medicinal potentials of ginseng and ginsenosides on cardiovascular diseases. *J. Ginseng Res*. **38:** 161-166.

3. Park HJ, Kim DH, Park SJ, Kim JM, Ryu JH. 2012. Ginseng in traditional herbal prescriptions. *J. Ginseng Res*. **36:** 225-241.

4. Christensen LP. 2008. Ginsenosides. Chemistry, biosynthesis, analysis, and potential health effects. *Adv. Food Nutr. Res*. **55:** 1-99.

5. Corbit RM, Ferreira JF, Ebbs SD, Murphy LL. 2005. Simplified extraction of ginsenosides from American ginseng (*Panax quinquefolius* L.) for high-performance liquid chromatography-ultraviolet analysis. *J. Agric. Food Chem*. **53:** 9867-9873.

6. Nguyen HT, Song GY, Kim JA, Hyun JH, Kang HK, Kim YH. 2010. Dammarane-type saponins from the flower buds of *Panax ginseng* and their effects on human leukemia cells. *Bioorg. Med. Chem. Lett*. **20:** 309-314.

7. Qu C, Bai Y, Jin X, Wang Y, Zhang K, You J, *et al*. 2009. Study on ginsenosides in different parts and ages of *Panax quinquefolius* L. *Food Chem*. **115:** 340-346.

8. Shi W, Wang Y, Li J, Zhang H, Ding L. 2007. Investigation of ginsenosides in different parts and ages of *Panax ginseng*. *Food Chem*. **102:** 664-668.

9. Hasegawa H. 2004. Proof of the mysterious efficacy of ginseng: basic and clinical trials: metabolic activation of ginsenoside: deglycosylation by intestinal bacteria and esterification with fatty acid. *J. Pharmacol. Sci*. **95:** 153-157.

10. Akao T, Kanaoka M, Kobashi K. 1998. Appearance of compound K, a major metabolite of ginsenoside Rb1 by intestinal bacteria, in rat plasma after oral administration − measurement of compound K by enzyme immunoassay. *Biol. Pharm. Bull*. **21:** 245-249.

11. Liu Y, Zhang JW, Li W, Ma H, Sun J, Deng MC, Yang L. 2006. Ginsenoside metabolites, rather than naturally occurring ginsenosides, lead to inhibition of human cytochrome P450 enzymes. *Toxicol. Sci*. **91:** 356-364.

12. Baek NI, Kim DS, Lee YH, Park JD, Lee CB, Kim SI. 1995. Cytotoxicities of ginseng saponins and their degradation products against some cancer cell lines. *Arch. Pharm. Res*. **18:** 164-168.

13. Popovich DG, Kitts DD. 2004. Generation of ginsenosides Rg3 and Rh2 from North American ginseng. *Phytochemistry* **65:** 337-344.

14. Park CS, Yoo MH, Noh KH, Oh DK. 2010. Biotransformation of ginsenosides by hydrolyzing the sugar moieties of ginsenosides using microbial glycosidases. *Appl. Microbiol. Biotechnol*. **87:** 9-19.

15. Siddiqi MZ, Siddiqi MH, Kim YJ, Jin Y, Huq MA, Yang DC. 2015. Effect of fermented red ginseng extract enriched in ginsenoside Rg3 on the differentiation and mineralization of preosteoblastic MC3T3-E1 cells. *J. Med. Food* **18:** 1-7.

16. Wang Y, Choi KD, Yu H, Jin F, Im WT. 2016. Production of ginsenoside F1 using commercial enzyme Cellulase KN. *J. Ginseng Res*. **40:** 121-126.

17. An DS, Cui CH, Lee HG, Wang L, Kim SC, Lee ST, *et al*. 2010. Identification and characterization of a novel *Terrabacter ginsenosidimutans* sp. nov. beta-glucosidase that transforms ginsenoside Rb1 into the rare gypenosides XVII and LXXV. *Appl. Environ. Microbiol*. **76:** 5827-5836.

18. Yoo DS, Rho HS, Lee YG, Yeom MH, Kim DH, Lee SL, *et al*. 2011. Ginsenoside F1 modulates cellular responses of skin Melanoma Cells. *J. Ginseng Res*. **35:** 86-91.

19. Lee EH, Cho SY, Kim SJ, Shin ES, Chang HK, Kim DH, *et al*. 2003. Ginsenoside F1 protects human HaCaT keratinocytes from ultraviolet-B-induced apoptosis by maintaining constant levels of Bcl-2. *J. Invest. Dermatol*. **121:** 607-613.

20. Ko SR, Choi KJ, Suzuki K, Suzuki Y. 2003. Enzymatic preparation of ginsenosides Rg2, Rh1, and F1. *Chem. Pharm. Bull*. **51:** 404-408.

21. Kim YS, Yoo MH, Lee GW, Choi JG, Kim KR, Oh DK. 2011. Ginsenoside F1 production from ginsenoside Rg1 by a purified β-glucosidase from *Fusarium moniliforme* var. *subglutinans*. *Biotechnol. Lett*. **33:** 2457-2461.

22. Wang D, Yu H, Song J, Xu Y, Jin F. 2012. Enzyme kinetics of ginsenosidase type IV hydrolyzing 6-*O*-multi-glycosides of protopanaxatriol type ginsenosides. *Process Biochem*. **47:** 133-138.

23. Wei Y, Zhao W, Zhang Q, Zhao Y, Zhang Y. 2011. Purification and characterization of a novel and unique ginsenoside Rg 1-hydrolyzing β-D-glucosidase from *Penicillium sclerotiorum*. *Acta Biochim. Biophys. Sin*. **43:** 226-231.

24. An DS, Cui CH, Sung BH, Yang HC, Kim SC, Lee ST, *et al*. 2012. Characterization of a novel ginsenoside-hydrolyzing α-L-arabinofuranosidase, AbfA, from *Rhodanobacter ginsenosidimutans* Gsoil 3054[T]. *Appl. Microbiol. Biotechnol*. **94:** 673-682.