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ORIGINAL PAPER

Isolation and characterization of novel ginsenoside-hydrolyzing glycosidase from *Microbacterium esteraromaticum* that transforms ginsenoside Rb2 to rare ginsenoside 20(*S*)-Rg3

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Abstract Ginsenoside Rb2 was transformed by recombinant glycosidase (Bgp2) into ginsenosides Rd and 20(*S*)-Rg3. The *bgp2* gene consists of 2,430 bp that encode 809 amino acids, and this gene has homology to the glycosyl hydrolase family 2 protein domain. SDS-PAGE was used to determine that the molecular mass of purified Bgp2 was 87 kDa. Using 0.1 mg ml^{-1} of enzyme in 20 mM sodium phosphate buffer at 40 °C and pH 7.0, 1.0 mg ml^{-1} ginsenoside Rb2 was transformed into 0.47 mg ml^{-1} ginsenoside 20(*S*)-Rg3 within 120 min, with a corresponding molar conversion yield of 65 %. Bgp2 hydrolyzed the ginsenoside Rb2 along the following pathway: Rb2 → Rd → 20(*S*)-Rg3. This is the first report of the biotransformation of ginsenoside Rb2 to ginsenoside 20(*S*)-Rg3 using the recombinant glycosidase.

Keywords Biotransformation · Glycosidase · Ginsenoside Rb2 · Ginsenoside 20(*S*)-Rg3

Introduction

Ginseng, the root of *Panax ginseng* C. A. Meyer, which belongs to the Araliaceae family, is a traditional herbal medicine that has been used for thousands of years in Asian countries for its various medicinal functions, including anti-inflammatory (Wu et al. 1992), anti-tumor (Mochizuki et al. 1995), anti-fatigue (Lee et al. 2005), anti-diabetic (Ni et al. 2010) and anti-cancer activities (Chae et al. 2009). Many of its medicinal effects are attributed to the triterpene glycosides that are also known as ginsenosides (Attele et al. 1999; Yuan et al. 2002). The production of deglycosylated ginsenosides, including F2, Rg3, Rh1, Rh2, F1, and compound K, has attracted great interest

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because these compounds are present at low concentrations or are absent in wild ginseng but have more profound pharmaceutical activities than glycosylated major ginsenosides Rb1, Rb2, Re, and Rg1 (Tawab et al. 2003). Ginsenoside Rb2 is one of the major components of ginseng, as it comprises 2–17 % of the total ginsenosides found in ginseng root (Hu and Kitts. 2001). Because ginsenoside Rb2 has an arabinopyranose moiety and three glucose moieties, hydrolyzing arabinopyranose and glucose moieties at C-20 can easily be transformed the ginsenoside Rb2 into ginsenoside 20(*S*)-Rg3.

The deglycosylated ginsenoside 20(*S*)-Rg3, which is absent in ginseng root, reportedly has anti-metastatic (Mochizuki et al. 1995), tumor-suppressing (Shinkai et al. 1996), hepatoprotective (Lee et al. 2005) and vasodilating effects (Kim et al. 2003). Its production has been achieved from major ginsenosides Rb1, Rb2, RC and Rd by using enzymatic methods including the use of purified β-glucosidase from *Paecilomyces Bainier* sp. 229 (Yan et al. 2008), lactase from *Penicillium* sp. (Ko et al. 2007) and crude enzyme from *Microbacterium* sp. GS514 (Cheng et al. 2008). However, these enzymes exhibited low selectivity and poor productivity, and the sequence has yet to be fully clarified. Recently, recombinant glycosidases have been used for ginsenoside Rb2 transformation (Lee et al. 2011; Noh and Oh 2009; Quan et al. 2012a), these mainly convert ginsenoside Rb2 to ginsenoside Rd, compound Y and compound K. But conversion of ginsenoside Rb2 to ginsenoside Rg3 has not yet been reported.

We report here the cloning and characterization of the novel glycosyl hydrolase family 2 ginsenoside-hydrolyzing glycosidase (Bgp2) for the biotransformation of ginsenoside Rb2. The enzyme is able to hydrolyze the outer and inner arabinopyranose and glucose moieties at the C-20 position of the ginsenoside Rb2, effectively converting it into ginsenosides Rd and 20(*S*)-Rg3.

Materials and methods

Materials

Isopropyl-β-D-thiogalactopyranoside (IPTG), *p*-nitrophenyl (*p*NP)-β-D-glucopyranoside, *p*NP-α-D-glucopyranoside, *p*NP-β-D-galactopyranoside, *p*NP-α-L-arabinofuranoside, *p*NP-α-L-arabinopyranoside, *o*-nitrophenyl (*o*NP)-β-D-glucopyranoside, and *o*NP-β-D-galactopyranoside were purchased from Sigma. The pure ginsenosides Rb1, Rb2, Rd, 20(*S*)-Rg3, 20(*S*)-Rh2, F2 and compound O were obtained from the ginseng genetic resource bank at Kyung-Hee University (Yongin, Korea).

Fosmid library construction and fosmid sequencing

Microbacterium esteraromaticum GS514(KACC 16318, Korean Agricultural Culture Collection, Suwon, Korea) was isolated from a ginseng field. Among the isolated strains, this strain transformed the major ginsenosides to minor ginsenosides time dependently with crude ginseng saponins. Based on the 16S rDNA gene sequences (GenBank accession number EU036992.1), it showed 98.7 % identity with *M. esteraromaticum* DSM 8609^T. To clone the ginsenoside-hydrolyzing glycosidase gene from *M. esteraromaticum*, a fosmid library was constructed using a CopyControl fosmid library production kit (Epicentre, Madison, WI, USA), according to the manufacturer's protocol. Infected *Escherichia coli* EPI300-T1^R transferred onto Luria–Bertani (LB) plates containing 12.5 μg ml^{-1} chloramphenicol and 27 μg ml^{-1} X-Glu, and incubated at 37 °C. After 16 h putative clones producing β-glucosidase were selected based on their blue color. A single clone putatively containing a ginsenoside β-glucosidase gene was selected by TLC assay for ginsenoside-hydrolyzing activity. The fosmid DNA was purified with a Fosmid MAX DNA purification kit (Epicentre). The transposon was inserted using a HyperMu KAN-1 insertion kit (Epicentre), according to the manufacturer's protocol. Sequences were assembled using the SeqMan program in the DNASTAR package (DNASTAR, Madison, WI, USA), yielding a 37.9 kb contig.

Molecular cloning, expression, and purification of recombinant glycosidase (Bgp2)

The assembled DNA sequence was analyzed using the ORF Finder program on the NCBI website (www.ncbi.nlm.nih.gov/gorf). Predicted ORFs were subjected to a similarity search using BLASTP, which identified a putative open reading frame of a glycosidase belonging to glycosyl hydrolase family 2. To clone this gene (*bgp2*), the gene was amplified from

the genomic DNA as a template by polymerase chain reaction using *Pfu* DNA polymerase. The *bgp2* gene (GenBank accession number JN852950) was amplified using the following primers (with *Nde*1 and *Eco*R1 restriction sites underlined): *bgp2*F (5′-CCC ATA TGC GAT CCC TGC CAC TGC TGA-3') and *bgp2*R (5′-GGA ATT CTC ATG AGG GGC TCA CCG TCA-3′). The amplified fragment was then digested with *Nde*1 and *Eco*R1 and inserted into pMAL-c5X in order to generate the maltose-binding protein (MBP)-*bgp2* gene fusion. The Bgp2 protein expression construct was verified by DNA sequencing. *E. coli* BL21 (DE3), which had been transformed with recombinant pMAL-*bgp2*, was grown in LB-ampicillin medium at 37 °C to an optical density of 0.4 at 600 nm (OD_{600}). Protein expression was induced by adding 0.5 mM of IPTG. Bacteria were incubated for an additional 9 h at 28 °C and harvested by centrifugation at 5,000×*g* for 30 min at 4 °C. The cells were washed twice with 20 mM sodium phosphate buffer (pH 7.0, 1 mM EDTA, and 1 mM NaCl) and then resuspended in 20 mM sodium phosphate buffer (pH 7.0). Cells were disrupted by five repetitions of 3-min of sonication at 1-s intervals on an ultrasonic processor that was set to a 70 % output and then allowed to cool on ice. Debris was removed by centrifugation (12,000×*g*, at 4 °C for 30 min). The crude extract from 5 ml of cell lysate was applied to the pre-equilibrated amylose column (5 ml, NEB, USA), followed by washing with 12 column volumes of elution buffer (20 mM sodium phosphate buffer pH 7.0, 1 mM EDTA, and 1 mM NaCl). The bound proteins were eluted with elution buffer supplemented with 10 mM maltose. The cleaved proteins were applied to the second amylose column, and Bgp2 was eluted with flow-through and purified. The protein homogeneity was assessed using a 10 % SDS–PAGE that was stained with Coomassie blue. Protein concentration was determined using the SMART BCA protein assay kit (Intron, Korea).

Enzyme characterization

The specific activity of purified Bgp2 was determined using *p*NP-α-L-arabinopyranoside as substrate in 20 mM sodium phosphate buffer, pH 7.0 at 37 °C. Reactions were stopped after 5 min by adding Na_2CO_3 to produce a concentration of 50 mM. The released *p*NP was immediately measured using a microplate reader at 405 nm. One unit of activity was defined as the amount of protein required to generate 1 μmol of *p*NP per min. The standard reaction to investigate the specific activity of purified Bgp2 and the effects of pH and temperature on the enzyme activity was performed as previously described (Quan et al. 2012b). Substrate preference was examined using 10.0 mM chromogenic *o*NP and *p*NP substrates at 40 °C for 5 min with one unit of enzyme activity defined as the release of 1 μmol of either *o*NP or *p*NP per min. The following substrates were tested: *p*NP-β-D-glucopyranoside, *p*NP-α-D-glucopyranoside, *p*NP-β-D-galactopyranoside, *p*NP-α-L-arabinofuranoside, *p*NP-α-L-arabinopyranoside, *o*NP-β-D-glucopyranoside, and *o*NP-β-D-galactopyranoside.

Enzymatic transformation of ginsenosides

In order to measure the enzymatic transformation of the ginsenosides, 0.1 mg ml^{-1} of the enzyme (Bgp2) dissolved in 20 mM sodium phosphate buffer (pH 7.0) was incubated with an equal volume of the ginsenosides Rb2, Rb1, Rd and compound O at 1 mg ml^{-1} in 20 mM sodium phosphate buffer (pH 7.0) at 40 °C. Samples were withdrawn at timed intervals. An equal volume of water-saturated *n*-butanol was added to each sample in order to stop the reaction. The *n*-butanol fraction was then evaporated to dryness, which allowed the dissolved methanol to be analyzed by TLC, HPLC, and LC/MS.

Analytical methods of ginsenosides

TLC was performed with silica gel plates ($60F_{254}$, Merck, Darmstadt, Germany), and the developing solvent was $CHCl_3$:CH_3OH:H_2O (65:35:10, *v*/*v*, lower phase). Spots were detected on the TLC plates by spraying with 10 % (*v*/*v*) H_2SO_4 and then heating to 110 °C for 10 min. The reaction mixture was extracted with *n*-butanol saturated with H_2O and evaporated in a vacuum. The residue was dissolved in methanol and analyzed by HPLC using a C_{18} (250 × 4.6 mm, particle size 5 μm) column with acetonitrile (solvent A) and distilled water (solvent B) as mobile phases at 85 % B for 5 min, 79 % B for 20 min, 42 % B for 55 min, 10 % B for 12 min, and 85 % B for 18 min, which were all collected at a rate of 1.6 ml min^{-1}. Detection was at 203 nm. In regard to the LC/MS, ginsenosides were analyzed using the

Agilent ESI–MS with negative polarity and an ion trap analyzer. The ion spray was operated at 5 l N_2/min, 3.5 kV, 25 psi, and 300 °C.

Results

Molecular cloning, expression, and purification of recombinant glycosidase (Bgp2)

Genomic DNA from *M. esteraromaticum* was extracted using the genomic DNA extraction kit. The insert of the positive clone was a 37.9-kb genomic fragment, and the nucleotide sequence revealed a total of 15 putative ORFs. An ORF was consisting of 2,430 bp encoding 809 amino acids, with homology to the protein domain of the glycosyl hydrolase family 2. This gene termed *bgp2* was amplified via PCR and then inserted into the pMAL-c5X vector and expressed in *E. coli*. Bgp2 is homologous to the glycosyl hydrolase family 2 based on its amino acid sequence similarity to glycosidases in *Modestobacter* sp. 42H12-1 (53 %, GenBank accession number YP_006366885), *Frankia* sp. CN3 (50 %, GenBank accession number ZP_09167636) and *Paenibacillus* sp. HPL-003 (48 %, GenBank accession number YP_005077324).

In order to maximize yield of the fusion protein, we tested the protein induction under different conditions. Induction with 0.5 mM IPTG at 28 °C for 9 h produced the maximum level of soluble active fusion enzyme. The MBP-Bgp2 fusion protein was purified from cell-free lysate by affinity chromatography using an amylose resin. Most of the fusion protein eluted within the first few fractions after the addition of maltose to the elution buffer. SDS–PAGE of the eluted protein fractions showed a single band of 129.5 kDa (Fig. 1). After digestion of the purified fusion protein

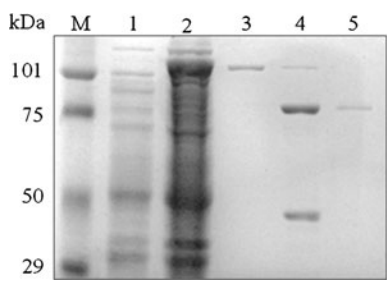

Fig. 1 Purification of recombinant Bgp2: *M* molecular mass marker, *lane 1* crude extract of uninduced BL21 (DE3) cells carrying pMAL-Bgp2, *lane 2* crude extract of induced recombinant BL21 (DE3) cells carrying pMAL-Bgp2, *lane 3* amylose affinity purified pMAL-Bgp2, *lane 4* factor Xa digested fusion protein showing Bgp2 and MBP, *lane 5* purified Bgp2 protein eluted from the second amylose column

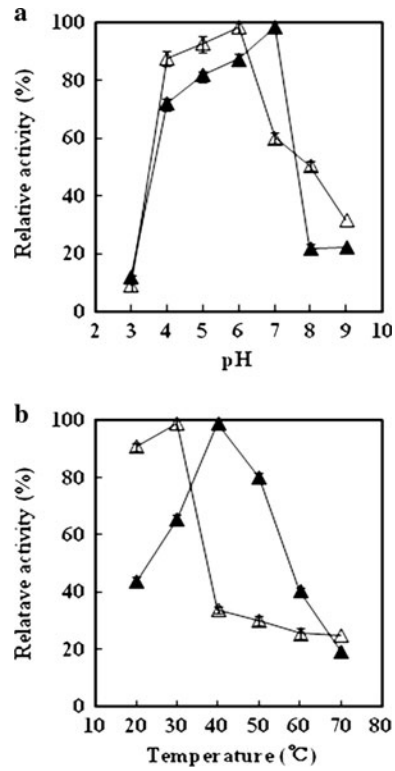

Fig. 2 Effect of pH and temperature on the stability and activity of recombinant Bgp2, which was determined using *p*NP-α-L-arabinopyranoside as a substrate. **a** Effect of pH on the stability and activity of recombinant Bgp2, which was determined using *p*NP-α-L-arabinopyranoside as a substrate. The following buffers (20 mM) were tested: glycine–HCl buffer (pH 3.0), citric acid-sodium citrate buffer (pH 4.0–5.0), sodium phosphate buffer (pH 6.0–7.0), and Tris–HCl buffer (pH 8.0–9.0). *Filled triangle* enzyme activity. Maximum activity was observed at pH 7.0 and was defined as 100 % = 35 U (mg protein)$^{-1}$. *Open triangle* stability. Enzyme activity was assayed under standard conditions (pH 7.0, 40 °C) after incubation at 40 °C for 4 h at different pH levels. The initial activity prior to incubation was defined as 100 %. **b** Effect of temperature on the stability and activity of recombinant Bgp2, which was determined using *p*NP-α-L-arabinopyranoside as a substrate. *Filled triangle* the thermo-dependence of enzyme activity was assayed in 20 mM sodium phosphate buffer (pH 7.0) at various temperatures ranging from 20 to 70 °C. Maximum activity was observed at 40 °C and was defined as 100 % = 35 U (mg protein)$^{-1}$. *Open triangle* thermostability was tested by incubating aliquots of the enzyme in 20 mM sodium phosphate buffer (pH 7.0) for 4 h at different temperatures

with the factor Xa protease, two protein bands appeared, which migrated with apparent molecular weights (Mw) of 87.0 and 42.5 kDa by SDS–PAGE, corresponding to Bgp2 and MBP, respectively (Fig. 1). These digested proteins were again purified by chromatography on a second amylose column, which bound the cleaved MBP tag, while unbound pure Bgp2 was collected in the flow-through. The Mw of the purified Bgp2 was 87.0 kDa as determined by SDS–PAGE (Fig. 1).

Enzyme characterization

The optimum pH for enzyme activity was pH 7.0 in 20 mM sodium phosphate buffer (Fig. 2a). The effect of pH on enzyme stability is shown in Fig. 2a. After incubation in solutions ranging in pH 4.0–7.0 at 40 °C for 4 h, the enzyme retained more than 60 % of its original activity. The optimal temperature for Bgp2 was 40 °C (Fig. 2b). The effect of temperature on enzyme stability is shown in Fig. 2b. After a 4 h incubation at pH 7.0 at 30 °C, the enzyme retained full activity. When the incubation temperature was increased to 40 °C, the enzyme retained only 40 % of its activity under the same conditions. The substrate specificity of Bgp2 was tested using 10.0 mM *p*NP and *o*NP glycosides with α and β configurations. The results, which are summarized in Table 1, demonstrate that Bgp2 was highly active for the *p*NP-α-L-arabinopyranoside and then *p*NP-β-D-glucopyranoside. The enzyme exhibited no activity toward *p*NP-α-D-glucopyranoside, *p*NP-α-L-arabinofuranoside, *p*NP-β-D-galactopyranoside, *o*NP-β-D-galactopyranoside or *o*NP-β-D-glucopyranoside.

Table 1 Relative activity of purified recombinant Bgp2 toward various chromogenic substrates as measured by *o*NP or *p*NP release at 40 °C

Substrate[a]	Relative activity ± SD (%)[b]
*p*NP-α-L-arabinopyranoside	100 ± 3.3
*p*NP-α-D-glucopyranoside	0
*p*NP-α-L-arabinofuranoside	0
*p*NP-β-D-galactopyranoside	0
*p*NP-β-D-glucopyranoside	23 ± 2.6
*o*NP-β-D-glucopyranoside	0
*o*NP-β-D-galactopyranoside	0

[a] Final concentration: 10.0 mM

[b] The relative enzyme activity against *p*NP-α-L-arabinopyranoside was assumed to be 100 %

Biotransformation pathway of ginsenoside Rb2 by Bgp2

The time-course experiment was performed and the hydrolyzed product was periodically analyzed by TLC and HPLC(Figs. 3a, 4). Hydrolysis of the ginsenoside Rb2 produced two different metabolites: ginsenoside Rb2 was transformed to metabolite 1 and then to

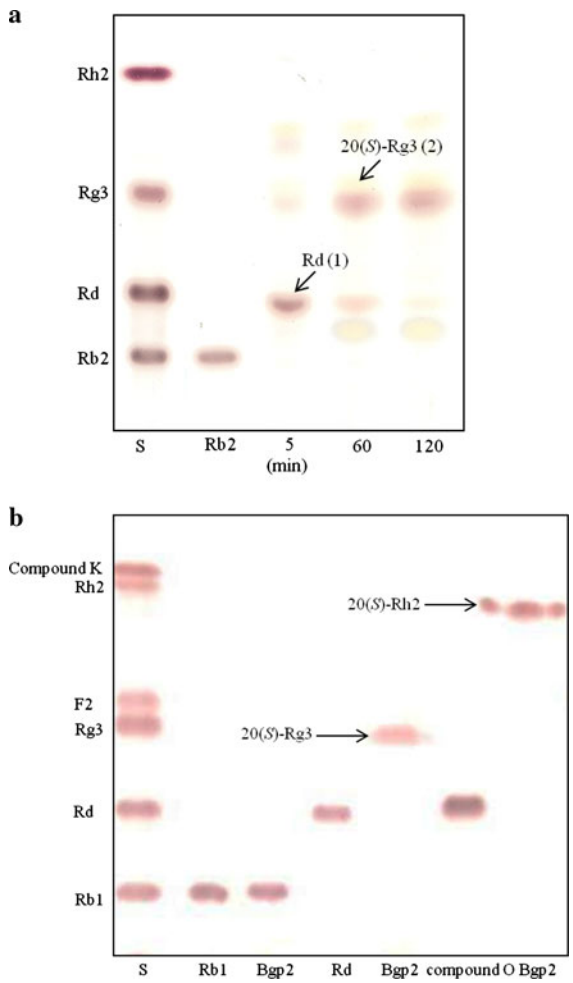

Fig. 3 TLC analysis of the transformation of ginsenosides. **a** TLC analysis of the time-course transformation of ginsenoside Rb2 by Bgp2. *S* standard, *Rd (1)* metabolites 1, *Rg3 (2)* metabolites 2. **b** Transformation of ginsenosides Rb1, Rd and compound O by Bgp2 under the optimum conditions of 40 °C and a pH 7.0. The reaction time was 6 h

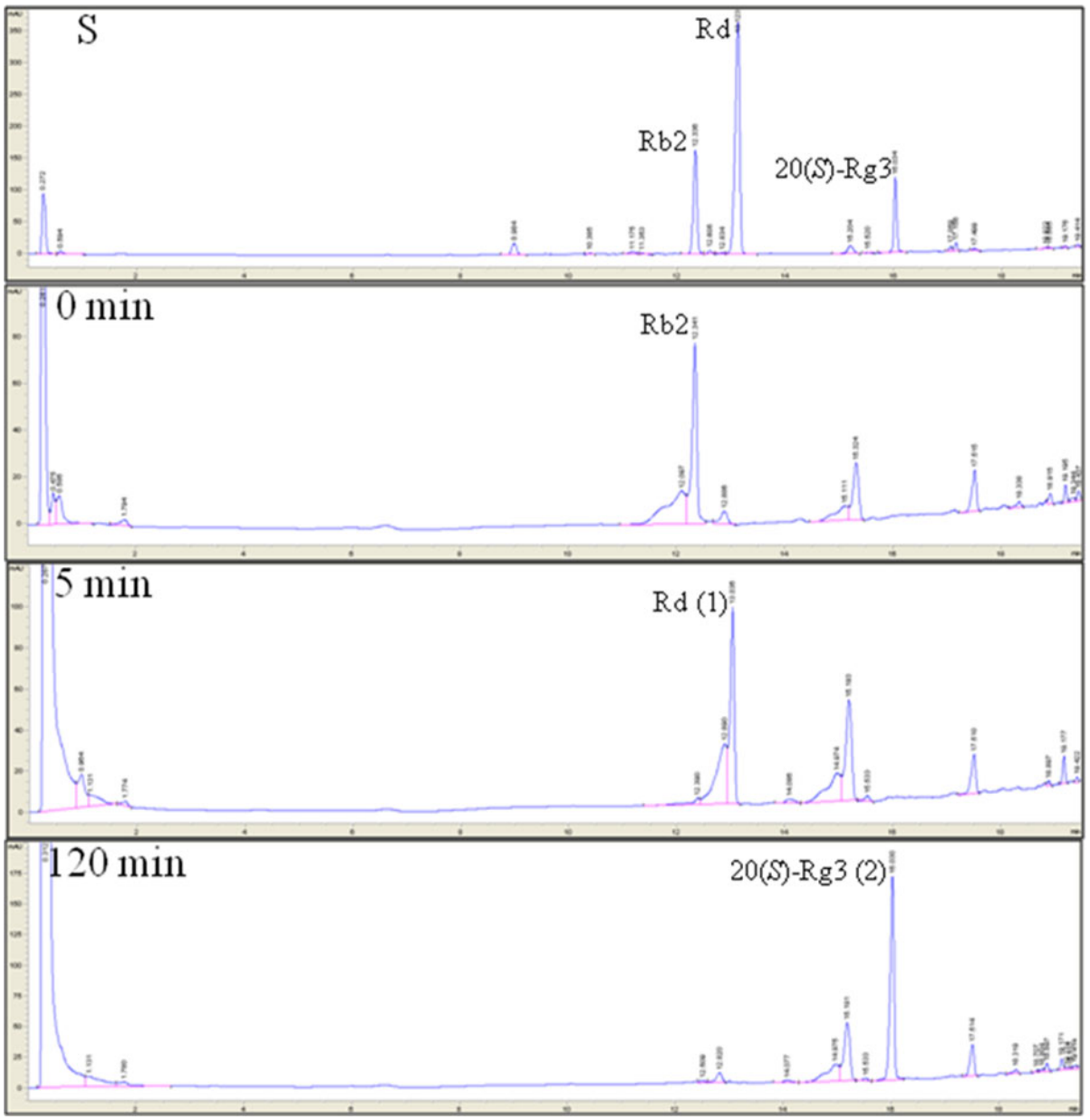

Fig. 4 HPLC analysis of the time course of the transformation of ginsenoside Rb2 by Bgp2. *S* ginsenoside standards, *Rd (1)* metabolite 1, 20(*S*)-Rg3 *(2)* metabolite 2

metabolite 2. By the end of the reaction, almost all of the ginsenoside Rb2 and metabolite 1 were transformed to metabolite 2. This indicates that metabolite 1 was an intermediate metabolite, and metabolite 2 was the final product. Based on a comparison of retention factor values (determined by TLC) and metabolite retention times (determined by HPLC) with those of standard ginsenosides, metabolites 1 and 2 were identified as ginsenosides Rd and 20(*S*)-Rg3, respectively.

Metabolites 1 and 2 from the transformation were subjected to LC/MS in order to determine their Mw. Metabolite 1 was obtained as a white powder and displayed a pseudomolecular ion peak [M + formic acid] at *m/z* 992 in the MS–ESI, which corresponds to the elemental formula $C_{48}H_{82}O_{18}$ (Mw, 947). Thus,

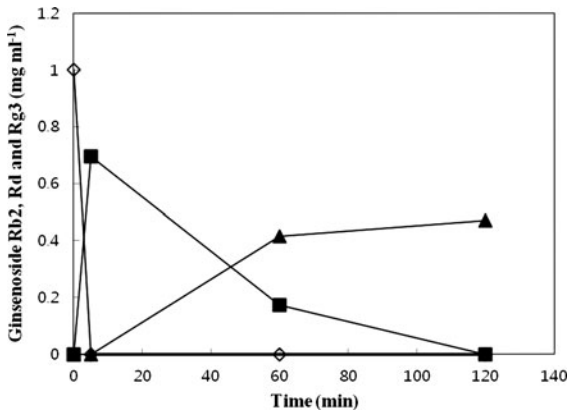

Fig. 5 Time course for ginsenosides Rd and 20(*S*)-Rg3 production from ginsenoside Rb2 by Bgp2 under optimum conditions at 40 °C and pH 7.0. Rb2 (*open diamond*) → Rd (*filled square*) → 20(*S*)-Rg3 (*filled triangle*)

metabolite 1 was confirmed as ginsenoside Rd (Supplementary Fig. 1a). The molecular formula of metabolite 2 was determined to be $C_{42}H_{72}O_{13}$ based on the protonated molecular ion peak [M + formic acid] at *m/z* 830 (Mw, 785) in the MS–ESI. This result confirmed that metabolite 2 was ginsenoside 20(*S*)-Rg3 (Supplementary Fig. 1b).

The conversion of ginsenoside Rb2 by Bgp2 was confirmed quantitatively by HPLC analysis. As shown in Fig. 5, 1.0 mg ml^{-1} ginsenoside Rb2 was transformed into 0.47 mg ml^{-1} ginsenoside 20(*S*)-Rg3 within 120 min, with a corresponding molar conversion yield of 65 %. Hence, Bgp2 hydrolyzed the ginsenoside Rb2 along the following pathway: Rb2 → Rd → 20(*S*)-Rg3 (Fig. 6).

To investigate whether Bgp2 had the same specificity and selectivity for glycone residues attached at the C-3 and C-20 positions of other PPD ginsenosides, the ginsenosides Rb1, Rd, and compound O were also used as substrates (Fig. 3b). Ginsenoside Rd was converted to ginsenoside 20(*S*)-Rg3 by hydrolysis of the glucose moiety at position C-20, while compound O was converted to ginsenoside 20(*S*)-Rh2 by hydrolysis of the outer and inner arabinopyranose and glucose moieties at the C-20 position. However, when ginsenoside Rb1 was used as the substrate, Bgp2 did not hydrolyze the outer glucose moiety at position C-20. These results indicate that Bgp2 has substrate specificity for PPD type ginsenosides, and that it has specific affinity to the outer arabinopyranose or inner glucose moiety at the C-20 position (Fig. 6).

Discussion

We report our findings regarding two recombinant β-glucosidases (*bgp1* and *bgp3*) from *M. esteraromaticum* that are able to effectively transform ginsenosides belonging to glycosyl hydrolase family 3: a *bgp1* gene that consists of 2,496 bp and encodes 831 amino acids (Quan et al. 2012c) and a *bgp3* gene that consists of 2,271 bp that encode 756 amino acids (Quan et al. 2012b). The glycosidase gene (*bgp2*) from *M. esteraromaticum* was cloned and expressed in *E. coli* BL21 (DE3). The enzyme (Bgp2) had optimal conditions for 40 °C and pH 7.0. Ginsenoside-hydrolyzing β-glucosidase (Bgp3) from *M. esteraromaticum* (Quan et al. 2012b), β-glycosidase from *Sulfolobus solfataricus* (Noh et al. 2009), purified β-glucosidase from *Paecilomyces Bainier* sp. 229 (Yan et al. 2008), and β-glucosidase (Bgp1) from *M. esteraromaticum* (Quan et al. 2012c) were optimal at pH 7.0, 5.5, 3.5 and 7.0 and at a temperature of 40, 90, 55 and 40 °C, respectively.

Ginsenoside Rb2 is one of the major PPD-type ginsenosides and accounts for 2–17 % of the total ginsenoside in ginseng root (Hu and Kitts. 2001). Because ginsenoside Rb2 has an arabinopyranose moiety and three glucose moieties, hydrolyzing arabinopyranose and glucose moieties can easily be transformed the ginsenoside Rb2 into rare ginsenosides. Based on an analysis of the hydrolysis products of ginsenoside Rb2, Bgp2 first hydrolyzed the outer arabinopyranose moiety attached to the C-20 position of ginsenoside Rb2 and then the inner glucose moieties at the C-20 position, thereby effectively converting ginsenoside Rb2 to ginsenosides Rd and 20(*S*)-Rg3 (Fig. 6). Recently, several genes of glycosidase that convert major ginsenosides into minor ginsenosides have been cloned and expressed in *Escherichia coli*. For example β-glucosidase (Bgp3 and Bgp1) from *M. esteraromaticum* transforms ginsenoside Rb2 into compound Y and compound K, ginsenoside Rb1 into compound K, and ginsenoside Rb1 into ginsenoside Rg3, respectively (Quan et al. 2012a, b, c). Thermophilic β-glycosidase from *S. solfataricus* transforms ginsenosides Rb1 or Rc to compound K (Noh et al. 2009), and β-glycosidase from *Sulfolobus acidocaldarius* transforms ginsenoside Rb1 into compound K, ginsenoside Rb2 into compound Y, and ginsenoside Rc into compound Mc, respectively (Noh and Oh 2009).

In the transformation of ginsenosides by β-D-glucosidase, the substrate has been used mostly

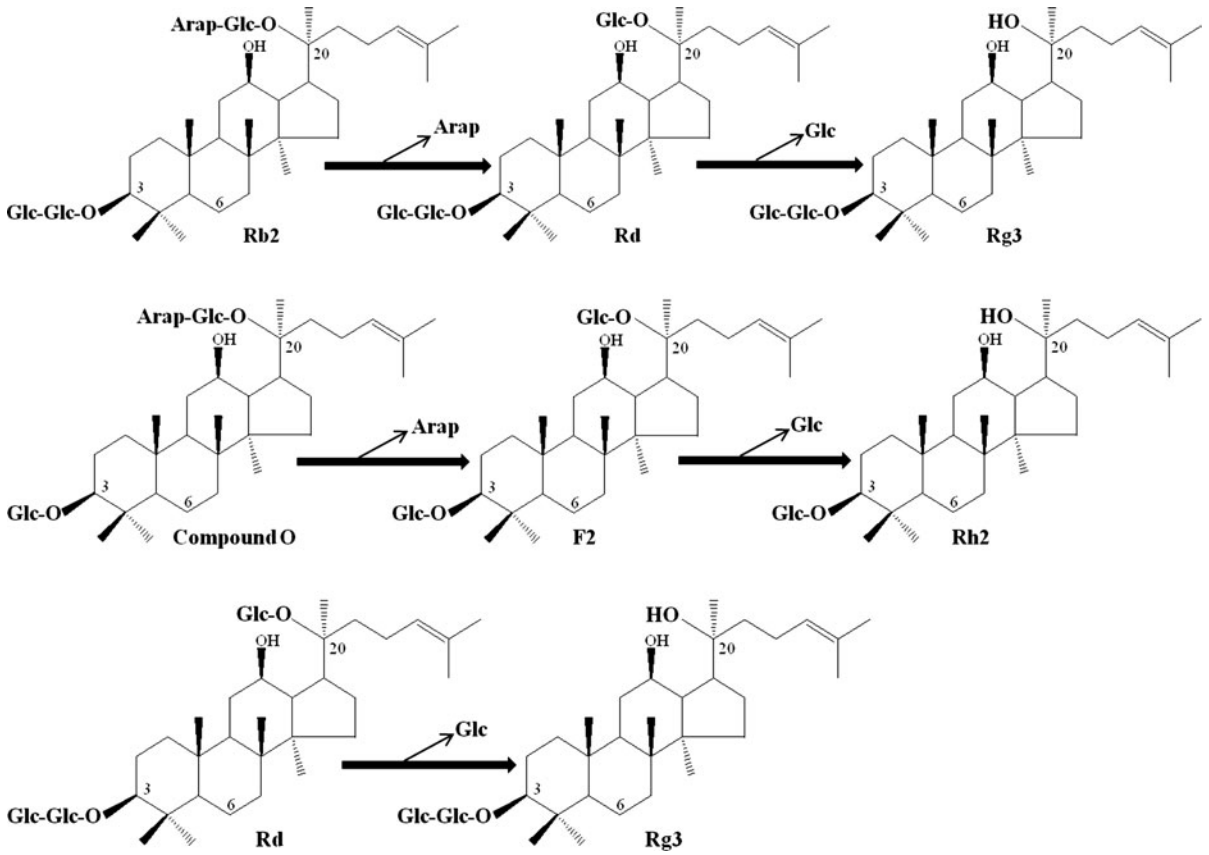

Fig. 6 Transformation pathway of ginsenosides Rb2, compound O and Rd by Bgp2. *Glc* β-D-glucopyranosyl and *Arap* α-L-arabinopyranosyl

ginsenoside Rb1 because ginsenoside Rb1 contains four molecules of glucose. The application of β-D-glucosidase to ginsenoside hydrolysis has serious limitation owing to little or no activity for major ginsenosides Rb2, Rc and Re which harbor L-arabinopyranoside, L-arabinofuranoside and L-rhamnose, respectively. To transform these major ginsenosides Rb2, Rc and Re effectively, the construction of multi-expression system of the genes encoding α-L-arabinopyranosidase, α-L-arabinofuranosidase and α-L-rhamnosidase with β-D-glucosidase gene is necessary. These multi-enzymes, which are obtained from multi-expression system, can be used in the transformation of ginsenosides via multiple-step processes.

In conclusion, a recombinant ginsenoside hydrolyzing-glycosidase (Bgp2) that belongs to the glycosyl hydrolase family 2 was constructed from *M. esteraromaticum* for the biotransformation of major ginsenosides. Bgp2 is able to convert the major ginsenosides Rb2 and Rd into rare ginsenoside 20(*S*)-Rg3 through the selective hydrolysis of the arabinopyranose and glucose moieties. This is the first study to report on the biotransformation of ginsenoside Rb2 to ginsenoside 20(*S*)-Rg3 using the recombinant glycosidase.

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