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ORIGINAL PAPER

Bioconversion of ginsenoside Rc into Rd by a novel α-L-arabinofuranosidase, Abf22-3 from *Leuconostoc* sp. 22-3: cloning, expression, and enzyme characterization

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Abstract A novel α-L-arabinofuranosidase (Abf22-3) that could biotransform ginsenoside Rc into Rd was obtained from the ginsenoside converting *Leuconostoc* sp. strain 22-3, isolated from the Korean fermented food kimchi. The gene, termed *abf*22-3, consisting of 1,527 bp and encoding a protein with a predicted molecular mass of 58,486 Da was cloned into the pMAL-c2x (TEV) vector. A BLAST search using the Abf22-3's amino acid sequence revealed significant homology to that of family 51 glycoside hydrolases. The over-expressed recombinant Abf22-3 in *Escherichia coli* BL21 (DE3) catalyzed the hydrolysis of the arabinofuranoside moiety attached to the C-20 position of ginsenoside Rc under optimal conditions of pH 6.0 and 30 °C. This result indicated that Abf22-3 selectively converts ginsenoside Rc into Rd, but did not catalyze the hydrolysis of glucopyranosyl groups from Rc or other ginsenosides such as Rb_1 and Rb_2. Over-expressed recombinant enzymes were purified by two steps with amylose-affinity and DEAE-cellulose chromatography and then characterized. The kinetic parameters for α-L-arabinofuranosidase showed apparent K_m and V_{max} values of 0.95 ± 0.02 μM and 1.2 ± 0.1 μmol min^{-1} mg of $protein^{-1}$ against *p*-nitrophenyl-α-L-arabinofuranoside, respectively. Using a purified MBP–Abf22-3 (10 μg/ml), 0.1 % of ginsenoside Rc was completely converted to ginsenoside Rd within 20 min.

Keywords Ginsenoside Rc · Biotransformation · α-L-Arabinofuranosidase · *Leuconostoc*

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Introduction

Ginsenosides are the major components in ginseng and are regarded as the main constituents responsible for the biological and pharmacological effects of ginseng (Kim and Park 2011; Lee et al. 2011; Sticher 1998). Thus far, more than 180 kinds of naturally occurring saponins have been isolated (Christensen 2009). However, ginsenosides contained in very small quantities in ginseng, called minor ginsenosides, are demanded because of their stronger anti-tumor, anti-wrinkle, anti-inflammatory, and anti-diabetic effects

as compared to major ginsenosides (Kim 2009; Choi et al. 2011). The production of large amounts of these minor ginsenosides from the major ginsenosides can be accomplished through a number of physio-chemical methods such as heating (Kim et al. 2000), acid treatment (Bae et al. 2004), and alkali treatment (Yun et al. 2001) or biotransformation methods such as fermentation using bacterial or fungal strains directly (Quan et al. 2011) and enzymatic methods (Liu et al. 2010; Wang et al. 2012; Yu et al. 2007, 2009). Because physio-chemical approaches produce non-specific racemic mixtures of rare ginsenosides, enzymatic methods have been explored in efforts to produce more specific minor ginsenosides (Rd, F_2, F_1, C–K) that cannot be produced by the former methods (Ko et al. 2000; Kim et al. 2006; Park et al. 2010).

To date, three types of glycoside hydrolases, β-D-glucosidase (Yan et al. 2008), α-L-arabinopyranosidase, and α-L-arabinofuranosidase (Shin et al. 2003), have been found to be involved in the biotransformation of protopanaxadiol type ginsenosides. Recently, several genes of glycosidase that convert major ginsenosides into minor ginsenosides have been cloned and expressed in *Escherichia coli*: (1) β-glucosidase from a soil metagenome transforms Rb_1 to Rd (Kim et al. 2007), (2) thermophilic β-glycosidase from *Sulfolobus solfataricus* transforms Rb_1 or Rc to C–K (Noh et al. 2009), (3) β-glycosidase from *Sulfolobus acidocaldarius* transforms Rb_1 into C–K, Rb_2 into C–Y and Rc into C–Mc, respectively (Noh and Oh 2009), (4) β-glucosidase from *Terrabacter ginsenosidimutans* Gsoil 3082^T could transform Rb_1 into C–K via Gypenoside XVII and Gypenoside LXXV (An et al. 2010), (5) β-glucosidase from *Sphingomonas* sp. 2F2 transforms Rb_1 to Gypenoside XVII, Rb_2 into C–O, Rc into C–Mc_1, and Rd into F_2 (Wang et al. 2011), and (6) α-L-arabinofuranosidase and α-L-arabinopyranosidase from *Bifidobacterium longum* H-1 transform Rc into Rd and Rb_2 into Rd, respectively (An et al. 2012; Lee et al. 2011).

Among the major ginsenosides, ginsenoside Rc comprises 3–16 % of the total ginsenosides in ginseng root or leaf (Qu et al. 2009; Shi et al. 2007), thus offering a sizable amount for conversion into Rd which is the important divergent position of the biotransformation of ginsenoside: Rd → $Rg_3(S)$ → $Rh_2(S)$ pathway or Rd → F_2 → compound K pathway (Fig. 1). One solution to this then is to obtain recombinant α-L-arabinofuranosidase to biotransform ginsenoside Rc into Rd with high activity.

Herein, we report the cloning and characterization of a novel ginsenoside-transforming α-L-arabinofuranosidase (Abf22-3) from *Leuconostoc* sp. 22-3, obtained from the Korean fermented food kimchi. This recombinant enzyme could efficiently catalyze the conversion of ginsenoside Rc to Rd.

Materials and methods

Chemicals, bacterial strains, vectors, and media

The ginsenosides Rb_1, Rc, Rd, F_2, and C–K were purchased from Nanjing Zelang Medical Technology

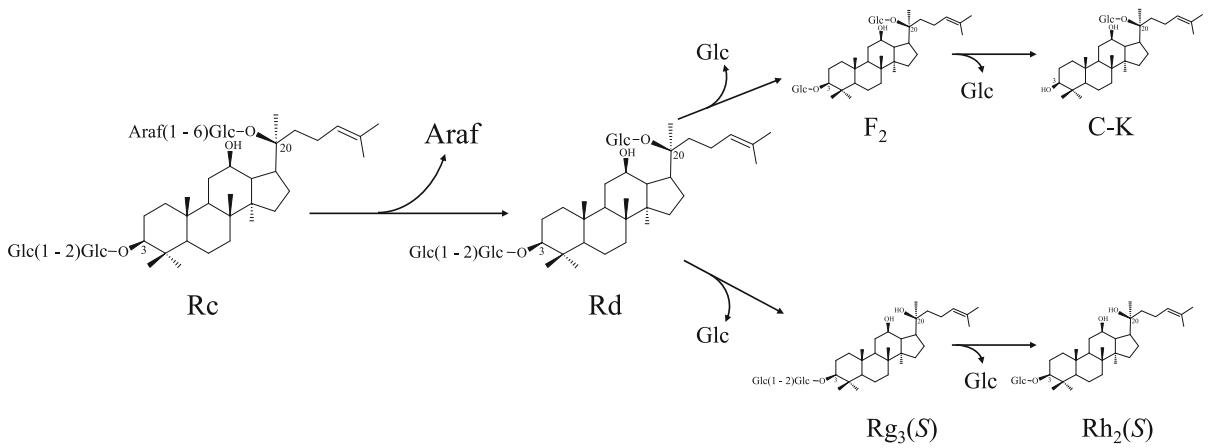

Fig. 1 Biotransformation pathway of ginsenoside Rc into Rd which is the important divergent position of the biotransformation of ginsenoside

Co., Ltd. (Nanjing, China). PPD mixture was kindly supplied by Prof. Jin in Dalian Polytechnic University in China. Chromogenic substrates for an enzyme activity assay were obtained from Sigma. *Leuconostoc* sp. 22-3, which has ginsenoside-hydrolyzing activity, was isolated from kimchi, a Korean fermented food, and cultivated on MRS agar (BD, USA) under aerobic conditions at 30 °C and used for the gene cloning experiment. *E. coli* BL21 (DE3) with pMAL-c2x (TEV) vector was used to express maltose binding protein (MBP) fused recombinant enzymes. All *E. coli* cells with plasmids were grown in LB broth (BD, USA) or on a LB agar plate at 37 °C supplemented with ampicillin (100 mg/l). The other chemicals used in this study were at least of analytical reagent grade, and the sources are noted individually in the methods section below.

Fosmid library construction and fosmid sequencing

A CopyControl™ Fosmid Production kit (Epicentre Technologies, WI) was used to clone the ginsenoside hydrolyzing glycosidase gene from *Leuconostoc* sp. 22-3. A fosmid library was constructed according to the manufacturer's protocol. Infected *E. coli* was transferred onto LB plates supplemented with 40 μg/ml X-Glc (5-bromo-4-chloro-3-indolyl β-D-glucopyranoside) and 12.5 μg/ml chloramphenicol and then incubated at 37 °C for 16 h. The blue color clones were selected as putative ginsenoside hydrolyzing clones. After confirmation of the ginsenoside-hydrolyzing activity by a TLC assay, one clone was selected for fosmid sequencing. Fosmid DNA was purified according to the manufacturer's protocols (Fosmid MAX DNA purification kit, Epicentre, WI) and was sequenced by Macrogen Co. Ltd (Korea). The final sequences assembly procedure was conducted by the SeqMan program in the DNASTAR package (DNASTAR, WI), yielding 35.5 kb contig.

Cloning, expression, and purification of recombinant Abf22-3

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 two putative open reading frames of a β-glucosidase belonging to glycosyl hydrolase family 1 and α-L-arabinofuranosidase belonging to glycosyl hydrolase family 51. To clone this gene (*abf22-3*), the gene was amplified by PCR using primers containing *Eco*RI and *Hin*dIII restriction sites, respectively, as shown in bold: abf22-3 F 5′-CG**G AAT TC**A AAG TTA AAA TAG AAA AAC GTC AAA ATG-3′ and abf22-3 R 5′-CCC **AAG CTT** CTA ACC TAA TAC AAT TTT GAA TAC AG-3′. The amplified fragment was digested with *Eco*RI and *Hin*dIII and inserted into a pMAL-c2x (TEV) vector.

Escherichia coli BL21(DE3) was transformed with the resulting recombinant pMAL-c2x-TEV-Abf22-3. BL21 (DE3) harboring the recombinant plasmid was grown in a LB-ampicillin medium at 37 °C until the culture reached an OD_{600} of 0.6, at which point protein expression was induced by the addition of 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Bacterial cells incubated further for 24 h at 18 °C were harvested by centrifugation at 10,000× rpm for 15 min at 4 °C. The cells were washed twice with a solution consisting of 50 mM sodium phosphate, 5 mM EDTA, and 1 % Triton X-100 (pH 7.0) and then resuspended in 50 mM sodium phosphate (pH 7.0). The cells were disrupted by ultrasonication (Vibra-cell, Sonics & Materials, CT) on ice at 5 min. Intact cells and debris were removed by centrifugation at 13,000× rpm for 30 min at 4 °C to obtain a crude cell extract. After washing the amylose resin column (GE Healthcare, USA) with 5 CV (column volume) of wash buffer (20 mM Tris–Cl, 1 mM EDTA, 1 mM DTT, 500 mM NaCl, pH 7.4), the supernatants was loaded on an amylose resin chromatography column. And the column was washed with 3 CV of wash buffer and 4 CV of binding buffer (20 mM Tris–Cl, 1 mM EDTA, 1 mM DTT, pH 7.4). MBP fusion protein was eluted with 2 CV of elution buffer (20 mM Tris–Cl, 1 mM DTT, 100 mM maltose, pH 7.4). The MBP tag was removed by incubation with TEV protease (Life Technologies, USA). After TEV treatment, the sample was loaded on DEAE-anion-exchange chromatography column (Whatman). The column was washed with 5 CV of 13 % buffer B (20 mM Tris–Cl, 1 mM EDTA, 1 mM DTT, 1000 mM NaCl, pH 7.4) and 87 % of buffer A (20 mM Tris–Cl, 1 mM EDTA, 1 mM DTT, pH 7.4). The bound protein was eluted with 2 CV of 30 % buffer B mixed with 70 % of buffer A. The homogeneity of the protein was assessed by 10 % SDS-PAGE and Coomassie blue staining.

Enzyme characterization and determination of kinetic parameters

The specific activity of purified Abf22-3 was determined using pNPAf (pNP-α-L-arabinofuranoside) as a surrogate substrate in 50 mM sodium phosphate buffer, pH 7.0 at 37 °C. Reactions were stopped after 5 min by the addition of Na_2CO_3 at a final concentration of 0.5 M, and the release of *p*-nitrophenol was measured immediately using a microplate reader at 405 nm (model 680; Bio-Rad, Hercules, CA). One unit of activity was defined as the amount of protein required to generate 1 μmol of *p*-nitrophenol per min. Specific activity was expressed as units per milligram of protein. Protein concentrations were determined using the bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL), with bovine serum albumin (Sigma) as the standard. All assays were performed in triplicate. The standard reaction to investigate the specific activity of purified Abf22-3 and the effects of pH, temperature, metals, and chemical reagents on the enzyme activity was performed as previously described (An et al. 2010; Wang et al. 2012). Kinetic studies were performed with freshly purified enzyme (20 μg/ml) using 0.1 mM to 2 mM pNPAf as substrates. For the former, the absorbance of *p*-nitrophenol was monitored at 405 nm for 20 min at 37 °C. The obtained data were used to determine K_m and V_{max} using the enzyme kinetics program reported by Cleland (1979).

Biotransformation ability of recombinant Abf22-3

Initial biotransformation experiments using ginsenoside Rc as a substrate revealed that the presence of MBP fused to Abf22-3 did not affect the enzyme activity. Therefore, the MBP fusion protein was used to determine the specificity and selectivity of Abf22-3 for the hydrolysis of arabinofuranosyl moiety attached at the C-20 positions of ginsenoside Rc. Purified recombinant Abf22-3 (10 μg/ml) in 50 mM sodium phosphate buffer (pH 7.0) was reacted with an equal volume of Rc solution at a concentration of 0.1 % (w/v) in 50 mM sodium phosphate buffer (pH 6.0) at 37 °C. Also, the conversion of a 50 mg/ml concentration PPD mixture (protopanaxadiol type ginsenosides) (comprised of Rb_1: 56.0 %, Rc: 16.0 %, Rb_2: 2.8 %, Rb_3: 4.9 %, Rd: 14.9 %) solution was investigated for the application of an industrial scale usage reacted with 10 μg/ml soluble purified MBP–Abf22-3 in 50 mM sodium phosphate buffer (pH 6.0) at 37 °C. Samples were withdrawn at regular intervals. For TLC analyses, an equal volume of water-saturated *n*-butanol was added to stop the reaction, and the reactant present in the *n*-butanol fraction was analyzed after pretreatment. For HPLC analyses, an equal volume of methanol was added to stop the reaction. After centrifugation at 13,000× rpm for 10 min, the supernatant was used for analyses.

Analytical methods of ginsenosides

The TLC analysis was performed using $60F_{254}$ silica gel plates (Merck, Germany) with $CHCl_3$–CH_3OH–H_2O (65:35:10, v/v, lower phase) as the developing solvent. The spots on the TLC plates were detected by spraying with 10 % (v/v) H_2SO_4 followed by heating at 110 °C for 5 min. A HPLC analysis of the ginsenosides was performed using an HPLC system (Younglin Co. Ltd, Korea), 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 32 % solvent A and 68 % solvent B and was changed to: A from 32 to 65 %; 0–8 min, A from 65 to 100 %; 8–12 min, A 100 %; 12–15 min, A from 100 to 32 %; 15–25 min. The flow rate was 1.0 ml/min, and detection was performed by monitoring absorbance at 203 nm and with an injected volume of 25 μl.

Nucleotide sequence accession number

The sequences for *abf22-3* gene from *Leuconostoc* sp. 22-3 were deposited into GenBank/EMBL/DDBJ under accession number JQ065683.

Results and discussion

Fosmid library construction and cloning of *abf22-3*

An α-L-arabinofuranosidase gene was isolated from a ginsenoside-hydrolyzing bacterium, *Leuconostoc* sp. 22-3, isolated from the Korean fermented food kimchi. Since strain 22-3 converted ginsenosides Rb_1, Rb_2, and

Rc into $Rg_3(S)$ via Rd (data not shown), it could have three types of glycoside-hydrolase activity: β-D-glucosidase, α-L-arabinopyranosidase, and/or α-L-arabinofuranosidase. To isolate and clone the respective genes for the ginsenoside-hydrolyzing enzymes from strain 22-3, we created a fosmid library, screened a clone containing the gene encoding the enzymatic activity, and determined the full sequence of the fosmid vector. An ORF FINDER (www.ncbi.nlm.nih.gov/gorf) analysis of the full sequence of the 35.5 kb clone insert revealed that it encoded a total of 17 putative ORFs of longer than 300 codons (data not shown). One of the ORFs was a homologue of glycoside hydrolase genes in glycoside hydrolase family 51. This ORF, termed *abf*22-3 of 1,527 bp in length, was homologous to α-L-arabinofuranosidase of *Leuconostoc mesenteroides* subsp. *mesenteroides* ATCC 8293^T of glycoside hydrolase family 51 (99 % similarity). The putative α-L-arabinofuranosidase was amplified by PCR, and then subcloned into the pMAL-c2x (TEV) vector to generate MBP-fused Abf22-3 under the control of an IPTG-inducible promoter.

Expression and purification of recombinant Abf22-3

The MBP–Abf22-3 was expressed in *E. coli* BL21 (DE3). To maximize the yield of the fusion protein in a soluble form, we tested different induction conditions and found that induction with 0.1 mM IPTG at 18 °C for 24 h cultivation produced the half level of soluble active fusion enzyme (Fig. 2). The MBP–Abf22-3 fusion protein purified by amylose-affinity column chromatography was digested by TEV protease at room temperature for 4 h to remove the MBP tag, and the resulting Abf22-3 was further purified by chromatography on DEAE-cellulose and the single band was determined by SDS-PAGE (Fig. 2). This procedure resulted in 82-fold purification of Abf22-3 and a recovery of 33 % from crude extract (Table 1). Furthermore, the results of SDS-PAGE agreed with size estimates based on the translated polypeptide sequence (58,486 Da).

Enzyme characterization

Abf-22-3's optimum pH was 6.0 in potassium phosphate buffer (Fig. 3a). The enzyme retained over 78 % of its optimal activity at pH 7.0; it exhibited residual

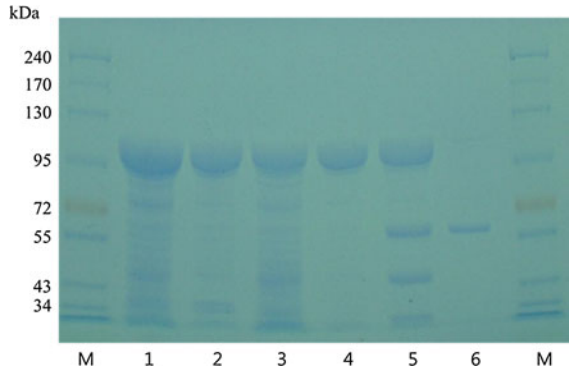

Fig. 2 SDS-PAGE analysis of recombinant Abf22-3. *Lanes*: *M* molecular mass standard, *1* crude extract of BL21 carrying pMAL-c2x-TEV-Abf22-3 after adding IPTG, *2* insoluble fraction of crude extract after sonication, *3* soluble fraction of crude extract after sonication, *4* MBP–Abf22-3 enzyme fraction after amylose column, *5* MBP–Abf22-3 enzyme after treat with TEV, *6* recombinant Abf22-3 fraction after DEAE cellulose column chromatography

activity at pH 5.0, 6.0, 7.5, 9.0, and 10.0 and no activity below pH 4.0. The enzyme activity culminated at 30, 37, and 45 °C. The enzyme was stable at temperatures lower than 37 °C, but lost activity at temperature higher than 37 °C within 2 h (Fig. 3b). The effects of metal ions, EDTA, β-mercaptoethanol, and SDS on Abf-22-3 activity were also investigated (Table 2). Abf22-3 activity was not affected by β-mercaptoethanol, which is well known thiol group inhibitors. These results suggested that sulfhydryl groups may not be involved in the catalytic center of the enzyme, but rather may be essential for maintenance of the three-dimensional structure of the active protein. The enzyme did not require Mg^{2+} for activity and was enhanced by 1 mM Na^+, K^+, Mg^{2+}, Mn^{2+}, Ca^{2+}, Zn^{2+}, and Co^{2+}. However, Abf22-3 activity was significantly inhibited by 10 mM Zn^{2+}, Cu^{2+}, and SDS. The chelating agent EDTA did not inhibit Abf22-3 activity, which indicated that divalent cations are not required for enzymatic activity. Enzymatic activity was markedly inhibited in the presence of 10 mM SDS. The substrate specificity of Abf-22-3 was tested using 1 mM PNP- and ONP-glycosides with α and β configurations described by An et al. (2010). The results showed that the enzyme was only active against pNPAf; the other substrates, including pNP-β-D-glucopyranoside, pNP-β-D-galactopyranoside, pNP-β-D-fucopyranoside, pNP-*N*-acetyl-β-D-glucosaminide, pNP-β-L-arabinopyranoside, pNP-β-D-man-

Table 1 Purification scheme of Abf22-3

Purification steps	Total protein (mg)	Specific activity (U/mg)	Total activity (U[a])	Purification (Fold)	Yield (%)
Crude extract	1620.0	0.3	502.2	1.0	100
Amylose	35.3	11.2	395.6	36.1	79
DEAE-cellulose	6.4	25.5	163.8	82.2	33

[a] One unit (U) of α-L-arabinofuranosidase was defined as the of enzyme liberating 1 μmol/min of *p*-nitrophenol

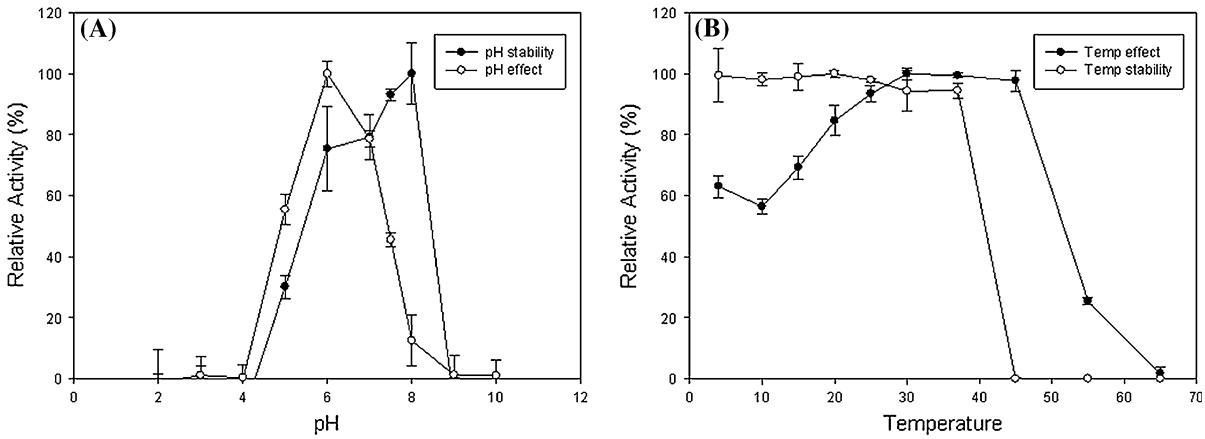

Fig. 3 Effects of pH (**a**) and temperature (**b**) on the stability and activity of recombinant Abf22-3

Table 2 Effect of metals and other chemicals on the activity of recombinant Abf22-3

Metal ion or reagent	Relative activity ± SD (%)[a]	
	1 mM	10 mM
NaCl	120.3 ± 1.07	95.6 ± 1.45
KCl	131.6 ± 2.77	103.0 ± 1.28
$MgCl_2$	121.6 ± 2.19	107.8 ± 2.32
$MnCl_2$	127.9 ± 3.24	116.2 ± 2.83
$CaCl_2$	125.0 ± 2.69	101.6 ± 1.60
$ZnCl_2$	131.0 ± 2.15	63.5 ± 1.07
$CoCl_2$	126.9 ± 2.27	115.7 ± 1.96
$CuCl_2$	102.8 ± 1.63	22.1 ± 1.28
SDS	97.1 ± 3.55	19.5 ± 1.47
EDTA	102.1 ± 0.63	101.4 ± 1.43
β-Mercaptoethanol	100.8 ± 0.26	95.8 ± 2.97
Control	100.0 ± 0.92	100.0 ± 1.75

[a] The specific activity at 100 % was 44.3 U/mg protein

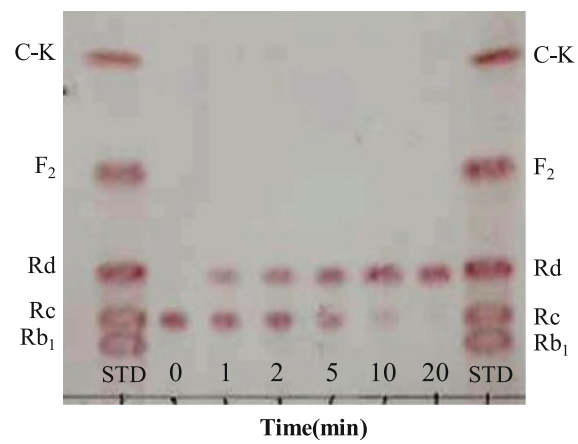

Fig. 4 Time-course thin layer chromatography (TLC) analyses of ginsenoside Rc by MBP–Abf22-3 at an enzyme concentration of 10 μg/ml and ginsenoside Rc concentration of 1.0 mg/ml. *Lanes*: *STD* standard; *0–20* reaction time (min)

nopyranoside, pNP-β-D-xylopyranoside, pNP-α-D-glucopyranoside, pNP-α-L-arabinopyranoside, pNP-α-L-rhamnopyranoside, pNP-α-D-mannopyranoside, pNP-α-D-xylopyranoside, oNP-β-D-glucopyranoside, oNP-β-D-galactopyranoside, oNP-β-D-fucopyranoside, and oNP-α-D-galactopyranoside, were not hydrolyzed. The K_m and V_{max} for the hydrolysis of pNPAf by α-L-arabinofuranosidase were 0.95 ± 0.02 μM and 1.2 ± 0.1 mmol min^{-1} mg of $protein^{-1}$, respectively.

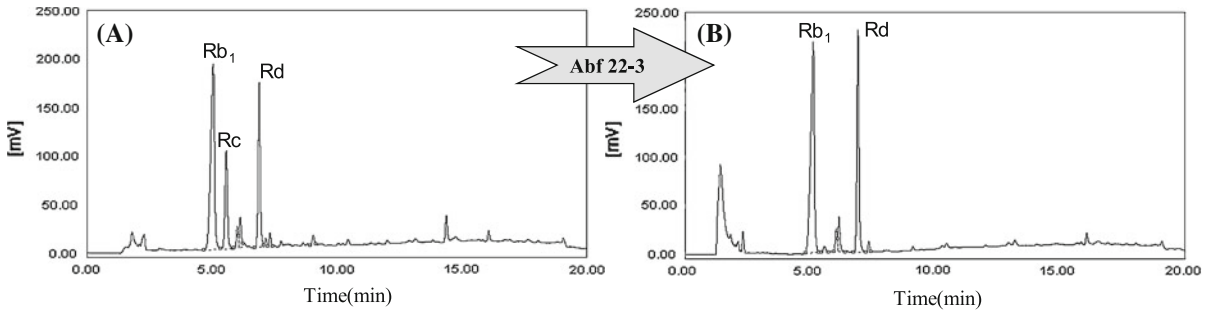

Fig. 5 HPLC analysis results of transformation of PPD mixture by MBP–Abf22-3. **a** Chromatogram of PPD mixture; **b** chromatogram of reaction mixture after 12 h

Biotransformation of ginsenoside Rc

For verification of the bioconversion of ginsenoside Rc into Rd by MBP–Abf22-3, TLC and HPLC analyses were carried out at regular intervals. As shown Fig. 4, it is clear that MBP–Abf22-3 could completely transform the ginsenosides Rc into ginsenoside Rd within 20 min at a concentration of 0.1 % (w/v) ginsenoside Rc solution reacted with 10 μg/ml soluble purified MBP–Abf22-3 in 100 mM sodium phosphate buffer (pH 6.0) at 37 °C. As shown in Fig. 5, the HPLC chromatogram of a PPD mixture comprised of major ginsenosides (Rb_1: 56.0 %, Rc: 16.0 %, Rb_2: 2.8 %, Rb_3: 4.9 %, Rd: 14.9 %) was changed because of biotransformation caused by MBP–Abf22-3. 95 % of ginsenoside Rc was converted to Rd within 12 h and over 99.5 % of Rc was converted to Rd after 24 h. These HPLC results indicated that Abf22-3 selectively converts ginsenoside Rc into Rd, but does not catalyze the hydrolysis of glucopyranosyl groups of Rc or other ginsenosides such as Rb_1 and Rb_2, Rb_3, and Rd. Consequently, the enzyme can produce ginsenoside Rd at a rate of 80 mg ml^{-1} day^{-1} mg of $protein^{-1}$ transforming ginsenoside Rc.

Because ginsenoside Rc is one of the major PPD-type ginsenosides and accounts for 3–16 % of the total ginsenoside in ginseng root or hair (Qu et al. 2009; Shi et al. 2007), a sizable amount that can potentially be exploited for the production of minor ginsenosides by biotransformation methods. Furthermore, ginsenoside Rd is a very important divergent position of the biotransformation of ginsenosides: Rd → $Rg_3(S)$ → $Rh_2(S)$ pathway or Rd → F_2 → compound K pathway generated by hydrolysis of the terminal or inner glucose moiety attached to the C3 or C20 carbon of ginsenoside Rd using β-glucosidase (An et al. 2010; Wang et al. 2012; Quan et al. 2012). Thus, biotransformation of ginsenoside Rc to Rd by recombinant Abf22-3 finally would lead to the transformation of ginsenoside Rc to its most deglycosylated form, namely F_2, $Rg_3(S)$, C–K or $Rh_2(S)$, if appropriate β-glucosidase is used in combination with it.

In conclusion, we reported on the cloning of an enzyme that selectively produces Rd through the biotransformation of the major ginsenoside Rc using a recombinant enzyme characterized as α-L-arabinofuranosidase belonging to glycoside hydrolase family 51. The enzyme has optimal reaction conditions in pH 6.0 at 30 °C. Under these optimum conditions, the enzyme showed nearly a 100 % conversion rate and productivity of 80 mg ml^{-1} day^{-1} mg of $protein^{-1}$ to convert ginsenoside Rc into Rd by selectively hydrolyzing outer arabinofuranoside moiety at the C20 position. Biotransformed ginsenoside Rd and other minor ginsenosides derived from it would be adopted in the cosmetic and pharmaceutical industry.

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