Food Chemistry 141 (2013) 1369–1377

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Mass production of the ginsenoside $Rg_3(S)$ through the combinative use of two glycoside hydrolases

Jin-Kwang Kim [a,d], Chang-Hao Cui [b], Qingmei Liu [a,c], Min-Ho Yoon [d], Sun-Chang Kim [a,b,c], Wan-Taek Im [a,*]

[a] KAIST Institute for Biocentury, Korea Advanced Institute of Science and Technology, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea
[b] Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea
[c] Intelligent Synthetic Biology Center, 373-1, Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea
[d] Department of Bio-Environmental Chemistry, College of Agriculture and Life Sciences, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764, Republic of Korea

ARTICLE INFO

Article history:
Received 15 August 2012
Received in revised form 1 March 2013
Accepted 4 April 2013
Available online 18 April 2013

Keywords:
Ginsenoside $Rg_3(S)$
β-Glucosidase
α-L-arabinofuranosidase
Biotransformation
Mass production

ABSTRACT

The ginsenoside $Rg_3(S)$, which is one of the exceptional components of Korean red ginseng extract, has been known to have anti-cancer, anti-metastatic, and anti-obesity effects. An enzymatic bioconversion method was developed to obtain the ginsenoside $Rg_3(S)$ with a high specificity, yield, and purity. Two glycoside hydrolases (BglBX10 and Abf22-3) were employed to produce $Rg_3(S)$ as a 100 g unit. The conversion reaction transformed ginsenoside Rc to Rd using Abf22-3, followed by Rb_1 and Rd to $Rg_3(S)$, using BglBX10. It was performed in a 10 L jar fermenter at pH 6.0 and 37 °C for 24 h, with a high concentration of 50 mg/ml of purified ginsenoside mixture obtained from ginseng roots. Finally, 144 g of $Rg_3(S)$ was produced from 250 g of root extract with 78 ± 1.2% chromatographic purity. These results suggest that this enzymatic method would be useful in the preparation of ginsenoside $Rg_3(S)$ for the functional food and pharmaceutical industries.

1. Introduction

Ginseng is a greatly valued herb in Asian countries and it is one of the most prominent medicinal plants in the world (Attele, Wu, & Yuan, 1999). It has been used for thousands of years to heal diseases, provide nutrition, and to stay healthy (Qi, Wang, & Yuan, 2011). The ginseng root consists of ginsenosides, polysaccharides, peptides, polyacetylenic alcohols, and fatty acids (Lee, 1992). Ginsenosides are the major active components in terms of the biological and pharmacological effects of ginseng (Park, Rhee, & Lee, 2005). In general, the ginsenoside family exhibits a variety of pharmaceutical functions: immunomodulatory, anti-tumour, anti-carcinogenic, anti-inflammatory, anti-allergic, anti-atherosclerotic, anti-stress, anti-diabetic, anti-proliferation, anti-genotoxic, and anti-hypertensive functions, as well as neurotransmission modulation effects (Jia & Zhao, 2009; Sun, 2004).

In particular, ginsenoside Rg_3 has anti-cancer and various pharmacological effects that have been proven to inhibit lung metastasis. This anti-metastatic effect was associated with the inhibition of the invasion and adhesion by tumour cells, as well as the suppression of tumour-induced angiogenesis (Mochizuki et al., 1995). Furthermore, the ginsenoside Rg_3 is known to have an anti-tumour effect, possibly mediated through the down-regulation of the NF-κB and AP-1 transcription factors (Keum et al., 2003). In another study, it was reported that Rg_3 decreased intracellular calcium levels (Iishi et al., 1997) and it was also shown to reduce the cytotoxicity in colon cancer, dependent on several mechanisms, including apoptosis (Lee et al., 2009). In other studies, Rg_3 was reported to significantly inhibit the growth and angiogenesis of ovarian cancer when used alone or combined with cyclophosphamide (Xu, Xin, Cui, Jiang, & Gu, 2007), and it was also demonstrated to inhibit the proliferation of prostate cancer cells (Kim et al., 2004).

It is also known to inhibit the opening of MPTP through free radical-scavenging action in the brain, and this implies that the inhibition of MPTP may contribute to its neuroprotective effect (Tian, Zhang, Li, Liu, & Xu, 2009). Ginsenoside Rg_3 is known to have anti-obesity effects involving the AMPK signalling pathway and PPAR-γ inhibition (Hwang et al., 2009). These effects demonstrate the use of ginsenoside Rg_3 as a pharmaceutically active compound.

20(*S*)-Ginsenoside Rg_3 [hereafter $Rg_3(S)$] is a native ginsenoside found in Korean ginseng (*Panax ginseng*, C.A. Meyer), but the amount in Korean ginseng is relatively low (Choi, 2008). Ginsenoside $Rg_3(S, R)$ is an exceptional component of Korean red ginseng that is generated during heating and mild acid treatment processes (Ko et al., 2005). Korean red ginseng has evolved over hundreds of

* Corresponding author. Tel.: +82 42 3504450; fax: +82 42 3504451.
E-mail address: wandra@kaist.ac.kr (W.-T. Im).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.04.012

years as a result of conservation and the increased awareness of its pharmaceutical effects (Nam, 2005). Korean red ginseng is produced through the process of steaming raw ginseng at 96–98 °C for 18 h (In, Kim, Lee, Park, & Yang, 2006). Then, more pharmacological minor ginsenosides, such as Rg_3, Rg_2, Rh_1, Rk_1, Rg_5, Rg_4, and Rg_6, are produced through the loss of the glycosyl moieties at the C20 position of the major ginsenosides (Rg_1, Re, Rb_1, Rb_2, Rc, and Rd). Thus, $Rg_3(S, R)$ can be easily produced through the weak acid-high temperature process, i.e. the red ginseng process. However, this process creates by-products, such as $Rg_3(R)$, Rk_1, and Rg_5. Consequently, $Rg_3(S)$ has a low concentration in Korean red ginseng (Han et al., 1982; Park, 2004).

Another method of obtaining $Rg_3(S)$ is to use microbes or enzymatic processes. One example is *Microbacterium* sp. strain GS514 which has β-glucosidase activity and shows a strong ability to convert ginsenoside Rb_1 or Rd into $Rg_3(S)$ (Cheng, Na, Bang, Kim, & Yang, 2008). The other is commercial Cellulase-12T which could biotransform ginsenoside saponin of white ginseng extract into $Rg_3(S)$ by more than 2.7 times that of untreated white ginseng extract (Chang et al., 2009). Until now, several ginsenoside-hydrolysing recombinant enzymes have been constructed, but the majority of these only have hydrolysis ability for the outer glucose moiety at the C6 position of the aglycone. Only one enzyme has the same ability as BglBX10 (Quan, Min, Yang, Kim, & Yang, 2012). However, Quan et al. conducted a simple enzyme characterisation, without further scale-up or process engineering. Thus, the experiment in this study was designed to meet the industrial need, which is the mass production of ginsenoside $Rg_3(S)$, in order to fulfil the original purpose of application as a recombinant enzyme.

In the present study, an enzymatic method for deglycosylation was used to convert the major ginsenosides present in prepared ginseng root extracts into $Rg_3(S)$ with a high efficiency and purity. In order to achieve this objective, two glycoside hydrolases [BglBX10 (recombinant β-glucosidase from *Flavobacterium johnsoniae* UW101^T) and Abf22-3 (recombinant α-L-arabinofuranosidase from *Leuconostoc* sp. 22-3)] were used. After determining the optimum conditions in terms of the pH, temperature, and substrate specificities of the recombinant BglBX10, the scaled-up production of $Rg_3(S)$ was performed, in order to convert ginsenoside Rc to Rd, using Abf22-3, followed by the conversion of Rb_1 and Rd to $Rg_3(S)$, using BglBX10 (Fig. 1). This is the first report to produce a 100 g unit scale production of $Rg_3(S)$, using two recombinant glycoside hydrolases in series.

2. Methods

2.1. Materials

The PPD-type ginsenosides mixture (PPDGM) from the root of *Panax quinquefolius* (comprised of Rb_1: 53.8%, Rc: 15.8%, Rb_2: 2.8%, Rb_3: 4.8%, Rd: 16.7%, $Rg_3(S)$: 1.7% and other ginsenosides: 4.4%, based on mole ratio) from Hongjiou Biotech Co. Ltd. (China), was used as the substrate in the current investigation. Ginsenoside standards which are over 98% pure, such as Rb_1, Rc, Rb_2, Rd, $Rg_3(S)$, $Rh_2(S)$, $Rh_2(R)$, F_2, compound K (C-K), protopanaxadiol (PPD), Rg_1, Re, $Rg_2(S)$, $Rh_1(S)$, F_1, and protopanaxatriol l (PPT), were purchased from Nanjing Zelang Medical Technology Co. Ltd. (China). Methanol and acetonitrile, of HPLC grade were obtained from Merck (Darmstadt, Germany). The other chemicals used in this study were of a minimum analytical reagent grade, and the sources are noted individually in Section 2. Recombinant α-L-arabinofuranoside (Abf22-3) was prepared as described by Liu et al. (2013). The genomic DNA, from *F. johnsoniae* KACC 11414^T, *Escherichia coli* BL21 (DE3), and pGEX 4T-1 plasmid (GE Healthcare, USA), were used as β-glucosidase gene, host, and expression vector sources, respectively. *F. johnsoniae* KACC 11414^T was grown under aerobic conditions at 30 °C on R2A agar (BD, USA). The recombinant *E. coli* for protein expression was cultivated in a Luria–Bertani (LB) medium supplemented with ampicillin (100 mg/l).

2.2. Molecular cloning, expression, and purification of recombinant BglBX10

The genomic DNA from *F. johnsoniae* KACC 11414^T was extracted using a genomic DNA extraction kit (Solgent, Korea). The gene encoding β-glucosidase was amplified from the genomic DNA as a template via a polymerase chain reaction (PCR), using *Pfu* DNA polymerase (Solgent, Korea). The sequence of the oligonucleotide primers used for the gene cloning was based on the DNA

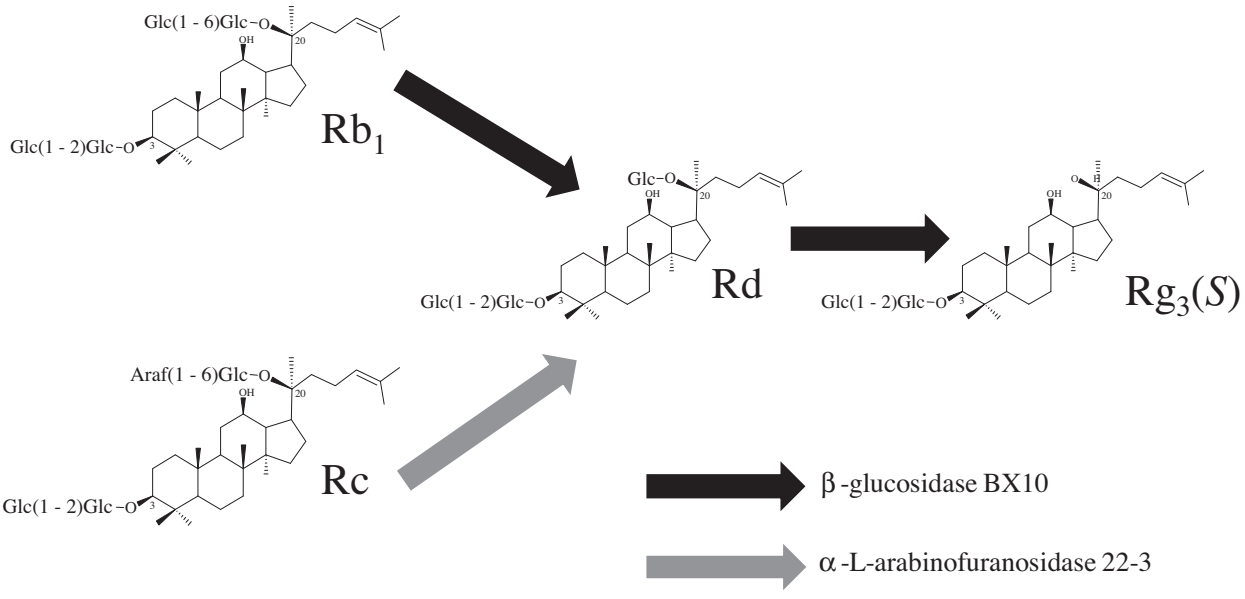

Fig. 1. Biotransformation pathway to produce Rg3(S) by two glycoside hydrolases.