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Biotransformation of Ginsenosides (Ginseng Saponins)

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ABSTRACT

Ginseng is a famous herbal medicine. The major active ingredient of ginseng is ginsenoside, a ginseng saponin. After oral intake of ginseng, the major ginsenosides are hydrolyzed in the human intestinal tract into the more active minor ginsenosides, and the converted minor ginsenosides are absorbed. The minor ginsenosides such as ginsenoside C-K, Rh2, Rh1, Rg3, and Rg2 have special physiological and therapeutic activities that are readily used for ginseng medicines and health foods. This review introduces the biotransformation of ginsenosides into minor ginsenosides and introduces four newly developed types of ginsenosidases (ginseng saponin-glycosidases) *i.e.*, ginsenosidase type I, which can hydrolyze multi-20-*O*-glycosides and 3-*O*-glycosides of the protopanaxadiol (PPD) type ginsenosides; ginsenosidase type II, which can hydrolyze multi-20-*O*-glycosides of the ginsenosides; ginsenosidase type III, which can hydrolyze 3-*O*-glucoside of the multi-PPD type ginsenosides; ginsenosidase type IV, which can hydrolyze multi-6-*O*-glycosides of the protopanaxatriol (PPT) type ginsenosides.

Keywords: ginseng, ginsenoside, ginsenoside biotransformation, ginsenosidase type I, II, III and IV, novel enzyme

CONTENTS

INTRODUCTION

Ginseng, the root of species of the *Panax* genus [including *Panax ginseng* C. A. Meyer, *Panax quinquefolium* L. (American ginseng), *Panax notoginseng* (Sanchi ginseng, or Tienchi ginseng), *Panax japonicus*], has been widely used as a ''magic'' herbal medicine for thousands of years.

The major active ingredients of ginseng are ginsenosides (ginseng saponins) of which over 50 components have been identified. Ginsenosides are categorized into three types: protopanaxadiol type ginsenosides (PPD), protopanaxatriol type ginsenosides (PPT), and oleanonic acid type ginsenoside.

Ginseng's major ginsenosides, including ginsenoside Rb1, Rb2, Rc, Rd, Re and Rg1, are found in ginseng, while minor ginsenosides such as 20(*S*) and 20(*R*)-Rg3, 20(*S*) and 20(*R*)-Rg2, 20(*S*) and 20(*R*)-Rh2, 20(*S*) and 20(*R*)-Rh1; Rg5 and Rk1; Rg4 (F4) and Rg6; Rh3 and Rk2; Rh4 and Rk3 are found in red ginseng.

Researches over the last decade have shown that the minor ginsenosides such as Rg3, Rg2, Rh2, Rh1, Rg5 and Rh3 showing strong antitumor, antimetastatic, hepatoprotective, neuroprotective, immune-stimulating, anti-diabetes and vasodilating activities. It is generally agreed that the minor ginsenosides have high therapeutic and nutritional value (Jin 2009). Therefore, the minor ginsenosides are readily used for ginseng medicines and health foods.

However, it is very difficult to extract these minor ginsenosides from red and wild ginseng roots because the contents of rare ginsenosides in red and wild ginseng roots

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Invited Review

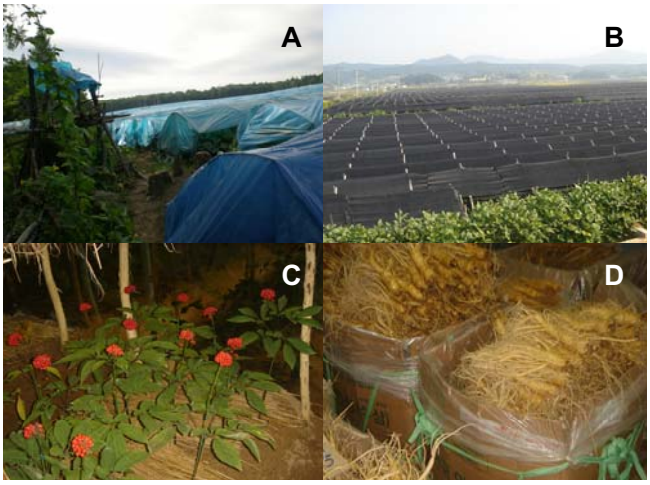


Fig. 1 A ginseng field and fresh ginseng. Pictures by F. Jin (2006). (**A**) China ginseng field; (**B**) Korea ginseng field; (**C**) Ginseng leaf and bud; (**D**) Fresh ginseng.

Fig. 2 The main marketability ginseng roots. Pictures by F. Jin (2009). (**A**) White ginseng; (**B**) Red ginseng; (**C**) American ginseng; (**D**) Notoginseng.

are extremely low (Kitagawa *et al*. 1983). Moreover, after ginseng is taken orally, the ginseng saponins are hydrolyzed in the human intestinal tract by intestinal bacteria, where the major ginsenosides are converted into active forms of minor ginsenosides, which are subsequently absorbed. However, transformation of these minor ginsenosides in the human body is very low (Kanaoka *et al.* 1994).

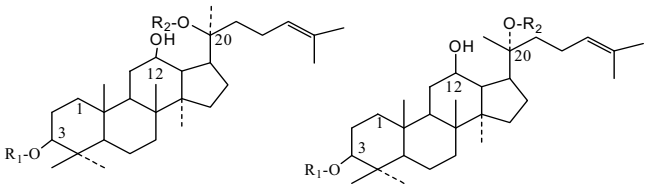
Therefore, biotransformation of major ginsenosides such as Rb1, Rb2, Rc, Rd, Re and Rg1 to produce more active minor ginsenosides is a key factor for ginseng medicine and health foods.

To obtain more active minor ginsenosides, our lab developed new special ginsenosidases, which can be divided into four types: ginsenosidase type I, II, III and IV. These ginsenosides are the focus of this review.

GINSENG AND GINSENOSIDES

Ginsengs

The main, widely used ginsengs include ginseng (Korea ginseng, *Panax ginseng* C. A. Meyer), American ginseng (*Panax quinquefolium* L.) and notoginseng (Sanchi ginseng, or Tienchi ginseng, *Panax notoginseng*). Korea ginseng is mainly planted in China, Korea, Russia and Japan, American ginseng in China, America and Canada and notoginseng mainly in Yunnan, Guizhou and Sichuan Provinces,



20(*S*)-PPD Type Ginsenoside **20(*R*)-PPD Type Ginsenoside**

Ginsenosides	R_1	R_2 (*S*, *R* Isomer)
Ra1	Glc-(1→2)-Glc-	Xyl-(1→4)-Ara(*p*)-(1→6)-Glc- (*S*)
Ra2	Glc-(1→2)-Glc-	Xyl-(1→2)-Are(*f*)-(1→6)-Glc- (*S*)
Ra3	Glc-(1→2)-Glc-	Xyl-(1→3)Glc-(1→6)-Glc- (*S*)
Rb1	Glc-(1→2)-Glc-	Glc-(1→6)-Glc- (*S*)
Rb2	Glc-(1→2)-Glc-	Ara(*p*)-(1→6)Glc- (*S*)
Rb3	Glc-(1→2)-Glc-	Xyl-(1→6)-Glc- (*S*)
Rc	Glc-(1→2)-Glc-	Ara(*f*)-(1→6)-Glc- (S)
Rd	Glc-(1→2)-Glc-	Glc- (*S*)
F2	Glc-	Glc- (*S*)
Rg3	Glc-(1→2)-Glc-	H- (*R, S*)
Rh2	Glc-	H- (*R, S*)
C-K	H-	Glc- (*S*)
Quinquenoside R1	6-Ac-Glc-(1→2)-Glc-	Glc-(1→6)-Glc- (*S*)
Rs1	6-Ac-Glc-(1→2)-Glc-	Ara(*p*)-(1→6)Glc- (*S*)
Rs2	6-Ac-Glc-(1→2)-Glc-	Ara(*f*)-(1→6)Glc- (*S*)
Rs3	6-Ac-Glc-(1→2)-Glc-	H- (*R, S*)
Malonyl-Rb1	6-Ma-Glc-(1→2)-Glc-	Glc-(1→6)-Glc- (*S*)
Malonyl-Rb2	6-Ma-Glc-(1→2)-Glc-	Ara(*p*)-(1→6)Glc- (*S*)
Malonyl-Rc	6-Ma-Glc-(1→2)-Glc-	Ara(*f*)-(1→6)-Glc- (S)
Mmalonyl-Rd	6-Ma-Glc-(1→2)-Glc-	Glc- (*S*)
Natoginsenoside-R4	Glc-(1→2)-Glc-	Xyl-(1→6)Glc-(1→6)-Glc- (*S*)
Natoginsenoside-Fa	Xyl-(1→2)Glc-(1→2)-Glc-	Glc-(1→6)-Glc- (*S*)

Glc, β-D-glucopiranoside; Xyl, β-D-xylopirannoside; Ara*p*, α-L-arabinopiranoside; Ara*f*, α-L-arabinofuranoside; Ac, Acetyl; Ma, Malonyl

Fig. 3 Protopanaxadiol (PPD) type ginsenosides. Modified from Jin (2009).

China.

Other ginsengs such as *Panax japonicus* is rarely produced in Japan; wild *P. trifolius* L. is produced in Canada and America; *P. pseudonginseng* var. *angustifolus* Burk, *P. pseudongingseng* var. 'Major Burk', *P. pseadonginseng* var. Zingbrtmsis C. Y. Wv. et. K. M. Feng, *P. pseudonginseng* var. *bipinnatifidus* Seen and *P. stipuleanatus* Seem are produced to a small extent to the south of China, Vietnam and India (Wang 2001).

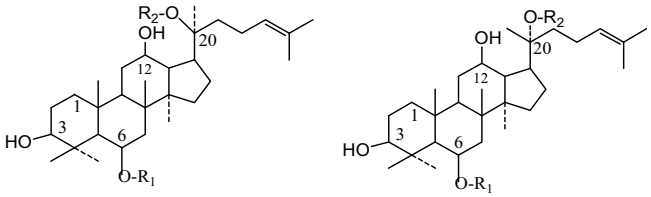
The planting of ginseng and main ginseng roots are shown in **Figs. 1** and **2**.

Ginsenosides

One of physiologically active materials of ginseng plants is a saponin, ginsenoside, 50 of which are known. Ginsenosides are divided into three types, namely protopanaxadiol (PPD) type and protopanaxatriol (PPT) type ginsenosides which are dammarane saponins, and oleanonic acid type saponin such as ginsenoside Ro. The ginsenosides Ra1, Ra2, Ra3, Rb1, Rb2, Rb3, Rc, Rd, F2, Rg3, Rg5, Rh2, and Rh3 are PPD type ginsenosides; Re, Rg1, Rg2, Rg4, Rh1, Rh_4 are PPT type ginsenosides. Ginsenoside Ra1, Ra2, Ra3, Rb1, Rb2, Rb3, Rc, Rd, F2, Re, Rg1 are dammarane 20(*S*)-saponins, but ginsenosides Rg3, Rh2, Rg2, Rh1 have 20(*S*) and 20(*R*)-forms (Wang 2001). The common structures of several compounds belonging to the ginsenosides are shown in **Figs. 3-5**.

Ginsenoside Rs1, Rs2, Rs3, malonyl-Rb1, malonyl-Rb2, malonyl-Rc and malonyl-Rd are common in green parts of ginseng; Quinquenoside R1 acetyl, ginsenoside Rs1, Rs2 and Rs3; the manonyl of malonyl-Rb1, malonyl-Rb2, malonyl-Rc and malonyl-Rd are easily hydrolyzed from dry green parts of ginseng into Rb1, Rb2, Rc and Rd (Wang 2001).

The structure of PPT type ginsenosides is shown in **Fig. 4** while the oleanonic acid type ginsenoside Ro is shown in



20(*S*)-PPT Type Ginsenoside **20(*R*)-PPT Type Ginsenoside**

Ginsenosides	R_1	R_2 (S, R) isomer)
Re	Rha-(1→2)-Glc-	Glc- (*S*)
Rf	Glc-(1→2)-Glc-	H- (*S,R*)
20-Glc-Rf	Glc-(1→2)-Glc-	Glc- (*S*)
Rg1	Glc-	Glc- (*S*)
Rg2	Rha-(1→2)-Glc-	H- (*S,R*)
Rh1	Glc-	H- (*S,R*)
F1	H-	Glc-
Ppt-F2	H-	Ara*p*-(1→6)-Glc-
F5	H-	Ara*f*-(1→6)-Glc-
Natoginsenoside-R1	Xyl-(1→2)-Glc-	Glc- (*S*)
Natoginsenoside-R2	Xyl-(1→2)-Glc-	H-
Natoginsenoside-R3	Glc-	β-D-Glc-(1→6)-Glc-
Natoginsenoside-R6	Glc-	α-D-Glc-(1→6)-Glc-

Rha, α-L-Rhamnopiranoside; Ara*p*, α-L-Arabinopiranoside; Ara*f*, α-L-Arabinofuranoside; Glc, β-D-Glucopiranoside; Xyl, β-D-xylopiranoside

Fig. 4 Protopanaxatriol (PPT) type ginsenosides. Modified from Jin (2009).

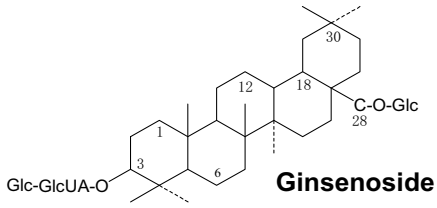


Fig. 5 Oleanonic acid type ginsenoside Ro. Modified from Jin (2009).

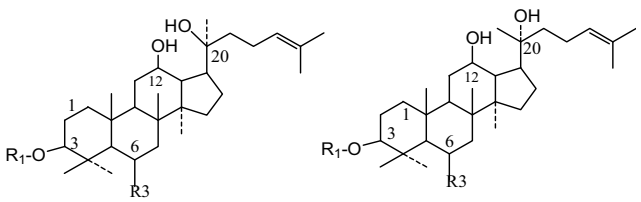
Fig. 5.

Although over 50 kinds of ginsenosides are known, only several contain the main, higher content ginsenosides in the drug ginseng. For example, over 90% of ginsenosides in Korea ginseng root consist of ginsenoside Rb1, Rb2, Rc, Rd, Re and Rg1, while the content of other ginsenosides is low; the main ginsenosides in American ginseng roots are ginsenoside Rb1, Re, Rc, Rd and Rg1; the main ginsenosides in notoginseng roots are ginsenoside Rb1, Rg1, Rd and R1.

The content of ginsenosides differs depending on the breed, production area and planting year of ginseng. In our laboratory, ginsenoside content was examined using the roots of the marketable ginseng drug such as ginseng and American ginseng produced in Fusong, Jilin Province, China and notoginseng produced in Yunnan Province, China. The main ginsenoside content in ginseng drug is shown in **Table 1**.

There is a high content of ginsenoside Rb1, Rb2, Rc, Rd, Re and Rg1 in ginseng, a high content of ginsenoside Rb1 and Re in American ginseng and a high content of ginsenoside Rb1 and Rg1 in notoginseng (**Table 1**).

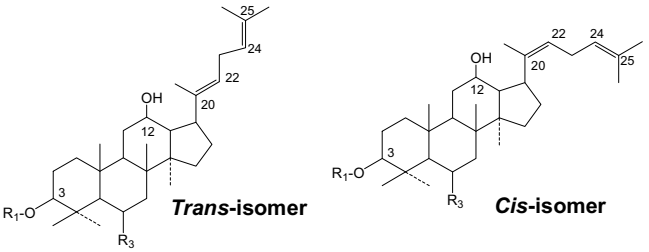
The Pharmacopoeia of the People's Republic of China (2005) prescribes the main ginsenoside contents of ginseng drug when analysed by HPLC: ginsenoside Rg1 and Re contents should not be < 0.3%, ginsenoside Rb1 should not be < 0.2%, the total content of ginsenoside Rg1, Re and Rb1 in American ginseng drug should not be < 2.0% and the total content of ginsenoside Rg1, Rb1 and R1 of notoginseng drug should not be < 2.0%.



20(*S*)-Ginsenoside **20(*R*)-Ginsenoside**

Ginsenosides	R_1	R_3
Rg3	Glc-(1→2)-Glc-	H
Rh2	Glc-	H
Rs3	6-Ac-Glc-(1→2)-Glc-	H
Rg2	H	Rha-(1→2)-Glc-*O*-
Rh1	H	Glc-*O*-

Fig. 6 20(*S*) and 20(*R*)-forms minor ginsenoside Rg3, Rh2, Rg2, Rh1Rs3 in red ginseng. Modified from Park *et al.* (1998).



Ginsenoside	R_1	R_3
***Trans*-isomer**		
Rg5	Glc-(1→2)-Glc-	H
Rh3	Glc-	H
Rs4	6-Ac-Glc-(1→2)-Glc-	H
Rg4 (F4)	H	Rha-(1→2)-Glc-*O*-
Rh4	H	Glc-*O*-
***Cis*-isomer**		
Rk1	Glc-(1→2)-Glc-	H
Rk2	Glc-	H
Rs5	6-Ac-Glc-(1→2)-Glc-	H
Rg6	H	Rha-(1→2)-Glc-*O*-
Rk3	H	Glc-*O*-

Fig. 7 20-C and 20-C ethylene isomer of minor ginsenosides in red ginseng. Modified from Park *et al.* (1998).

Minor ginsenosides of red ginseng

Ginseng is generally used in the form of white ginseng which is prepared by drying fresh ginseng at room temperature, used in the form of red ginseng which is prepared by steaming fresh ginseng at 98-100°C for 1.5-2.5 h.

In red ginseng, when preparing fresh steamed ginseng, the 20(carbon)-*O*-glycoside of 20(*S*)-ginsenosides Rb1, Rb2, Rc, Rd, Re, Rg1, 6-Ac-Rb, 6-Ac-Rb2, 6-Ac-Rc and 6-Ac-Rd of fresh ginseng are hydrolyzed to 20(*S*) and 20(*R*)-forms Rg3, Rh2, Rg2, Rh1, Rs3; in addition, the 20-C(carbon)-OH of minor ginsenoside 20(*S*) and 20(*R*)-forms Rg3, Rh2, Rg2, Rh1, Rs3 is further dehydrated to form an ethylene band between 20-carbon and 22-carbon which have a *cis*- and *trans*-ethylene band isomer that changes into minor ginsenosides such as Rg5 and Rk1, Rh3 and Rk3, Rg4 (F4) and Rg6, Rh4 and Rk3, Rs4 and Rs5 (Park *et al.* 1998).

The structure of minor ginsenosides is shown in **Figs. 6**

Table 1 Major classes of ginsenosides in ginsengs (%).

Ginseng species	Rb1	Rb2	Rc	Rd	Re	Rg1	R1
Korea ginseng (4 year)	0.71	0.42	0.37	0.21	0.63	0.60	-
American ginseng (4 year)	1.84	L	0.31	0.45	1.1	0.20	-
Notoginseng (3 year)	3.5	L	-	0.60	0.40	3.8	0.65

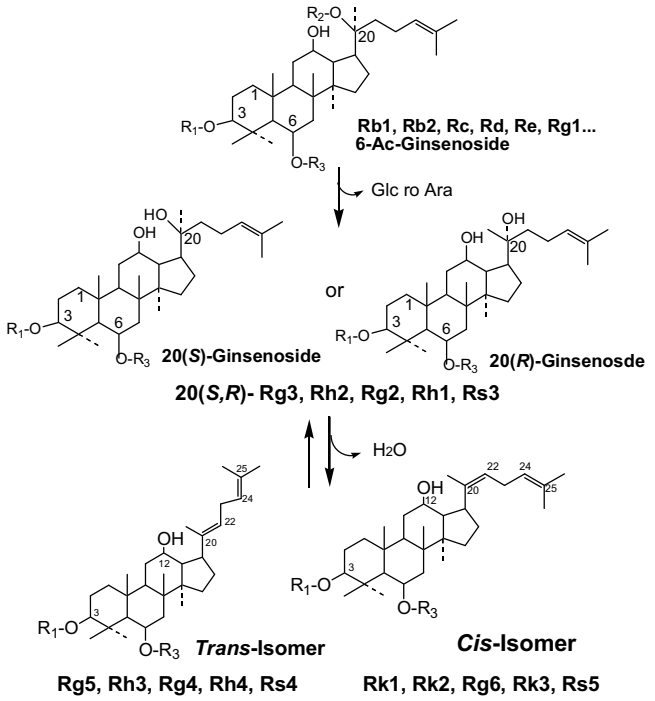


Fig. 8 Changes in ginsenoside during red ginseng preparation.

and **7**. The transformation of ginsenoside in the preparation of fresh steamed red ginseng is shown in **Fig. 8**.

However, the content of minor ginsenosides in traditional red ginseng is very low. For example, the content of ginsenoside Rh2 is only 0.001% in red ginseng (Kitagawa *et al*. 1983).

RHAEMACOLOGICAL ACTIVITIES OF MINOR GINSENOSIDES

Those ginsenosides in the drug ginseng with a high content are Rb1, Rb2, Rc, Rd, Re and Rg1. The pharmacological activities of the major ginseng ginsenosides have already been recognized (Jin 2009).

Ginsenoside Ro has significant anti-inflammation, anti-toxicity, anti-fermentation effects, and can activate thrombin cells. Ginsenoside Rb1 has anti-tiredness and anti-virus activity; it also affects the modulation of the central nervous system (CNS), anti high blood lipid, hypnogenesis, antalgic, ataractic, and promotes the secretion of hormones. Ginsenoside Rb2 is an effective anti-cancer, anti-tiredness, anti-virus, anti-platelet aggregation, anti-glucosuria, and anti-melanoma substance. Ginsenoside Rc enhances the ability to treat anti-cardiotach disorder, anti-tiredness, anti-oxidation and anti-hepatotoxin by suppressing the CNS. Ginsenoside Rd also has anti-virus and anti-cardiotach disorder effects and restrains the multiplication of HSV-1, although it can induce the synthesis of serum protein. Ginsenoside Re has anti-pain and anti-tiredness activity, accelerates DNA and RNA synthesis, and inhibits the CNS (The Korean Society of Ginseng 1997; reviewed in Jin 2009).

Ginsenoside Rg1 promotes "intelligence" (acuteness?) of the CNS and improves memory and learning in mouse models (Chen *et al.* 2008; Qi *et al.* 2009). Ginsenoside Rg1 can cure Alzheimer's disease (Wang *et al.* 2009), can be used as a neuroprotector at the cellular level (Li *et al.* 2009), may attenuate neurotoxicity in human neuroblastoma cells (Gao *et al.* 2009), can be used as an adjuvant therapy in the treatment of colorectal cancer (Fishbein *et al*. 2009) and has an antiproliferative effect on human colorectal cancer cells (Wang *et al.* 2009). Ginsenoside Rg1 and Rb1 have mediative effect in HepG2 cells (Wang *et al*. 2008). Ginsenoside Rg1 inhibits rat left ventricular hypertrophy (Deng *et al.* 2009). Ginsenoside Rg1, Rh1 and Rg3 showed relatively higher antimicrobial and antioxidant activities than other ginsenosides (Lim *et al.* 2009).

However, after ginseng is consumed orally, ginseng saponins are hydrolyzed in human intestinal tract by intestinal bacteria, and the major ginsenosides are converted into the active forms of minor ginsenosides and are subsequently absorbed (Kanaoka *et al.* 1994; Bae *et al*. 2000). Therefore, the pharmaceutical activities of the minor ginsenosides such as ginsenoside C-K, Rh2, Rh1, Rg3, and Rg2 have received special attention recently, and are widely investigated as shown in the following sections.

Ginsenoside Rh2 and Rh3

Ginsenosides Rh2 and Rh3 have anti-cancer activity (Toda *et al.* 1993; Nakata *et al.* 1998). Ginsenosides Rh2 and Rh3 induced differentiation of HL-60 cells into morphological and functional granulocytes during leukaemia therapy (Kim *et al.* 1998). Ginsenosides Rh2 and Rh3 significantly inhibited the proliferation of human cervical adenocarcinoma HeLa cells (Fei *et al.* 2003; Yi *et al.* 2009), and prostate cancer LNCaP cells (Xie *et al.* 2006). Ginsenoside Rh2 induced a phenotypic reverse transformation in B16 melanoma cells (Fujikawa *et al.* 1987; Matsui *et al.* 1995) and arrested the activity of kinase 1 during apoptosis in human breast cancer cells, the MCF7 cells (Ham e*t al.* 2006; Choi *et al.* 2009). Ginsenosides Rh2 and Rh3 had a differentiation-inducing capability on SK-HEP-1 human hepatoma cells *in vitro* and/or *in vivo* (Lee *et al.* 1996; Wu and Xie 2008). Ginsenoside 20(*R*)-Rh2 showed selective osteoclastogenesis inhibitory activity in RAW264 cells *in vitro* (Liu *et al.* 2009).

Ginsenosides Rh3 and Rh2 have an anti-inflammatory effect: they inhibited microglial cell activation in neurodegenerative diseases (Park *et al.* 2009). The anti-inflammatory activity of ginsenosides Rh3 and Rh2 was shown by the inhibited production of inflammatory mediators by suppressing the activation of tumor nuclear factor (TNF)-B and its upstream signaling cascade (Park and Cho 2009).

Furthermore, ginsenosides Rh2 and Rh3 also have other activities such as anti-obesity, anti-anaphylaxis, anxiolytic, anti-dementia and anti-CNS disease. Ginsenoside Rh2 is the most effective candidate for preventing metabolic disorders such as obesity and acts via the AMPK signaling pathway (Hwang *et al.* 2007). Ginsenoside Rh2 could promote the differentiation of preadipocytes by activating glucocorticoid receptor in 3T3-L1 cells (Niu *et al.* 2009). The anti-pruritic effects of Rh2 inhibit scratching behavior and vascular permeability in mice (Trinh *et al.* 2008). Ginsenosides Rh2 and Rh3 showed potent inhibition of mouse passive cutaneous anaphylaxis (Bae *et al.* 2006) and showed anxiolytic-like effects by antagonizing GABA/benzodiazepines in mice in the elevated plus-maze model (Kim *et al.* 2009).

Ginsenoside C-K

Compound K (C-K) enhances insulin secretion with beneficial metabolic effects in *db*/*db* mice (Shin and Kim 2005; Han *et al.* 2007; Yoon *et al*. 2007). C-K offers protection from liver injury (Lee *et al.* 2005); ginsenoside Rh2 and C-K may improve ischemic brain injury (Bae *et al.* 2004); C-K has immunomodulatory effects (Yang *et al.* 2008).

Ginsenoside C-K has anti-cancer effects, specifically anti-tumor effects (Chai *et al.* 2007). C-K and Rh2 inhibit TNF and exert anti-inflammatory effects in human astroglial cells (Choi *et al.* 2007). Ginsenoside C-K is effective for the treatment of septicemia caused by lipopolysaccharides of Gram-negative bacteria, and can inhibit cell proliferation by inducing apoptosis and cell cycle arrest at the G_1 phase in human monocytic leukemia cells (Kang *et al.* 2005, 2006).

C-K might prevent or improve deteriorations in health such as xerosis and wrinkles, partly ascribed to the age-dependent decrease of the hyaluronan (HA) content in human skin (Kim *et al.* 2004). C-K can improve scratching behaviors and chronic oxazolone-induced dermatitis or pso-

riasis of mouse models (Shin *et al.* 2005; Yong *et al.* 2005). C-K suppresses ultraviolet radiation-induced apoptosis by inducing DNA repair in human keratinocytes; C-K can also prevent skin aging and is superior in inhibiting the decompaction of the epidermal-dermal junction (Cai *et al.* 2008).

Ginsenoside Rh1

Ginsenoside Rh1 is an antioxidant inducing hemolysis (Sun *et al.* 2005, 2006). Ginsenoside Rh1 has anti-platelet aggregation activity in the treatment of cardio- and cerebrovascular diseases (Wang *et al.* 2008).

Ginsenoside Rh1 activated the transcription of the estrogen-responsive luciferase reporter gene showing the greatest estrogenic effect in human breast carcinoma MCF-7 cells (Lee *et al.* 2003; Dong and Kiyama 2009). Ginsenoside Rh1 possesses characteristic effects on the proliferation of human leukemia cells (THP-1) (Popovich and Kitts 2002).

Ginsenoside Rh1 also showed anti-allergic, anti-inflammatory, immunomodulatory, and improving memory effects. The antiallergic action of ginsenoside Rh1 originated from its cell membrane-stabilizing and anti-inflammatory activities, which could improve the inflammation caused by allergies (Park *et al.* 2004; Kim *et al.* 2008). Ginsenoside Rh1 improved chronic dermatitis and psoriasis in mouse ear dermatitis models (Shin *et al.* 2006). Ginsenoside Rh1 significantly ameliorated memory-impaired models induced by scopolamine in mice and increased hippocampal excitability in the dentate gyrus of anesthetized rats, improving memory and hippocampal excitability (Lee *et al.* 2000; Wang *et al.* 2009).

Ginsenoside Rg3 and Rg5

Ginsenosides Rg3, Rg5 and Rk1 have various anti-cancer properties. Ginsenoside Rg3 inhibits angiogenesis and growth of lung cancer (Lu *et al.* 2008), TNF-B, and enhances the susceptibility of colon cancer cells to docetaxel and other chemotherapeutics (Kim *et al.* 2009; Lee *et al*. 2009; Xie *et al*. 2009). Ginsenosides Rg3, Rg5 and Rk1 arrest the cell cycle at the G_1 phase in HeLa cells (Lee *et al.* 2009).

Ginsenosides Rg3, Rg5 and Rk1 distinctly inhibit lipid accumulation and are suitable for the therapy of hypercholesterolemia and triglyceridemia; Rg3 ginsenoside is the most effective at inhibiting lipid accumulation (Kim *et al*. 2009); ginsenoside Rg3 and C-K have distinct anti-ischemic effects (Kim 2007); Rg3 inhibits platelet aggregation via the modulation of downstream signaling components such as cAMP and ERK2 (Lee *et al*. 2007). 20(S)-ginsenoside Rg3, a neuroprotective agent, inhibits mitochondrial permeability transition pores in the rat brain (Tian *et al*. 2009), useful for treating patients suffering from Alzheimer's disease (Yang *et al.* 2009). Ginsenoside Rk1 and Rg5 inhibited arachidonic acid (AA)-induced platelet aggregation in a dose-dependent manner, inhibited U46619 (thromboxane A2 mimetic agent)-induced platelet aggregation; and the ginsenoside 20(*S*)-Rg3 and 20(*R*)-Rg3 shoed mild inhibitory activity against AA- and U46619-induced aggregation (Lee *et al*. 2009).

Ginsenoside Rg3 has an anti-diabetes effect by improving insulin signaling and glucose uptake primarily by stimulating the expression of IRS-1 and GLUT4 (Kim *et al.* 2009); Rg3 of red ginseng displays beneficial effects in the treatment of diabetes at least in part via the stimulation of insulin release in a glucose-independent manner (Kim and Kim 2008); 20(*S*)-Rg3 has beneficial effects on diabetic renal damage with an inhibitory effect against NMDA receptor-mediated nitrosative stress (Kang *et al.* 2008).

Ginsenoside Rg2

Ginsenoside Rg2 can protect human erythrocytes against hemin-induced hemolysis (Li *et al.* 2008) and as an antioxidant, it can also prolong the lag time of hemolysis (Liu *et al.* 2002).

Rg2 induces gap junction-mediated intercellular communication (Zhang *et al.* 2001), exerts effects on the immune responses to ovalbumin (OVA) in mice (Sun *et al.* 2007) and can act as a prooxidant (Liu *et al.* 2003). Ginsenoside-Rg2 protects against memory impairment (Zhang *et al.* 2008), and protects cells against UVB-induced genotoxicity by increasing DNA repair and decreasing apoptosis (Jeong *et al.* 2007) and hydrogen peroxide-induced cytotoxicity of human umbilical cord vein endothelial cells *in vitro* (Xin *et al.* 2005).

Rg2 might regulate the 5-HT3A receptors that are expressed in *Xenopus* oocytes and its regulation might be one of the pharmacological actions of *P. ginseng* (Lee *et al.* 2004). Rg2 also regulates glycine receptor which is expressed in *Xenopus* oocytes (Noh *et al.* 2003).

These studies indicate that the minor ginsenosides from red ginseng have pharmacological and physiological activities which can be readily used for ginseng medicine and health foods.

GINSENOSIDE METABOLISM BY INTESTINAL FLORA

After ginseng is consumed orally, the ginsenosides are hydrolyzed in the human intestinal tract by intestinal bacteria, and the major ginsenosides such as Rb1, Rb2, Rc, Rd, Re and Rg1 are converted into active forms of minor ginsenosides and are subsequently absorbed (Kanaoka *et al.* 1994; Bea *et al.* 2000, 2005; Chi *et al*. 2005).

The PPD type ginsenosides Rb1, Rb2, Rc and Rd are gradually hydrolyzed by intestinal bacteria (Kanaoka *et al.* 1994; Bea *et al.* 2000, 2005; **Fig. 9**). Ginsenosides Rb1, Rb2, Rc and Rd are gradually hydrolyzed: *i.e.*, the 20-*O*-β-D-glucoside of ginsenoside Rb1, 20-*O*-α-L-arabinopyranoside of ginsenoside Rb2, and the 20-*O*-α-L-arabinofunoside of ginsenoside Rc are hydrolyzed to ginsenoside Rd; then, the 3-*O*-β-D-glucoside of ginsenoside Rd is further hydro-

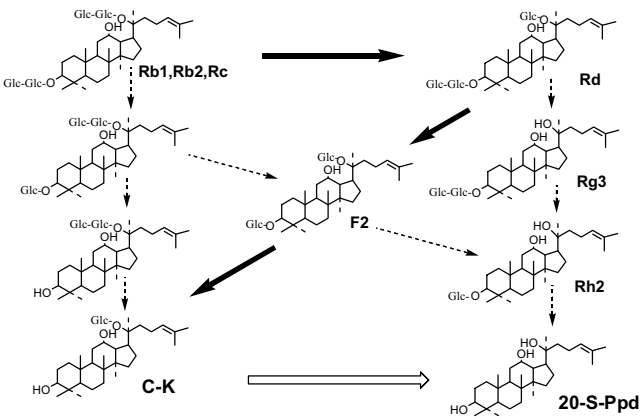


Fig. 9 Metabolism of ginsenoside Rb1, Rb2, Rc and Rd in intestinal tract by intestinal bacteria. Based on Kanaoka *et al.* (1994) and Bea *et al.* (2000, 2005).

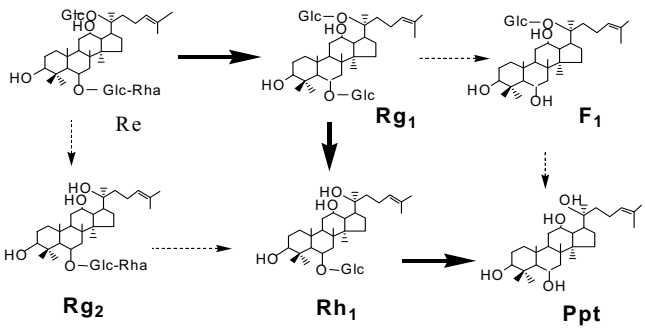


Fig. 10 Metabolism of ginsenoside Re and Rg1 in intestinal tract by intestinal bacteria. Based on Kanaoka *et al.* (1994) and Chi *et al*. (2005).

lyzed to ginsenoside F2; the 3-*O*-β-D-glucoside of F2 is further hydrolyzed to C-K; and C-K is hydrolyzed to aglycone.

The PPT type ginsenosides Re and Rg1 are also gradually hydrolyzed by intestinal bacteria (Kanaoka *et al.* 1994; Chi *et al*. 2005; **Fig. 10**). The 6-*O*-α-L-rhamnoside of ginsenoside Re is hydrolyzed to Rg1; the 6-*O*-β-D-glucoside of Rg1 is hydrolyzed to F1, or the 20-*O*-β-D-glucoside of Rg1 is hydrolyzed to Rh1; ginsenosides Rh1 and F1 are hydrolyzed to aglycone.

Therefore, after orally intake of ginseng, human bodies do not at first absorb the major ginsenosides such as Rb1, Rb2, Rc, Rd, Re and Rg1, but only absorb the converted minor ginsenosides. This proves that the pharmacological effects of ginseng on human bodies are the action of the converted ginsenosides, *i.e*., the action of the minor ginsenosides.

GINSENOSIDE BIOTRANSFORMATION

From the above it is clear that the minor ginsenosides have special physiological and therapeutic activities. Thus, the conversion of the major ginsenosides in ginseng into more active minor ginsenosides is very important for the production of ginseng medicines and health foods. To obtain highly active minor ginsenoside, the biotransformation of ginsenosides is explained in detail next.

Ginsenoside enzymes in ginseng plants

The ginsenoside enzymes of ginseng plants can be divided into two types: one type of enzymes is related to ginsenoside biosynthesis during ginseng growth; the other type of enzymes is related to the hydrolysis of the ginsenoside-sugar moiety.

Zhang and Yue (2005) reported the enzymes related to ginsenoside biosynthesis in ginseng cells, specifically a new enzyme UDP-glucose ginsenoside Rd glucosyltransferase from notoginseng (Yue and Zhang 2005) and protopanaxadiol 6-hydroxylase (Yue *et al.* 2008). These enzymes related to ginsenoside biosynthesis are very usable for ginseng cell growth since they allow ginseng cells with a high content of ginsenosides to be obtained.

Our laboratory previously purified and characterized new ginsenosidases hydrolyzing ginsenoside sugar-moieties from the fresh roots of ginseng (Zhang *et al*. 2001, 2002). A new ginsenosidase hydrolyzing the 3-*O*-β-(1→2)-glucoside of the ginsenoside Rg3 sugar moiety to ginsenoside Rh2 had a molecular weight of about 59 kDa. Another ginsenosidase hydrolyzing the 20-*O*-α-(1→6)-arabinofuranoside of ginsenoside Rc to ginsenoside Rd was isolated from ginseng roots, purified and characterized. The enzyme molecular weight was about 86 kDa; the enzyme also hydrolyzed the 20-*O*-glycoside of ginsenosides Rb1 and Rb2.

The possibility of utilizing the ginsenosidases of ginseng itself in ginseng product preparation is very valuable for increasing the content of the more active minor ginsenosides in ginseng products. In order to make the most of ginseng itself, the use of ginsenosidases in the red ginseng process and in the new red ginseng process have been patented (Jin *et al.* China Patent No. ZL200510136799.8; title, Preparation of new active red-ginseng). The new red ginseng and its products contained a higher content of more active and easy-to-absorb minor ginsenosides although the external appearance and color were as same as traditional red ginseng.

Ginsenoside hydrolysis by culture broth of microorganisms

Studies on the hydrolysis of ginsenoside-sugar moieties using concentrated cultures fermented by microorganisms (crude enzyme), mainly fungus and bacteria, were carried out 30 years ago: crude hesperidinase, naringinase, pectinase, amylase and cellulase, and a concentrated culture broth of the microorganisms from soil could hydrolyzed ginsenosides Rb1, Rb2, Rc to give C-K or its aglycone; hydrolysis of Rg1 gave its aglycone (Kohda and Tanaka 1975). The concentrated culture broth (or crude enzyme) of *Aspergillus oryzae* and *Penicillium* sp. strain also hydrolyzed ginsenoside Re into ginsenoside Rg1, Rh1 and F1, respectively (Ko *et al*. 2003). The culture broth of the *Mucilaginibacte composti* sp. nov could convert ginsenoside Re to ginsenoside Rg2 (Cui *et al*. 2011). The culture broth of the *Intrasporangium* sp. strain GS603, isolated from a ginseng field, could convert the ginsenoside Rb1 to minor ginsenoside F2 and gypenoside XVII (Cheng *et al.* 2007). The biotransformation of ginsenosides by microorganisms was reviewed by Park *et al*. (2010).

Our laboratory and the laboratory of Professor Sung-Taik Lee, KAIST (Korea Advanced Institute of Science and Technology), carried out a cooperation study on the hydrolysis of ginsenosides using new 20 kinds of strains isolated from soil as explained in some detail next (Shao *et al*. 2008; Yu *et al*. 2008; Wang *et al.* 2009; Wu *et al*. 2009).

The 16S rRNA gene sequence of 20 kind new strains showed different levels of similarity to the same sequence in other microorganisms. Specifically, sp. No. 1, GS0302: 98.4% similarity with *Arthrobacter chlorophenolicus* A-6; sp. No. 2, GS3043: 98.4% similarity with *Arthrobacter chlorophenolicus* A-6; sp. No. 3, GS0202: 98.4% similarity with *Arthrobacter chlorophenolicus* A-6; sp. No. 4, GS0314: 99.8% similarity with *Arthrobacter oxydans* DSM20119T; sp. No. 5, GS0207: 99.8% similarity with *Arthrobacter oxydans* DSM20119T; sp. No. 6, GS0586: 95.7% similarity with *Arthrobacter sachebrandtii* CCM 2783T; sp. No. 7, GS0501: 96.9% similarity with *Arthrobacter sachebrandtii* CCM 2783T; sp. No. 8, GS0557: 96.1% similarity with *Arthrobacter sachebrandtii* CCM 2783T; sp. No. 9, GS0251: 99.8% similarity with *Streptomyces exfoliantus* NRRL B-1237T; sp. No. 10, GS0213: 99.8% similarity with *Streptomyces polychromogenes* NRRL B-3032T; sp. No. 11, GS0090 W1-04: 99.0% similarity with *Mycobacterium mucogenicum* ATCC49650 T; sp. No. 12, GS0121 W3-12: 99.1% similarity with *Mycobacterium mucogenicum* ATCC49650 T; sp. No. 13, GS0053 M1-23: 98.7% similarity with *Rhodanobacter fulvus* Jip2 T; sp. No. 14, GS3054: 98.5% similarity with *Rhodanobacter fulvus* Jip2 T; sp. No. 15, GS0053 M2-06: 97.0% similarity with *Pedobacter himalayensis* HHS 22T; sp. No. 16, GS3078: 98.6% similarity with *Burkholderia caribiensis* MWAP64 T; sp. No. 17, GS0262: 97.9% similarity with *Lentzae waywayandensis* NRRL B-16159 T; sp. No. 18, *Terrabacter ginsenosidimutans* GS3082 (An *et al*. 2010); sp. No. 19, GS1547: 100% similarity with *Variovorax paradoxus* W-50 T; sp. No. 20, GS71: 98.71% similarity with *Enterobacter asburiae* JCM6051T.

To select good strains which produce the enzyme hydrolyzing sugar-moiety of ginsenosides, a culture broth of the 20 kinds of strains were cultured in the medium (tryptone, 10 g, yeast extract 5 g, NaCl 10 g, water 1000 ml) at 25°C for 2 days then reacted with 0.5% of ginsenoside Rb1, as shown in **Fig. 11** (Yu *et al*. 2008). Also shown in **Fig. 11** is that the culture broth of sp. No. 3, 14 and 18 strains obviously hydrolyzed ginsenoside Rb1; therefore, sp. No. 3, 14 and 18 strains were used in further studies. The culture broth of sp. No. 3, 14 and 18 strains in R2A medium cultured at 25°C for 6 days were also reacted with 0.5% of ginsenoside Rb1, as shown in **Fig. 12**. The culture broth of sp. No. 3 and 18 strains converted ginsenoside Rb1 into Rg3; the culture broth of sp. No. 14 converted the ginsenoside Rb1 into Rd. These facts prove that sp. No. 3 and 18 strains produce an enzyme capable of hydrolyzing two 20-*O*-glucosides of ginsenoside Rb1 into ginsenoside Rg3 while sp. No. 14 strain produces an enzyme hydrolyzing one 20-*O*-glucoside of Rb1 into ginsenoside Rd.

To assess the effects of fermentation conditions such as medium and fermentation temperature on enzyme production and to analyze the hydrolysis of culture broth on different ginsenosides, enzyme fermentation conditions and

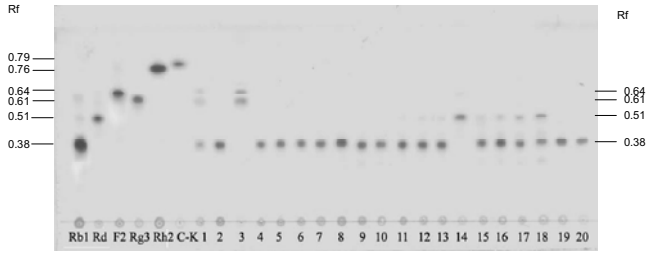


Fig. 11 Ginsenoside Rb1 hydrolysis by 20 new kinds of strains. Modified from Yu *et al.* (2008). Rb1, Rd, F2, Rg3, Rh2 C-K, standard ginsenoside; 1 to 20, sp. No. 1, sp. No. 2,…sp. No. 20 strain; 20 kind strains were cultured in R2A medium containing 3 mg/L of total ginsenoside at 25°C for 2 days; the cultures were reacted with 0.5% of ginsenoside Rb1; TLC silica gel F254, solvent, $CHCl_3$: methanol: water = 7: 2.5: 0.5 (under layer).

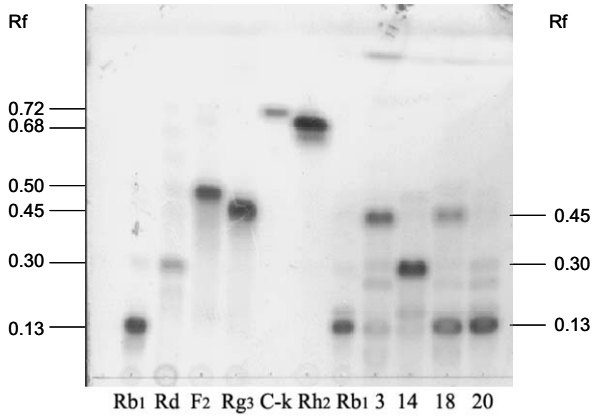


Fig. 12 Culture broth (at 25°C for 6 days) of sp. No. 3, No. 14, No. 18 and No. 20 strains hydrolysis on ginsenoside Rb1. Modified from Yu *et al.* (2008). Rb1, Rd, F2, Rg3, Rh2, C-K, standard ginsenoside; 3, 14, 18, 20, sp. No. 3, No. 14, No. 18 and No. 20 strains; the strains were cultured in R2A medium containing 3 mg/L of total ginsenoside at 25°C for 6 days; the cultures were reacted with 0.5% of ginsenoside Rb1; TLC silica gel F254, solvent, $CHCl_3$: methanol: water = 7: 2.5: 0.5 (under layer).

reactions on different ginsenosides were studied using sp. No. 3 and 18 strains. The results (Shao *et al*. 2008; Yu *et al*. 2008; Wang *et al.* 2009; Wu *et al*. 2009) were as follows.

Enzyme production by No. 3 and 18 strains in the medium containing 1% of protease peptone, 0.5% of yeast extract powder and 1% of NaCl was higher than that in the medium (defined above); the enzyme production was also high after adding 3 mg/l of total ginsenoside into the medium as an inducer of enzyme production. The culture broth not only hydrolyzed ginsenoside Rb1, but also hydrolyzed other PPD type ginsenosides such as Rb2, Rc and Rd.

Temperature affected enzyme fermentation. When the No. 3 and 18 strains were fermented at 25°C, the culture broths hydrolyzed ginsenosides Rb1, Rb2, Rc and Rd mainly into ginsenoside Rg3; when these strains were fermented at 35°C, the culture broths hydrolyzed ginsenosides Rb1, Rb2, Rc and Rd mainly into ginsenoside F2 and C-K, and ginsenoside Rh2 and gypenoside XVII production was also possible. The culture broth was also capable of hydrolyzing ginsenoside Rg1 into F1. Therefore, the culture broth of sp. No. 3 and 18 strains cultured at 25°C could hydrolyze the 20-*O*-glycoside of the PPD type ginsenosides, Rb1, Rb2, Rc and Rd, converting them mainly into Rg3 while the culture broth of sp. No. 3 and 18 strains cultured at 35°C could hydrolyze the 20-*O*-glycoside and 3-*O*-glycoside of the PPD type ginsenosides, Rb1, Rb2, Rc and Rd, converting them mainly into ginsenoside F2, C-K and gypenoside XVII, but mainly into F2 and C-K; also, 20-*O*-glucoside of PPT type ginsenoside Rg1 was hydrolyzed into ginsenoside F1.

New special ginsenosidases

To obtain active minor ginsenosides from a high content of major ginsenosides in ginseng, it is necessary to recognize ginsenosidases from the culture broth of microorganisms or crude enzymes from animals and plants. Since the crude enzyme or culture broth of microorganisms have a large number of enzymes, it is not possible to produce the desired minor ginsenoside from a major ginsenoside.

Our laboratory has been studying the special ginsenosidases hydrolyzing ginsenoside sugar moieties, their fermentation, isolation, purification and characterization (Yu *et al*. 1999, 2002; Zhang *et al*. 2001, 2002; Jin *et al*. 2003; Yu *et al*. 2004; Yang *et al*. 2007; Yu *et al*. 2007; Wang *et al*. 2009; Yu *et al*. 2009; Wang *et al*. 2011).

Others (Hu *et al.* 2007) reported a new special ginsenosidase from the China white jade snail (*Achatina fulica*) hydrolyzing ginsenoside Rb1 into Rd, F2 and C-K; Cheng *et al.* (2008) reported an enzyme converting major ginsenoside Rb1 to 20(*S*)-ginsenoside Rg3 from *Microbacterium* sp. GS514.

The new ginsenosidases can be divided into four types: Ginsenosidase type I, II, III and IV based on the hydrolyzing glycoside position of ginsenoside molecule as detailed next.

Ginsenosidase type I can hydrolyze multi-20-*O*-glycosides and 3-*O*-glycosides of PPD type ginsenosides such as Rb1, Rb2, Rb3, Rc, Rd, F2 and Rg3; *i.e*., ginsenosidase type I can hydrolyze the 20(carbon)-*O*-β-(1→6)-D-glucoside of Rb1, the 20-*O*-α-(1→6)-L-arabinoside(*p*) of Rb2, the 20-*O*-α-(1→6)-L-arabinoside(*f*) of Rc and the 20-*O*-β-(1→6)-D-xyloside of Rb3 to ginsenoside Rd; further, it can hydrolyze the 3-*O*-β-(1→2)-D-glucosides of Rd to F2 and the 3-*O*-D-glucosides of F2 mainly to C-K. The enzyme molecular weight from *Aspergillus* strain was about 80 kDa (Yu *et al.* 2007). The Ginsenoside type I reaction is shown in **Fig. 13**. It is shown in **Fig. 13** that ginsenosidase type I hydrolyzed multi-20-*O*-glycosides and 3-*O*-glycosides of the PPD type ginsenoside Rb1, Rb2, Rb3, Rc, Rd to mainly produce ginsenoside F2 and C-K; despite the enzyme faintly hydrolyzing ginsenoside F2 to Rh2, and faintly hydrolyzing C-K to its aglycone. Ginsenosidase type I also hydrolyzed the 3-*O*-β-(1→2)-D-glucosides of ginsenoside Rg3 to mainly produce ginsenoside Rh2, as shown in **Fig. 14**.

Ginsenosidase type II can hydrolyze multi-20-*O*-glycosides of ginsenosides.

The special ginsenosidase whose molecular weight is about 60 kDa, was purified and characterize from an *Aspergillus* strain (Yu *et al.* 2009) and specifically hydrolyzes multi-20-*O*-glycosides of PPD type ginsenosides such as ginsenoside Rb1, Rb2, Rb3 and Rc; *i.e.*, ginsenosidase type

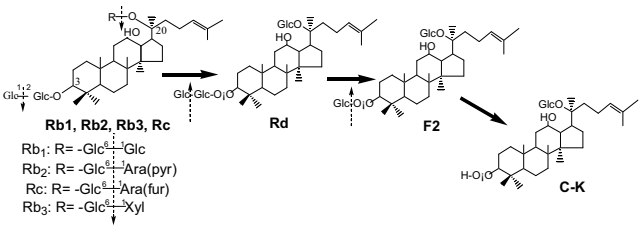


Fig. 13 Ginsenosidase type I reaction. Modified from Yu *et al.* (2007).

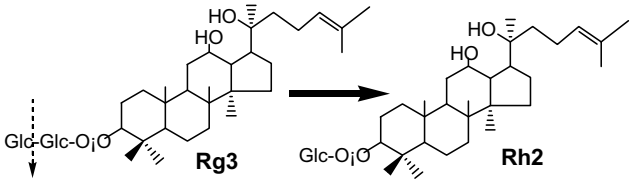


Fig. 14 Ginsenosidase type I hydrolysis on ginsenoside Rg3.

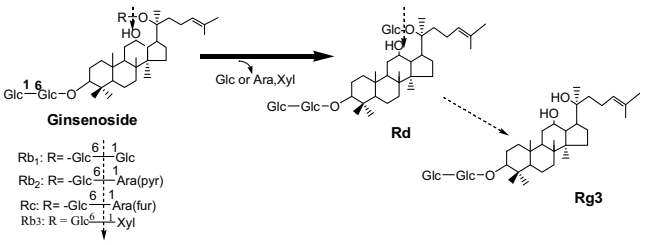


Fig. 15 Ginsenosidase type II from *Aspergillus* strain hydrolyzes 20-*O*-glycosides of PPD type ginsenosides. Modified from Yu *et al.* (2009).

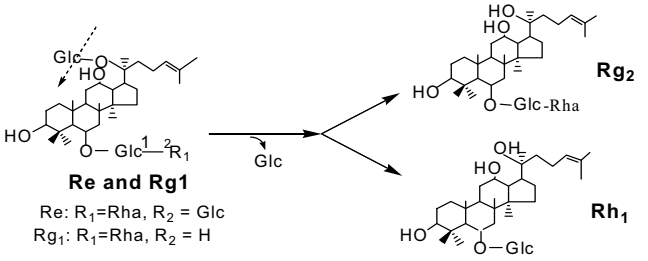


Fig. 16 Enzyme from bacteria hydrolyzes 20-*O*-glycosides of PPT type ginsenosides.

II can hydrolyze the 20(carbon)-*O*-β-(1→6)-D-glucoside of Rb1, the 20-*O*-α-(1→6)-L-arabinoside(*p*) of Rb2, the 20-*O*-α-(1→6)-L-arabinoside(*f*) of Rc and the 20-*O*-β-(1→6)-D-xyloside of Rb3 to ginsenoside Rd; the enzyme further faintly hydrolyzes the 20-*O*-β-D-glucosides of Rd to produce a small quantity of ginsenoside Rg3, as shown in **Fig. 15**. **Fig. 15** also shows that ginsenosidase type II from the *Aspergillus* strain hydrolyzed multi 20-*O*-glycosides of PPD ginsenosides such as Rb1, Rb2, Rc and Rb3 mainly to produce ginsenoside Rd; the enzyme further faintly hydrolyzed the 20-*O*-β-D-glucosides of Rd to produce a small quantity of ginsenoside Rg3; however, the enzyme could not hydrolyze the 20-*O*-glycoside of the PPT type ginsenosides Re, Rf and Rg1 (Yu *et al.* 2009).

However, the enzyme from bacteria such as *Microbacterium* sp. GS514 strain (Cheng *et al.* 2008), sp. No. 3, GS0202 (98.4% similarity of the 16S rRNA gene sequence with *Arthrobacter chlorophenolicus* A-6) and sp. No. 18, *Terrabacter ginsenosidimutans* sp. nov GS3082 (Shao *et al*. 2008; Yu *et al*. 2008; An *et al*. 2010) strains hydrolyzed multi-20-*O*-glycosides of PPD ginsenosides such as Rb1, Rb2, Rc and Rb3 mainly to produce ginsenoside Rg3 which could be used for Rg3 production.

Although ginsenosidase type II from the *Aspergillus* strain only hydrolyzed 20-*O*-glycoside of the PPD type ginsenoside, it did not hydrolyze the 20-*O*-glycoside of the PPT type ginsenosides Re and Rg1; however, our laboratory recognized that the special enzyme from bacteria such as *Microbacterium* sp. GS514 strain, sp. No. 3, GS0202 and sp. No. 18, *T. ginsenosidimutans* sp. nov GS3082 strains could hydrolyze the 20-*O*-glucoside of the PPT ginsenosides Re and Rg1 to respectively produce ginsenoside Rg2 and Rh1, as shown in **Fig. 16**.

Ginsenosidase type II might have two sub-enzymes: one that can only hydrolyze multi-20-*O*-glycosides of PPD type ginsenosides; the other that can hydrolyze 20-*O*-glucoside of PPT type ginsenosides Re and Rg1, but these needing further characterization.

Ginsenosidase type III can hydrolyze 3-*O*-glycosides of PPD type ginsenosides.

Our laboratory and the laboratory of Professor Sung-Taik Lee of KAIST (Korea Advanced Institute of Science and Technology) carried out a cooperation study on the special enzyme hydrolyzing 3-*O*-glycosides of PPD type ginsenosides. The enzyme gene (*bgl-gyp17*) comprising 1770 bp from *Terrabacter ginsenosidimutans* sp. nov GS3082 strain was cloned and overexpressed in *Escherichia coli*

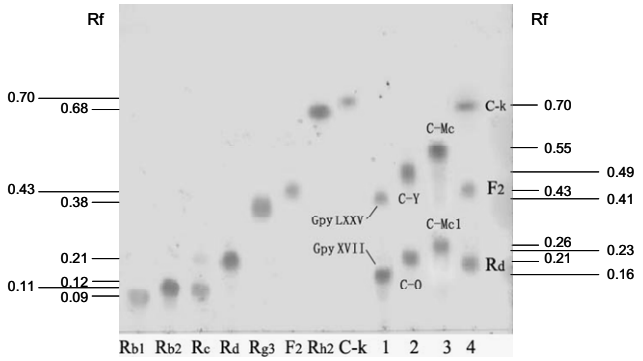


Fig. 17 Ginsenosidase type III hydrolysis on ginsenoside Rb1, Rb2, Rd and Rd. Rb1, Rb2, Rc, Rd, Rg3, F2, Rh2, C-K, standard ginsenoside; 1, product from reaction of 1% Rb1 by enzyme for 24 h; 2, product from reaction of 1% Rb2 for 24 h; 3, product from reaction of 1% Rc for 24 h; 4, product from reaction of 0.5% Rd for 72 h; TLC silica gel F254, solvent, $CHCl_3$: methanol: water = 7: 2.5: 0.5 (under layer).

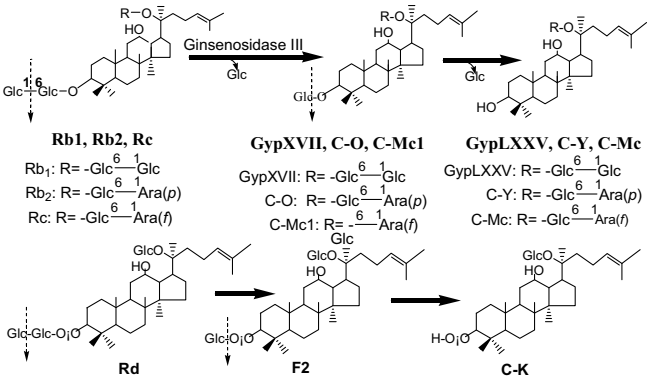


Fig. 18 Ginsenosidase type III hydrolysis of ginsenoside Rb1, Rb2 Rc and Rd.

cells. The recombinant enzyme of a polypeptide consisted of 589 amino acids (An *et al*. 2010).

The recombinant enzyme examined in our laboratory could selectively hydrolyze 3-*O*-sugar-moiety of protopanaxadiol (PPD) type ginsenosides such as Rb1, Rb2, Rc, Rd, F2, Rg3, and Rh2 but it could hardly hydrolyze the 20-*O*-sugar-moiety of PPT type ginsenosides. The enzyme hydrolysis of ginsenoside Rb1, Rb2, Rc and Rd is shown in **Fig. 17**, which indicates that recombinant ginsenosidase selectively hydrolyzed 3-*O*-glucosides of ginsenosides Rb1, Rb2, Rc and Rd; *i.e.*, the new enzyme hydrolyzed the 3-*O*-β-(1→2)-D-glucoside of ginsenoside Rb1 to gypenoside XVII and further hydrolyzed 3-*O*-β-D-glucoside of gyp XVII to gyp LXXV; it hydrolyzed the 3-*O*-β-(1→2)-D-glucoside of ginsenoside Rb2 to Compound-O (C-O) and further hydrolyzed the 3-*O*-β-D-glucoside of C-O to Compound-Y (C-Y); it hydrolyzed the 3-*O*-β-(1→2)-D-glucoside of ginsenoside Rc to Compound-Mc1 (C-Mc1) and further hydrolyzed the 3-*O*-β-D-glucoside of C-Mc1 to Compound-Mc (C-Mc); the enzyme also hydrolyzed the 3-*O*-β-(1→2)-D-glucoside of ginsenoside Rd to F2 and further hydrolyzed the 3-*O*-β-D-glucoside of Rd to C-K. The enzyme reaction is shown in **Fig. 18**.

Ginsenosidase type III also hydrolyzed the 3-*O*-β-D-glucoside of ginsenoside Rg3 to Rh2 and further hydrolyzed it to its aglycone.

In summary, the ginsenosidase type III enzyme could selectively hydrolyze 3-*O*-β-D-glucoside of PPD type ginsenosides such as Rb1, Rb2, Rc, Rd, F2 and Rg3, but could not hydrolyze the sugar-moiety of PPT type ginsenosides.

Ginsenosidase type IV can hydrolyze the multi-6-*O*-glycosides of PPT type ginsenosides.

Our laboratory previously purified and characterized ginsenoside-α-rhamnosidase from a fungal strain (Yu *et al*.

Table 2 Major ginsenosidases.

Enzyme type	Enzyme source	Hydrolyzing main glycoside position in ginsenoside	Hydrolyzing main glycoside
Ginsenosidase type I	Microorganism	3-*O*-(Carbon)-glycoside 20-*O*-(Carbon)-glycoside	Glc, Ara, Xyl
Ginsenosidase type II	Microorganism	20-*O*-(Carbon)-glycoside	Glc, Ara, Xyl
Ginsenosidase type III	Microorganism	3-*O*-(Carbon)-glycoside	Glc
Ginsenosidase type IV	Microorganism	6-*O*-(Carbon)-glycoside	Rha, Glc, Xyl

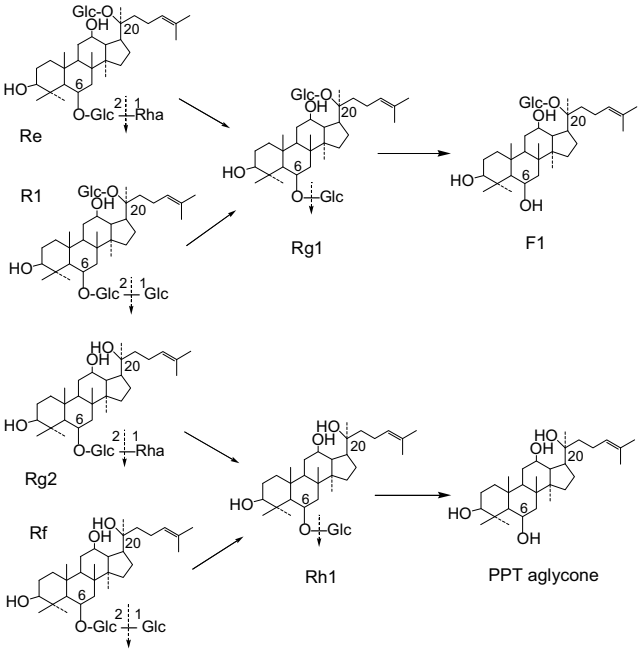


Fig. 19 Ginsenosidase type IV hydrolysis of ginsenoside Re, Rf, notoginsenoside R1 and Rg2. Based on Yu *et al*. (2002), Jin *et al*. (2003), and Wang *et al.* (2009, 2011).

2002). The enzyme not only hydrolyzed the 6-*O*-(1→2)-α-L-rhamnoside of ginsenoside Re to Rg1, but also hydrolyzed the 6-*O*-(1→2)-β-D-glucoside of ginsenoside Rf, and hydrolyzed the 6-*O*-(1→2)-β-D-xyloside of the noto-ginsenoside R1 to Rg1 (Jin *et al*. 2003; Wang *et al.* 2009, 2011); thus, the enzyme was a true ginsenosidase type IV hydrolyzing multi-6-*O*-glycosides of PPT type ginsenosides, as shown in **Fig. 19**.

Ginsenosidase type IV also hydrolyzed the 6-*O*-(1→2)-α-L-rhamnoside of ginsenoside Rg2 to Rh1; it also slightly hydrolyzed the 6-*O*-glucoside of ginsenoside Rg1 and Rh1 to respectively produce F4 and PPT aglycone. However, the enzyme from bacteria highly hydrolyzing Rg1 to F4 needs further studies.

In summarizing, new novel ginsenosidases hydrolyzing the sugar-moiety of ginsenosides selected the glycoside-position in the ginsenoside molecule but did not select glycosidic bonds; the ginsenosidases that hydrolyzed multi-glycosides of ginsenosides are shown in **Table 2**.

Ginsenoside type I can hydrolyze multi-20-*O* and 3-*O*-glycosides of PPD type ginsenosides; Ginsenosidase type II can hydrolyze multi-20-*O*-glycosides of the ginsenosides; Ginsenoside type III can hydrolyze 3-*O*-glycosides of PPD type multi-ginsenosides; Ginsenoside type IV can hydrolyze multi-6-*O*-glycosides of PPT type ginsenosides.

These properties of ginsenosidases differentiate them from those of glycosidases: one type of enzyme hydrolyzes one type of glycoside, as described in the Enzyme Nomenclature by NCIUBMB (Nomenclature Committee of the International Union Biochemistry and Molecular Biology described in http://www.qmul.ac.uk/iubmb/enzyme); therefore, ginsenosidases are a new novel class of glycosidases which can be used for the production of minor ginsenosides.

It is possible to produce active minor ginsenosides using the new special ginsenosidases by controlling the enzyme reaction time, temperature and substrate concentration.

CONCLUSION

This review shows that the major ginsenosides that exist in high concentrations in ginseng are ginsenosides Rb1, Rb2, Rc, Rd, Re and Rg1; the major minor-ginsenosides in red ginseng are Rg3, Rg5 and Rk1, Rh2, Rh3 and Rk2, Rg2, Rg4(F4) and Rg6, Rh1, Rh4 and Rk3; after ginseng is consumed orally, the human body does not first absorb the major ginsenosides such as Rb1, Rb2, Rc, Rd, Re and Rg1; rather, it only absorbs the converted minor ginsenosides. This proves that the pharmacological effects of ginseng on the human body are in fact the action of the converted ginsenosides, *i.e*., the action of the minor ginsenosides.

The minor ginsenosides from red ginseng have very good pharmacological and physiological activities. Therefore, biotransformation of the major ginsenosides into more active minor ginsenosides is very important for ginseng medicine and health food.

It is possible that the available active minor ginsenosides can be produced using new special ginsenosidases (Types I-IV) under controlled conditions.

The biotransformation of ginsenosides and new ginsenosidases indicate that it is potentially possible to biotransform thousands of other saponins or glycosides in herbs (*Kanpo*) into more active converted products.

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