International Journal of Biomedical and Pharmaceutical Sciences ©2012 Global Science Books

Biologically Active Proteins in Ginseng

Tzi Bun Ng* • **Jack Ho Wong** • **Randy Chi** • **Fai Chueng**

School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Sha Tin, New Territories, Hong Kong, China

Corresponding author: * **b021770@mailserv.cuhk.edu.hk**

ABSTRACT

Ginseng produces a variety of proteins and enzymes: alcohol dehydrogenase, catalase, chitnases, spermidine synthase, and glutamate decarboxylase, which are enzymes related with stress. Arginase and ginsenoside-β-glucosidase are hydrolytic enzymes whereas ribonuclease is an enzyme with defense activity. Polygalacturonase-inhibiting protein is a defense protein. Latex-like protein is a protein with osmoregulatory function. Medicinal peptide, radioprotective protein and antiproliferative tetrapeptides are proteins with medicinal activity. The PACAP-immunoreactive component increases cAMP in human neuroblastoma cells. Ginseng major protein and glutathione-related oligopeptides from ginseng do not have well defined functions as yet.

Keywords: enzymes, ginseng, proteins

Abbreviations: **CA**, chromosome aberration; **cDNA**, complementary DNA; **DNA**, deoxyribonucleic acid; **DTNB**, 5,5'-dithiobis-2-nitrobenzoic acid; **FPLC**, fast protein liquid chromatography; **GABA**, gamma aminobutyric acid; **GMP**, ginseng major protein; **DEAE-cellulose**, diethylaminoethyl-cellulose; **HIV**, human immunodeficiency virus; **Km**, Michaelis constant; **MTA**, 5'-deoxy-5'-methylthio-adenosine; **PCMB**, p-chloromercuribenzoate; **PgCATI**, *Panax ginseng* catalase I; **PgADH**, *Panax ginseng* alcohol dehydrogenase; **PgGAD**, *Panax ginseng* glutamate decarboxylase; **PGIP**, polygalacturonase inhibiting protein; **poly(A)**, poly (Adenine); **poly (C)**, poly (Cytosine); **poly (G)**, poly (Guanine); **poly (U)**, poly (Uracil); **PACAP**, pituitary adenyl cyclase activating peptide; **RIP**, ribosome inactivating protein; **RG**, red ginseng; **SG**, sun ginseng; **SP-Sepharose**, sulfopropyl-Sepharose; **SCE**, sister chromatid exchange; **tRNA**, transfer ribonucleic acid; **WG**, white ginseng

CONTENTS

INTRODUCTION

Ginseng belongs to the *Panax* genus in the family Araliaceae. It grows in northern China, Korea, and eastern Siberia. *Panax vietnamensis*, growing in Vietnam, is the southernmost ginseng. The word ginseng literally means "man root" (referring to ginseng root's characteristic bifuration, similar in shape to the legs of a man). The botanical/genus name *Panax* means "cure-all" in Greek (Chen *et al*. 2008).

Both Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius*) roots are adaptogens, aphrodisiacs, nourishing stimulants and useful in the treatment of type II diabetes (Ng and Yeung 1985), and sexual dysfunction (Murphy and Lee 2002). The root is usually sold in the

Received: 14 May, 2010. Accepted: 18 April, 2011.

Invited Review

Table 1 N-terminal sequences, molecular weights and subunit nature of enzymes and bioactive proteins from ginseng.

Enzyme/Bioactive protein	Molecular weight/subunit	N-terminal amino acid sequence
Alcohol dehydrogenase	29 kDa	MATPTKRVLLTSSGDEISQNIAY
Arginase	352 kDa/ decamer	NA
Catalase	57 kDa/ Precursor protein	MDPYQHRPSSAFNSSYWTTN
Ginsenoside-beta-glucosidase	59 kDa	NA
Glutamate decarboxylase	56 kDa	MVLSKTVSQSDVSISSTFGS
Ribonuclease from ginseng flowers	23 kDa	APNADGFR
Ribonuclease (Quinqueginsin)	53 kDa/ dimer	GAHGARVYNIDRNDV
Ribonuclease (Panaxagin)	52 kDa/ dimer	GAHGARVYNIFRAALXRALN
Ginseng major protein	56 kDa/ dimer	RDYPAAMFALRQQWP
Latex-like protein	16.9 kDa	MGLTGKLICQTGIKSDGDVFHEL
Medicinal peptide	5.9 kDa	NA
Polygalacturonase inhibiting proteins	40 kDa	MNAFFFITAILLASAAAVIC
Tetrapeptide with proliferative activity	NA	GREV

NA: information not available

Table 2 Enzymes from ginseng tissues.

Enzyme	Source	pI, optimum pH and temperature	Inducers, activators and inhibitors	Function	Reference
Alcohol dehydrogenase	*P. ginseng* leaves	pI 6.84	Induced by jasmonic acid, abscisic acid and salicylic acid	Protection against hormone-related environmental stress	Kim *et al*. 2009
Arginase	*P. ginseng* roots	pH 9.5 60°C	Activated by Mn^{2+} and Co^{2+} ions, inhibited by agmatine and 5'-deoxy-methylthioadenosine	Catalysis of conversion of arginine into ornithine and urea	Hwang *et al*. 2001
Catalase (PgCat1)	*P. ginseng* leaves, stems, roots, seedlings	NA	Induced by osmotic stress heavy metals, high light irradiation, plant hormones and osmotic agents	Protection against oxidant-associated environmental stresses	Purev *et al*. 2010
Ginsenoside-β-glucosidase	*P. ginseng* roots	pH 5 60°C	Activated by, Ca^{2+}ions, inhibited by Cu^{2+} ions	Hydrolysis of β-(1→2)-glucoside of ginsenoside Rg3 to ginsenoside Rh2	Zhang *et al*. 2001
Glutamate decarboxylase	*P. ginseng* leaves, roots, rhizomes, buds, seedlings	NA	Induced by temperature stress, osmotic stress, anoxia and mechanical damage	Catalysis of conversion of L-glutamate to γ-aminobutyric acid	Lee *et al*. 2010
Ribonuclease	*P. ginseng* flowers	pH 6-7 50°C	NA	Ribonucleolytic activity toward poly U, much lower activity toward poly C, poly A and poly G	Wang and Ng 2004
Ribonuclease (Quinqueginsin)	*P. quinquefolium* roots	pH 6.5	NA	RNase activity toward yeast tRNA and poly C, antifungal activity, and HIV-1 reverse transcriptase inhibitory activity	Wang and Ng 2000
Ribonuclease (Panaxagin)	*P. ginseng* roots	NA	NA	Same as Quinqueginsin	Ng and Wang 2001

NA: information not available

Table 3 Bioactive proteins from ginseng tissues.

Bioactive protein	Source	Inducers , activators and inhibitors	Function	Reference
Ginseng major protein	*P. ginseng* roots	NA	Resistance to pathogen infections	Yoon *et al*. 2002
Latex-like protein	*P. ginseng*	Induced by light and mannitol, reduced by exposure to darkness, H_2O_2, salicylic acid and wounding	Associated with light reaction of photosynthesis	Sun *et al.* 2010
Medicinal peptide	*P. ginseng*	NA	Hypoglycemic, antidiabetic	Yan *et al.* 2003
Polygalacturonase inhibiting protein	*P. ginseng* roots, leaves, buds and stem	NA	Reduces aggressive potential of fungal polygalacturonase	Sathiyaraj *et al*. 2010
Radioprotective protein	*P. ginseng*	NA	Increases survival and reduces chromosome aberration in UV-irradiated CHO-K1 cells	Kim and Park 1988; Kim and Choi 1988

NA: information not available

dried form. Dried ginseng leaves are less highly prized, but are sometimes used.

Panax ginseng is available in four forms: The form called fresh ginseng is the raw product. The form called white ginseng (WG) is fresh ginseng which has been cultivated for four to six years, peeled and dried to lower the water content to 12% or less. Ginseng is air dried in the sun and consequently turns to a yellowish-white colour. It may contain less of the therapeutic constituents. Some believe that enzymes in the root degrade these constituents during the course of drying. The form called red ginseng (RG) is harvested after six years of cultivation. The peel is not removed and is steam-cured at 100°C, endowing them with a shiny reddish-brown color. Steaming the root alters its biochemical composition, and minimizes degradation of its active ingredients. Subsequently, the roots are dried. RG is more commonly used as herbal medicine than WG (Fishbein *et al*. 2009). The form called sun ginseng (SG) is produced using a heat processing, which increases ginsenoside components such as ginsenoside-Rg3, -Rk1 and -Rg5 by steaming white ginseng at 120°C for 3 h. SG has higher nitric oxide, superoxide, hydroxyl radical and peroxynitrite scavenging activities compared with RG and WG. The increased steaming temperature produces an optimal amount of biological activity due to its ability to amplify specific ginsenosides. Wild ginseng is ginseng that is not cultivated domestically but rather grows naturally. It is relatively rare and has become increasingly endangered because of the high demand for the product. Wild ginseng can be either Asian or American and can be processed to be red ginseng.

Ginsenosides are the most extensively studied constituents of ginseng. They have diverse biological activities

(Chu and Zhang, 2009). Polysaccharide is another constituent. It possesses immunoenhancing activity (Yu *et al*. 2004). By comparison, the literature on ginseng proteins is meager. The purpose of the present article is to review the proteins produced by American ginseng and Asian ginseng.

The enzymes and other bioactive proteins produced by ginseng are summarized below.

STRESS-RELATED ENZYMES

Alcohol dehydrogenase

The cDNA of *P. ginseng* alcohol dehydrogenase (PgADH; EC 1.1.1.1) was isolated and characterized from leaves. The cDNA had an open reading frame of 801 bp, and a deduced amino acid sequence of 266 residues. The calculated molecular mass of the mature protein was approximately 29 kDa, and the predicted isoelectric point was 6.84. The deduced amino acid of PgADH closely resembled short-chain ADH proteins of other plants. Genomic DNA hybridization analysis indicated that PgADH represents a multi-gene family. Abscisic acid, salicylic acid and especially jasmonic acid significantly induced PgADH expression within 24 h of treatment. The positive response of PgADH to abiotic stimuli suggests that ginseng ADH may protect against hormone-related environmental stresses (Kim *et al*. 2009). It deserves mention that human hepatic ADH catalyzes the oxidation of alcohol to acetaldehyde and oxidation of methanol to formaldehyde.

Catalase

From *P. ginseng*, a cDNA clone containing a catalase (CAT1; EC 1.11.1.6) gene, designated as PgCat1, was obtained. PgCat1 presumably encoded a precursor protein with 492 amino acid residues, and its sequence was strikingly similar to a variety of other CAT1s. Genomic DNA hybridization analysis disclosed that PgCat1 represented a multi-gene family. Reverse transcriptase PCR results revealed different levels of expression of PgCat1 in different parts including leaves, stems, roots of ginseng seedlings. A variety of stresses including heavy metals, osmotic agents, high light irradiation, abiotic stresses and plant hormones brought about a marked induction of PgCat1, indicating that PgCat1 might play a protective role against oxidant-associated environmental stresses (Purev *et al*. 2010). It is noteworthy that there are nonpeptidic components of ginseng with antioxidant activity (Xu *et al*. 2010).

Glutamate decarboxylase

The enzyme glutamate decarboxylase (GAD; EC 4.1.1.15) catalyzes the conversion of L-glutamate to gamma-aminobutyric acid (GABA). A full-length cDNA 1881 bp long encoding GAD (designated as PgGAD) was isolated from ginseng root. The cDNA contained a 1491-bp open reading frame coding for a glutamate decarboxylase with 496 amino acids and possessing a Ser-X-X-Lys active site. The deduced amino acid sequence of PgGAD demonstrated 76-85% homology with other plant GADs, including those of *Arabidopsis*, petunia and tomato. The secondary structure of PgGAD was predicted by utilizing SOPMA software program. Southern blot analysis of genomic DNA indicated over one copy of the PgGAD gene. The stem of *P. ginseng* seedlings showed a higher level of expression than bud, leaf, rhizome and root. The ranking of expression level of PgGAD was in the order, stem > leaves > stems > roots > buds. Temporal analysis of gene expression disclosed a heightened expression after abiotic stresses like temperature stress, osmotic stress, anoxia, and mechanical damage. The enzyme activity of PgGAD was enhanced by 2-fold under cold stress (Lee *et al*. 2010).

PgGAD exhibited 85, 84, 81, and 76% of sequence identity with *Petunia x hybrid* GAD (Baum *et al*. 1993), *Arabidopsis thaliana* GAD1 and GAD2 (Arazi *et al*. 1995), *Lycopersicon esculentum* GAD (Gallego *et al*. 1995), respectively, but there was lower than 50% identity to microbial and animal GAD.

Results of secondary structure analysis disclosed that PgGAD was comprised of 224 α-helix and 35 β-turns jointed by 67 extended strands, and 168 random coils. In this aspect, PgGAD demonstrated remarkable resemblance to some other plant GADs. *P. hybrid* GAD had 225 α-helices, 30 β-turns jointed by 67 extended strands, and 172 random coils. *A. thaliana* (GAD1) had 187 α-helices, 32 β-turns jointed by 84 extended strands, and 191 random coils. *A. thaliana* (GAD2) had 216 α-helices, 30 β-turns jointed by 71 extended strands, and 177 random coils. *L. esculentum* GAD had 216 α-helices, 30 β-turns jointed by 71 extended strands, and 177 random coils.

Spermidine synthase (EC 2.5.1.16)

The 36-kDa enzyme possessed a predicted isoelectric point of 5.02 and a deduced amino acid sequence exhibiting remarkable homology to the *Lotus japonicus* enzyme. The enzyme was markedly induced by salt (41.5-fold increase), significantly induced by chilling, abscisic acid and jasmonic acid (>10-fold increase), and weakly induced by mannitol and $CuSO_4$ (Parvin *et al*. 2010)

Chitinases

Two 31-kDa chitinases (SBF1 and SBF2) that exhibited an optimum pH of 5-5.5 and an optimum temperature of 40-50°C were purifed from Korean ginseng (*Panax ginseng*) root. The N-terminal sequence of SBF1 closely resembled those of other plant chitinases but that of SBF2 was blocked. Their K_m values were 4.6 mM and 7.14 mM and their V_{max} values were 220 and 287 mmol/mg protein/h, respectively (Moon *et al*. 2010).

A class 1 chitinase (Pg Chi-1) is a 34.9 kDa protein expressed constitutively in all studied organs of ginseng plant. Various abiotic stresses e.g. Cu, H_2O, mannitol, jasmonic acid and NaCl and biotic stresses including bacterial , fungal and nematodes increased the expression of Pg-Chi (Pulla *et al*. 2011).

PACAP-immunoreactive component

A PACAP-immunoreactive component was purified from red ginseng (*Panax ginseng*). It elevated cAMP production in human neuroblastoma NB-ok-1 cells and oral administration of the component to rats produced an increase in the level of this component in blood (Takashima *et al*. 2006).

HYDROLYTIC ENZYMES

Arginase

A decameric 352-kDa arginase (EC 3.5.3.1) with relatively high thermostability was isolated from root tissues of three-year-old ginseng (*P. ginseng*). It manifested an optimal temperature and pH at 60°C and pH 9.5, respectively. Arginine analogues were not capable of substituting arginine as substrate, and a cysteine residue was located right at or in the vicinity of the active site. Mn^{2+} and Co^{2+} ions activated the enzyme. Agmatine and 5'-deoxy-methylthioadenosine were inhibitors. Specific activities of the enzyme in sliced ginseng roots were enhanced by gibberellic acid, indole-3-acetic acid, kinetin and putrescine, but not by putrescine. Increases in arginase activities induced by these plant hormones may regulate intracellular polyamine metabolism (Hwang *et al*. 2001).

Arginases have been purified from non-shade plants including soybean (Kang and Cho 1990), iris bulbs (Boutin 1982), peanut seedlings (Desai 1983), and *Evernia prunastri* (Martín-Falquina and Legaz 1984). Previously there were no accounts on shade plant arginases like that from ginseng. The specific activity of ginseng arginase rose prog-

ressively after germination, and attained peak level in 3-year-old roots. Beyond 3 years, the specific activity of the enzyme underwent a steady decrease. Leaves from 1 to 5-year-old ginsengs had very low activities as compared to that of roots. Heat treatment was employed to eliminate substantial amounts of extraneous proteins which were not removed or separated using various column chromatographic procedures. The conditions used in heat treatment did not denature arginase.

Ginseng arginase showed obvious differences from other arginases in molecular mass and number of subunits. The molecular masses of arginases from animals and microorganisms range from 98 kDa (Brusdeilins *et al*. 1983) to 278 kDa (Reddy and Campbell 1968). There are 3-10 subunits. *Saccharomyces cerevisiae* arginase is trimeric (Green *et al*. 1990), mouse liver arginase is tetrameric (Spolarics and Bond 1988), and iris bulb arginase (Boutin 1982) and *Bacillus brevis* arginase (Kanda *et al*. 1997) are hexameric. Carbonic anhydrase from *Vicia canencens* leaves (Demir *et al*. 1999) and arginine decarboxylase from *Escherichia coli* B (Boecker 1978) are decameric. Ginseng arginase as a decameric plant arginase is distinctive.

The isolated ginseng arginase demonstrated specificity for L-arginine and it was devoid of activity toward analogs such as L-homoarginine, agmatine, and canavanine. Similarly, arginases from iris bulb (Boutin 1982), rat small intestine (Fujimoto *et al*. 1976), and *Helicobacter pylori* (Mendz *et al*. 1998) were highly specific for L-arginine. Other arginases might differ in substrate specificity. Rat liver arginase (Reczkowski and Ash 1994) could employ L-arginine, argininamide, L-canavanine, and homoarginine as substrates.

Exposure of the ginseng arginase to the sulfhydryl-specific reagents, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and p-chloromercuribenzoate (PCMB) ensued in loss of activity. The effect of DTNB was reversed by treatment with dithiothreital (DTT), indicating that DTNB-induced inactivation was attributed to modification of an active-site cysteine residue. Arginine diminished the modification rate, signifying the direct involvement of cysteine residue in enzyme activity. In contrast, *N*-bromosuccinimide treatment of rat liver arginase disclosed the paramount importance of a histidine residue to the catalytic activity of the enzyme (Daghigh *et al*. 1996). X-ray diffraction study revealed that His, Asp, Glu, and Arg residues were involved in arginine binding and catalysis (Kanyo *et al*. 1996). The data were in keeping with results of Ber *et al*. (1978) and Muszyńska *et al*. (1972) demonstrating that the SH group of cysteine was crucial for the catalytic activity of non-shade plant arginases. The SH-group of cysteine was required for activity of arginases of non-shade plants and shade plants.

Agmatine and 5′-deoxy-5′-methylthioadenosine (MTA) exerted an inhibitory action on ginseng arginase, indicating that they could diminish levels of putrescine by decreasing ornithine. However, there was little information regarding the levels of these compounds. Thus, the significance of agmatine and MTA in ginseng roots awaits elucidation.

The K_m of ginseng arginase toward arginine was 82.7 mM, similar to that reported for soybean (83 mM) (Kang and Cho 1990), but differed from those reported for the embryo axis of *Arachis hypogea* seedlings (5.6 mM) (Desai 1983), *Bacillus brevis* (12.8 mM) (Kanda *et al*. 1997), *Saccharomyces cerevisiae* (15.7 mM) (Green *et al*. 1990), and rat small intestine (19 mM) (Fujimoto *et al*. 1976). Ginseng arginase exhibited the highest activity at pH 9.5, like arginases from iris bulbs (Boutin 1982), *Bacillus brevis* (Kanda *et al*. 1997), soybean (Kang and Cho 1990) and rat small intestine (Fujimoto *et al*. 1976), but very different from mouse liver arginase (Spolarics and Bond 1998). The optimum temperature for arginase from soybean (Kang and Cho 1990), iris bulb (Boutin 1982), *Bacillus brevis* (Kanda *et al*. 1997), rat small intestine (Fujimoto *et al*. 1976), *Saccharomyces cerevisiae* (Green *et al*. 1990), and human liver (Kuhn *et al*. 1995) resided in the range of 30 to 37°C. However, the optimum temperature for ginseng arginase was 60°C, which was high compared to those reported so far.

Ginsenoside-β-glucosidase

A 59-kDa ginsenoside-β-glucosidase (EC 3.2.1.21), which hydrolyzes β-(1→2)-glucoside of ginsenoside Rg3 to ginsenoside Rh2, was purified from ginseng roots. Its optimum temperature and optimum pH were 60°C, and pH 5.0, respectively. Mg^{2+}, Fe^{3+}, and Zn^{2+} ions did not affect the activity of the enzyme. Ca^{2+} ions activated whereas Cu^{2+} ions inhibited the enzyme. Ginsenoside-β-glucosidase may be a special β-glucosidase different from the original exocellulase such as β-glucosidase (Zhang *et al*. 2001).

The β-glucosidase present in ginseng hydrolyzes the β-(1→2)-glucoside bond of ginsenosides. Hence, it is called ginsenoside-β-glucosidase. Ginsenoside Rg3, a protopanaxadiol-type saponin, could be hydrolyzed by the enzyme (Zhang *et al*. 2001).

Ginsenoside-β-glucosidase from ginseng hydrolyzed more than 60% of Rg3 to Rh2. In contrast, the β-glucosidases from almonds (*Prunus amygdalus*) and *Clostridium thermocopriae* are not capable of hydrolyzing Rg3. This indicates that the saponin enzyme from ginseng is likely a special β-glucosidase, β-(1→2)-ginsenoside hydrolase (Zhang *et al*. 2001).

Comparison of the hydrolysis of different *p*-nitrophenyl-β-saccharosides such as glucoside, galactoside, arabinoside, xyloside, and rhamnoside revealed that *C. thermocopriae*, β-glucosidase could only hydrolyze *p*-nitrophenyl-β-glucoside, and the almond enzyme could hydrolyze all substrates tested except *p*-nitrophenyl-β-rhamnoside. However, the ginseng enzyme has only a slight activity toward hydrolyzes these substrates. This demonstrated that ginsenoside-β-glucosidase may be a special β-glucosidase distinct from the original exocellulase such as β-glucosidase (Zhang *et al*. 2001).

ENZYMES WITH DEFENSE ACTIVITY

Ribonuclease

A 23-kDa enzyme with much more potent ribonucleolytic activity toward poly(U) than toward poly(C), poly(A) and poly(G), was isolated from an aqueous extract of Chinese ginseng (*P. ginseng*) flowers. The purification procedure consisted of ion exchange chromatography and affinity chromatography. The ribonuclease (EC 3.1.27.5) was unadsorbed on the anion exchanger diethylaminoethyl-cellulose (DEAE-cellulose), and adsorbed on Affi-gel blue gel and the cation exchanger carboxymethyl-cellulose. The ribonuclease manifested an optimal pH at pH 6-7. There was a precipitous fall in enzymatic activity as the pH was decreased or elevated from pH 6.7. The enzyme exhibited an optimum temperature at 50°C. Just about 20% of the maximal activity was left when the temperature was lowered to 20°C or raised to 80°C. The characteristics of this ribonuclease were different from those of ribonuclease previously purified from ginseng roots (Wang and Ng 2004).

A homodimeric 53-kDa protein, designated as quinqueginsin, was isolated from the roots of American ginseng, *Panax quinquefolium*. It was unadsorbed on the anion exchanger DEAE cellulose in low ionic strength and neutral pH, and adsorbed on the affinity media Affigel blue gel and the cation exchanger SP-Sepharose under similar conditions. Its N-terminal amino acid sequence resembled those of plant ribosome inactivating proteins and fungal ribonucleases to some extent. The protein displayed a variety of biological activities. It exhibited ribonucleolytic activity toward yeast tRNA and specific RNase activity toward poly C. It inhibited translation in a cell-free rabbit reticulocyte lysate system with an IC_{50} of 0.26 nM, and inhibited mycelial growth in the fungi *Fusarium oxysporum*, *Rhizoctonia solani*, and *Coprinus comatus.* Its inhibitory action toward human immunodeficiency virus-1 reverse transcriptase was enhanced after chemical modification with succinic anhydride (Wang and Ng 2000).

Quinqueginsin demonstrated cell-free translation-inhib-

itory activity (IC_{50} = 0.26 nM) and RNase activity (1450 u/mg). The RNase activity was specific toward poly C: the activity toward poly A, poly G, poly U and poly C was 32.66, 0.21, 29.91 and 548.57 U/mg respectively. The pH optimum of the RNase activity of quinqueginsin was 6.5. The ribonuclease activity was high compared with the plant RIPs α- and β-momorcharins (Mock *et al*. 1996) while cell-free inhibitory activity was within the range reported for ribosome inactivating proteins (RIPs) (Ho *et al*. 1991). Quinqueginsin retarded mycelial growth in several fungal species including *Coprinus comatus*, *Fusarium oxysporum* and *Rhizoctonia solani.* It also exhibited anti-human immunodeficiency virus-1 transcriptase inhibitor activity. The biological activities of quinqueginsin are also typical of plant RIPs. The lack of activity of quinqueginsin, in the assay for N-glycosidase activity in which the activity typical of RIPs was evidenced by the presence of Endo's band, might be caused by the high ribonuclease activity of the protein which broke down the RNA.

Quinqueginsin exhibited chromatographic behavior on DEAE-cellulose, SP Sepharose and Affi-gel blue gel identical to that of RIPs. The N-terminal sequence (the first 15 residues) of quinqueginsin demonstrated sites of similarity to those of plant ribosome inactivating proteins (Ho *et al*. 1991; Barbieri *et al*. 1993). The N-terminal sequence of quinqueginsin also manifested resemblance to segments of the sequences of fungal RNases to some extent although there was little homology to the previously reported ginseng callus RNases (Moiseyev *et al*. 1997). Quinqueginsin induced a dose-dependent inhibition of HIV-1 reverse transcriptase which was augmented after succinylation. It also exhibited a slight suppressive action on α- and β-glucosidases.

A homodimeric 52-kDa ribonuclease designated as panaxagin, with an N-terminal sequence dissimilar to ribonucleases previously reported from ginseng calluses, was isolated from the roots of the Chinese ginseng, *P. ginseng*. The purification protocol entailed saline extraction, $(NH_4)_2SO_4$ precipitation, ion exchange chromatography on DEAE-cellulose, affinity chromatography on Affi-gel blue gel, ion exchange chromatography on SP-Sepharose, and FPLC gel filtration on Superdex 75 by fast protein liquid chromatography. Its N-terminal amino acid sequence showed resemblance to plant ribosome inactivating proteins and fungal ribonucleases. Panaxagin demonstrated ribonucleolytic activity toward yeast transfer RNA, inhibited translation in a cell-free rabbit reticulocyte lysate system, and hampered mycelial growth in the fungi *Coprinus comatus* and *Fusarium oxysporum*. However, there was no antifungal activity on *Rhizoctonia solani.* Its inhibitory activity against human immunodeficiency virus reverse transcriptase was enhanced after succinylation (Ng and Wang 2001).

GINSENG MAJOR PROTEIN

A major protein designated as ginseng major protein or GMP, composed of two 28-kDa subunits, was isolated from ginseng roots. The procedure involved ammonium sulfate fractionation, gel filtration chromatography, ion-exchange FPLC, and fast performance liquid chromatofocusing. The elution profile of GMP during gel filtration chromatography varied, depending on the ionic strength of buffers employed. In a buffer of low ionic strength, GMP existed as a complex with carbohydrate, which could be dissociated only at high ionic strength. Carbohydrate composition in GMP detected by gas chromatography varied, depending on the isolation method used. These results suggest that carbohydrates are bound non-covalently to GMP which has an abundance of acidic amino acids (Yoon *et al*. 2002).

GLUTATHIONE-RELATED OLIGOPEPTIDES

The isolation of six γ-glutamyl oligopeptides from aqueous methanol extracts of *P. ginseng* roots, by employing ion-exchange chromatography, gel filtration and reverse-phase high-performance liquid chromatography, was reported by Chen *et al*. (1998). Their structures, which are related to that of oxidizedglutathione, are as follows: P-I (N-γ-glutamylcystinyl-bis-glycine), P-ll (γ-glutamylcysteinylglycine disulfide, oxidized glutathione), P-III (*N*,*N*'-bis-γ-glutamylcystinylglycine), P-IV (γ-glutamylcysteinylglycinamide disulfide), P-V (*N*-γ-glutamylglycylcysteine disulfide), and P-VI(γ-glutamylarginine). The first five are related to oxidized glutathione. P-V (N-γ-glutamylglycylcysteine disulfide) is a peptide with somnogenic effect more potent than that of P-II. Whether the aforementioned oligopeptides possess activities characteristic of glutathione such as antioxidative activity remains to be elucidated.

PROTEIN WITH OSMOREGULATORY FUNCTION

Latex-like protein

The isolation and expression analysis of a major latex-like protein (MLP151) gene in *P. ginseng* has been reported. A full-length cDNA of MLP151 was 850 bp and contained a 456 bp open reading frame encoding a polypeptide of 151 amino acids. A theoretical pI value of MLP151 was 4.86 and calculated molecular weight was about 16.87 kDa. The MLP homolog proteins are found in various plants and neighbor-joining analysis disclosed that MLP151 had the closest distance with Sn-1 (bell pepper, MLP homolog gene). The expression of MLP151 was low in plantlets but induced by treatment with light and mannitol, and markedly reduced by exposure to darkness, H_2O_2 and salicylic acid and wounding (Sun *et al*. 2009).

MLP151 showed marked similarity to many other major latex-like proteins from *Arabidopsis thaliana* at the amino acid level and also high homology to those from peach (*Prunus persica*, Pp-MLP), bell pepper (*Capsicum annuum*, Sn-1), *A. thaliana* (MLP31), melon (*Cucumis melo*, MEL7), strawberry (*Fragaria vesca*, 3.1.R4), soybean (*Glycine max*, Msg), and opium poppy (*Papaver somniferum*, MLP15).

The secondary structure of MLP151 peptide showed 35.76% of alpha helices, 23.18% of extended strands, 7.95% of beta turns and 33.11% of random coils.

The major latex-like protein was up-regulated by high light intensity in ginseng leaves, but its role in plants is unclear (Nam *et al*. 2003). Expression of MLP151 was decreased by dark stress treatment. It is suggested that expression of MLP151 gene is associated with the light reaction of photosynthesis.

MLP151 gene was induced by mannitol. Since osmoregulatory genes including asparagine synthetase were induced by different osmotic stresses such as salts, mannitol or heavy-metals (Herrera-Rodriguez *et al*. 2007), but MLP151 was inhibited by NaCl treatment, the function of MLP151 gene may not be related to osmo-regulation. The light-induced gene, sigma factor (SIG5) (Nagashima *et al*. 2004), was induced by mannitol treatment. MLP151 gene was induced by both light and mannitol.

H_2O_2 stress did not up-regulate but rather suppressed the expression of MLP151 gene. Salicylic acid lowered catalase activity *in vitro* and brought about a rise in H_2O_2 concentration in tobacco plants (Chen *et al*. 1993) Addition of H_2O_2 upregulated catalase gene expression (Prasad *et al*. 1994). Both exogenous H_2O_2 and salicylic acid inhibited the expression of MLP151 in ginseng. Thus, MLP is not involved in the extinction of high concentrations of H_2O_2. Probably MLP151 protects plant cells by employing mechanisms distinct from that of catalase.

PROTEINS/PEPTIDES WITH MEDICINAL ACTIVITY

Medicinal peptide

Various non-peptidic constituents with hypoglycemic activity have been reported from ginseng (Ng and Yeung 1985; Liu *et al*. 2009). A medicinal peptide, Gsp (Ginseng peptide), with potential use as an antidiabetic drug was isolated

from ginseng. It was secreted in a recombinant form from the yeast *Pichia pastoris*. A DNA fragment, coding for four copies of Gsp each separated by a basic amino acid, was synthesized and inserted into the *P. pastoris* expression vector plasmid pPIC9. After electroporation of the resulting vector, pPIC9-Gsp, into the yeast, transformants were selected. Recombinant pre-Gsp secreted from *P. pastoris* had a molecular weight of 5.9 kDa and mature recombinant Gsp had an amino acid sequence identical to that of native Gsp. Optimization of the culturing process led to a yield of pre-Gsp as high as 800 mg/L in the clarified broth. A continuous batch fermentation process was developed that facilitated the same cell population to be reused five times without reduction of the level of expression. This continuous culturing process, which has the advantage of considerably reducing both time and cost in pharmaceutical production, may find application in the production of other recombinant proteins in *P. pastoris* (Yan *et al*. 2003).

Recombinant pre-Gsp was isolated from culture supernatants, following a 4-day induction, by a procedure that entailed precipitation at the predicted pI of Gsp (3.32) and gel filtration on Sephadex G-50. The purified pre-Gsp was then subjected to digestion with trypsin and carboxypeptidase B, in order to eliminate the extra C-terminal amino acid and generate mature monomeric Gsp. Amino acid sequencing and trypsin mapping revealed that the relative molecular mass and amino acid sequence of the recombinant product were the same as those of native Gsp. A test using hyperglycemic rats showed that recombinant and native Gsp were capable of lowering blood glucose levels by about 5 and 60%, respectively.

The best yield of recombinant pre-Gsp was obtained by using peptone instead of tryptone in the medium. Raising the peptone concentration beyond 345 mg/ml did not further increase the level of the recombinant protein levels. The optimum ratio of yeast extract to peptone in the medium was approximately 1: 2.

A process was developed for continuous production of recombinant pre-Gsp protein from the same batch of host cells. The process entailed batch culturing of the *P. pastoris* Gsp expression strain in a fermenter according to the standard procedure. Following methanol induction for 4 days, cultures were harvested and the supernatants used for purification of pre-Gsp. The cell pellets were resuspended in fresh methanol induction medium and cultured in the fermenter for 4 additional days to allow pre-Gsp synthesis and secretion. This process of harvesting and re-inoculation was repeated four times. This continuous batch fermentation process could be repeated at least five times with the same cells without major loss in the protein yield. With the use of this process, the time required to grow new batches of expression strain cells could be cut, and the cost of the growth medium could be reduced.

DEFENSIVE PROTEINS

Polygalacturonase inhibiting protein

Polygalacturonase inhibiting proteins (PGIPs) are the major defense proteins which play an important role in resistance to infection of pathogens. A putative novel gene encoding PGIP was isolated from *P. ginseng* C.A. Meyer, which manifested 70 and 68% similarity to chick pea and *Arabidopsis* PGIPs, respectively. The cDNA of PgPGIP was 1,275 bp long and its open reading frame encoded a polypeptide of 366 amino acids. The deduced amino acid sequence of PgPGIP had a characteristic PGIP topology. The level of transcription of PgPGIP was increased after wounding and infection with phytopathogenic fungi including *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Cylindrocarpon destructans*, *Fusarium oxysporum*, *Phythium ultimum* and *Rhizoctonia solani.* The constitutive PgPGIP expression of 4-years-old ginseng plants showed increased transcript level in roots, followed by buds, stems and leaves, suggesting developmental regulation of the gene. The crude PGIP extracts derived from fungus-infected plants directly reduced the aggressive potential of PGs from different fungi. Like other PGIPs, PgPGIP also exhibited a broad spectrum of inhibitory activity (Sathiyaraj *et al*. 2010). However, to date, a direct suppressive action of PGIPs on mycelial growth in fungi has not been demonstrated.

Radioprotective protein

The effects of a ginseng protein fraction with radio-protective action from Korean ginseng on relative survival and chromosome aberration were studied in UV-irradiated CHO-K1 cells by Kim and Park (1988). Treatment of the cells with the protein fraction (100 μg/ml), before UV irradiation at 4 J/m^2, increased the survival rate to 53.8% compared with 40.6% in control. Treatment of the cells with the protein (100 μg/ml) subsequent to UV irradiation at 4 and 8 J/m2 elevated the survival rate to 85.4 and 24.0% from 79.2 and 11.5% in control, respectively. When the ginseng protein (800 μg/ml) was added to cells exposed to UV light at 10, 20, 30 J/m^2, the incidence of chromosome aberration (CA) was decreased to almost the same level irrespective of the UV intensity of light. When the concentration of ginseng protein was increased from 200 to 800 μg/ml, at the UV doses of 10, 20, 30 J/m^2, the incidence of CA incidence was reduced as the dose of ginseng protein increased, at all UV doses tested. The results indicate that the protein may attenuate UV light-induced cell damage, especially damage to DNA, or play a role in repair processes of damaged DNA, to augment cell survival and diminish chromosome aberrations.

In order to unravel the mechanism of the antiradiation activity of ginseng protein, the effects of the protein on UV irradiation-induced sister chromatid exchanges (SCE) in CHO-Kl cells were examined. When cells were irradiated with 254 nm UV light at the dose of 0 to 80 erg/mm^2, there was an over two-fold increase in SCE. However, exposure of the cells to radio-protective ginseng protein prior to and following UV irradiation brought about a significant decrement in SCE at all UV doses in both cases with no significant differences. When the cells were treated with ginseng protein alone without UV irradiation, no changes in SCE frequency were detected regardless of when ginseng protein was added. These findings indicate that the mechanism of radioprotective activity of vadio-protective ginseng protein involves a reduction of DNA damage (Kim and Choi 1988). It is interesting to note that ginseng polysaccharide also exerts a radio protective action (Kim *et al*. 2007).

Tetrapeptide with proliferative activity

An alkaline fraction acquired by ion exchange chromatography of the aqueous extract of *P. ginseng* roots enhanced the proliferative response of baby hamster kidney-21 cells. After MCI-gel CHP 20P column chromatography, followed by dialysis and reversed-phase HPLC, the alkaline fraction was resolved into six fractions. An active colorless pure compound, Gly-Arg-gamma-Glu-Val-NH_2, was obtained from fraction 2. It produced 20% enhancement of proliferation of BHK-21 cells at a concentration of 3.40 μM (Yagi *et al*. 1994).

SUMMARY AND FUTURE PERSPECTIVES

The adaptogenic action of ginseng is well known (Nocerino *et al*. 2007). Some of the aforementioned proteins such as alcohol dehydrogenase, catalase, and radioprotective protein may partially account for the adaptogenic activity of ginseng. Other nonpeptidic ginseng constituents including ginsenosides polysaccharides may also contribute to anti-stress and antioxidant activities.

From the foregoing account, it can be seen that enzymes including arginase, ginsenoside glucosidase, catalase, glutamate decarboxylase, alcohol dehydrogenase and ribonucleases, are present in ginseng. In addition, a variety of bio-

active proteins comprising tetrapeptide with proliferative activity, glutathione-related oligopeptides, ginseng major protein, polygalacturonase inhibiting protein, latex-like protein, radioprotective protein and medicinal peptide have been isolated from ginseng. Some of the enzymes such as ribonucleases have other biological activities including antifungal and HIV-1 reverse transcriptase inhibitor activities. In fact, ribonucleases belong to the family of pathogenesis related proteins. A possible mechanism of the antipathogenic action of ribonucleases is the hydrolysis of RNA of invading pathogens. Ginsenoside-β-glucosidase is involved in ginsenoside metabolism. Catalase (Cat1), glutamate decarboxylase (GAD), alcohol dehydrogenase and ribonuclease are produced by ginseng as well as other organisms (Green 1994) Radioactive protein, ginseng major protein and ginseng medicinal peptide are unique to ginseng whereas PGIP and latex-like proteins are also found in other plants (Sun *et al*. 2010).

Comparison of the aforementioned ginseng enzymes and proteins with counterparts from other organisms discloses some unique features of the ginseng products. Ginseng ginsenoside-β-glucosidase manifests distinct substrate specificity. Ginseng ribonucleases differ from some of the known plant ribonucleases in N-terminal sequence and in possession of antifungal activity. In contrast, striking resemblance to its counterparts from other plants is demonstrated by glutamate decarboxylase, catalase, alcohol dehydrogenase and major latex-like protein. Ginseng radioprotective protein and ginseng medicinal peptide can account for some of the reported medicinal effects of ginseng although ginsenosides contribute to the bulk of the effects (Leung 2007). It is anticipated that more ginseng proteins with important functions will be revealed by future investigations. Proteomics is a tool of choice for identification of the functional proteins in ginseng.

Research on ginseng proteins is much less voluminous compared with research on ginsenosides. It is not known in detail whether there are differences in proteins produced by Korean ginseng and American ginseng. More research is necessary to address these questions.

REFERENCES

Arazi T, Baum G, Snedden WA, Shelp BJ, Fromm H (1995) Molecular and biochemical analysis of calmodulin: Interactions with the calmodulin-binding domain of plant glutamate decarboxylase. *Plant Physiology* **108**, 551-561

Barbieri L, Battelli MG, Stirpe F (1993) Ribosome-inactivating proteins from plants. *Biochimica et Biophysica Acta* **1154**, 237-282

Baum G, Chen Y, Arazi T, Takatsuji H, Fromm H (1993) A plant glutamate decarboxylase containing a calmodulin binding domain. Cloning, sequence, and functional analysis. *Journal of Biological Chemistry* **268**, 19610-19617

Ber E, Muszyńska G, Ĉechová D (1978) The lack of free SH groups in rat liver arginase. *Bulletin of the Academy of Polonerry Science. Series Science Biology* **26**, 665- 668

Boecker EA (1978) Arginine decarboxylase from *Escherichia coli* B: Mechanism of dissociation from the decamer to the dimer. *Biochemistry* **17**, 258-263

Boutin JP (1982) Purification, properties and subunit structure of arginase from iris bulbs. *European Journal of Biochemistry* **127**, 237-243

Brusdeilins M, Hapke C, Huberts HH, Schumacher K (1983) Identification of the apparently lymphocyte-specific human liver derived inhibitory protein (LIP) as cytoplasmic liver L-arginase. *Journal of Immunology* **131**, 2427-2431

Chen CF, Chiou WF, Zhang JT (2008) Comparison of the pharmacological effects of *Panax ginseng* and *Panax quinquefolium*. *Acta Pharmacologica Sinica* **29**, 1103-1108

Chen Z, Silva H, Klessig DF (1993) Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Science* **262**, 1883-1886

Chen ZK, Fan CX, Ye YH, Yang L, Jiang Q, Xing QY (1998) Isolation and characterization of a group of oligopeptides related to oxidized glutathione from the root of *Panax ginseng*. *Journal of Peptide Research* **52**, 137-142

Choi KT (2008) Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax ginseng* C A Meyer. *Acta Pharmacologica Sinica* **29**, 1109-1118

Chu SF, Zhang JT (2009) New achievements in ginseng research and its future prospects. *Chinese Journal of Integrated Medicine* **15**, 403-408

Daghigh F, Cavalli RC, Soprano DR, Ash DE (1996) Chemical modification and inactivation of rat liver arginase by N-bromosuccinimide: Reaction with His141. *Archives of Biochemistry and Biophysics* **327**, 107-112

Demir Y, Demir N, Kufrevioglu O (1999) Carbonic anhydrase from *Vicia canencens* leaves. *Preparative Biochemistry and Biotechnology* **29**, 235-244

Desai HV (1983) Purification and properties of arginase from *Arachis hypogea* L. seedlings. *Indian Journal of Biochemistry Biophysics* **20**, 236-237

Fishbein AB, Wang CZ, Li XL, Mehendale SR, Sun S, Aung HH, Yuan CS (2009) Asian ginseng enhances the anti-proliferative effect of 5-fluorouracil on human colorectal cancer: Comparison between white and red ginseng. *Archives of Pharmaceutical Research* **32**, 505-513

Fujimoto M, Kameji T, Kanaya A, Hagihira H (1976) Purification and properties of rat small intestinal arginase. *Journal of Biochemistry* **79**, 441-449

Gallego PP, Whotton L, Picton S, Grierson D, Gray JE (1995) A role for glutamate decarboxylase during tomato ripening: the characterisation of a cDNA encoding a putative glutamate decarboxylase with a calmodulin-binding site. *Plant Molecular Biology* **27**, 1143-1151

Green PJ (1994) The ribonucleases of higher plants. *Annual Reviews of Plant Physiology Plant Molecular Biology* **45**, 421-445

Green SM, Eisenstein E, McPhie P, Hensley P (1990) The purification and characterization of arginase from *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* **265**, 1601-1607

Herrera-Rodriguez MB, Perez-Vicente R, Maldonado JM (2007) Expression of asparagine synthetase genes in sunflower (Helianthus annuus) under various environmental stresses. *Plant Physiology and Biochemistry* **45**, 33-38

Ho WKK, Liu SC, Shaw PC, Yeung HW, Ng TB, Chan WY (1991) Cloning of the cDNA of alpha-momorcharin: a ribosome inactivating protein. *Biochimica et Biophysica Acta* **1088**, 311-314

Hwang HJ, Kim EH, Cho YD (2001) Isolation and properties of arginase from a shade plant, ginseng (*Panax ginseng* C.A. Meyer) roots. *Phytochemistry* **58**, 1015-1024

Kanda M, Ohgishi K, Hanawa T, Saito Y (1997) Arginase of *Bacillus brevis* Nagano: purification, properties, and implication in gramicidin S biosynthesis. *Archives of Biochemistry and Biophysics* **344**, 37-42

Kang JH, Cho YD (1990) Purification and properties of arginase from soybean, *Glycine max*, axes. *Plant Physiology* **93**, 1230-1234

Kanyo ZF, Scolnick LR, Ash DE, Christianson DW (1996) Structure of a unique binuclear manganese cluster in arginase. *Nature* **383**, 554-557

Kim C, Choi JE (1988) Effects of radioprotective ginseng protein on UV induced sister chromatid exchanges. *Archives of Pharmacal Research* **11**, 93-98

Kim HJ, Kim MH, Byon YY, Park JW, Jee Y, Joo HG (2007) Radioprotective effects of an acidic polysaccharide of *Panax ginseng* on bone marrow cells. *Journal of Veterinary Sciences* **8 (1)**, 39-44

Kim C, Park SY (1988) Effects of ginseng protein on relative survival and chromosome aberration of UV irradiated cells. *Archives of Pharmacal Research* **11**, 225-229

Kim YJ, Shim JS, Lee JH, Jung DY, Sun H, In JG, Yang DC (2009) Isolation and characterization of a novel short-chain alcohol dehydrogenase gene from *Panax ginseng*. *BMB Reports* **42**, 673-678

Kuhn NJ, Ward S, Piponski M, Young TW (1995) Purification of human hepatic arginase and its manganese (II)-dependent and pH-dependent interconversion between active and inactive forms: A possible pH-sensing function of the enzyme on the ornithine cycle. *Archives of Biochemistry and Biophysics* **320**, 24-34

Lee JH, Kim YJ, Jeong DY, Sathiyaraj G, Pulla RK, Shim JS, In JG, Yang DC (2010) Isolation and characterization of a Glutamate decarboxylase (GAD) gene and their differential expression in response to abiotic stresses from *Panax ginseng* C. A. Meyer. *Molecular Biology Reports* **37**, 3455-3563

Leung KW, Yung KK, Mak NK, Yue PY, Luo HB, Cheng YK, Fan TP Yeung HW, Ng TB, Wong RN (2007) Angiomodulaotry and nurological effects of ginsenosides. *Current Medicinal Chemistry* **14**, 1371-1380

Liu Z, Wang LJ, Li X, Hu JN, Chen Y, Ruan CC, Sun GZ (2009) Hypoglycemic effects of malonyl-ginsenosides extracted from roots of *Panax ginseng* on streptozotocin-induced diabetic mice. *Phytotherapy Research* **23 (10)**, 1426-1430

Lu JM, Yao Q, Chen C (2009) Ginseng compounds: An update on their molecular mechanisms and medical applications. *Current Vasccular Pharmacology* **7**, 293-302

Martín-Falquina A, Legaz ME (1984) Purification and properties of the constitutive arginase of *Evernia prunastri*. *Plant Physiology* **76**, 1065-1069

Mendz GL, Holmes EM, Ferrero RL (1998) *In situ* characterization of *Helicobacter pylori* arginase. *Biochimica et Biophysica Acta* **1388**, 465-477

Mock JWY, Ng TB, Wong RNS, Yao QZ, Yeung HW, Fong WP (1996) Demonstration of ribonuclease activity in the plant ribosome-inactivating proteins alpha- and beta-momorcharins. *Life Sciences* **59**, 1855-1859

Moiseyev GP, Fedoreyeva LI, Zhuravlov YN, Yasnetskaya E, Jekel PA, Beintema J J (1997) Primary structures of two ribonucleases from ginseng calluses. New members of the PR-10 family of intracellular pathogenesis-related plant proteins. *FEBS Letters* **407**, 207-210

Murphy LL, Lee TJ (2002) Ginseng, sex behavior and nitric oxide. *Annals of the New York Academy of Science* **962**, 372-377

Muszyńska G, Severina LO, Lobyreva LW (1972) Characteristics of arginases from plant, ureotelic and uricotelic organisms. *Acta Biochimica Polonica* **19**, 109-116

Nagashima A, Hanaoka M, Shikanai T, Fujiwara M, Kanamaru K, Taka-

hashi H, Tanaka K (2004) The multiple-stress responsive plastid sigma factor, SIG5, directs activation of the *psbD* blue light-responsive promoter (BLRP) in *Arabidopsis thaliana*. *Plant Cell Physiology* **45**, 357-368

Nam MH, Heo EJ, Kim JY, Kim SI, Kwon KH, Seo JB, Kwon O, Yoo JS, Park YM (2003) Proteome analysis of the responses of *Panax ginseng* C. A. Meyer leaves to high light: Use of electrospray ionization quadrupole-time of flight mass spectrometry and expressed sequence tag data. *Proteomics* **3**, 2351-2367

Ng TB, Yeung HW (1985) Hypoglycemic constituents of *Panax ginseng*. *General Pharmacology* **16**, 549-552

Ng TB, Wang H (2001) Panaxagin, a new protein from Chinese ginseng possesses anti-fungal, anti-viral, translation-inhibiting and ribonuclease activities. *Life Sciences* **68**, 739-749

Nocerino E, Amato M, Izzo AA (2000) The aphrodisiac and adaptogenic properties of ginseng. *Fitoterapia* **71 (Suppl 1)**, S1-5

Prasad TK, Anderson MD, Martin BA, Stewart CR (1994) Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. *Plant Cell* **6**, 65-74

Purev M, Kim YJ, Kim MK, Pulla RK, Yang DC (2010) Isolation of a novel catalase (*Cat1*) gene from *Panax ginseng* and analysis of the response of this gene to various stresses. *Plant Physiology and Biochemistry* **48**, 451-460

Reczkowski RS, Ash DE (1994) Rat liver arginase: Kinetic mechanism, alternate substrates, and inhibitors. *Archives of Biochemistry and Biophysics* **312**, 31-37

Reddy SR, Campbell JW (1968) A low molecular weight arginase in the earthworm. *Biochimica et Biophysica Acta* **159**, 557-560

Sathiyaraj G, Srinivasan S, Subramanium S, Kim YJ, Kim YJ, Kwon WS, Yang DC (2010) Polygalacturonase inhibiting protein: Isolation, developmental regulation and pathogen related expression in *Panax ginseng* C.A. Meyer. *Molecular Biology Reports* **37**, 3445-3454

Spolarics Z, Bond JS (1988) Multiple molecular forms of mouse liver arginase. *Archives of Biochemistry and Biophysics* **260**, 469-479

Sun H, Kim MK, Pulla RK, Kim YJ, Yang DC (2010) Isolation and expression analysis of a novel major latex-like protein (MLP151) gene from *Panax ginseng*. *Molecular Biology Reports* **37**, 2215-2222

Wang HX, Ng TB (2004) A ribonuclease from Chinese ginseng (*Panax ginseng*) flowers. *Protein Expression and Purification* **33**, 195-199

Wang HX, Ng TB (2000) Quinqueginsin, a novel protein with anti-human immunodeficiency virus, antifungal, ribonuclease and cell-free translation-inhibitory activities from American ginseng roots. *Biochemistry Biophysics Research Communication* **269**, 203-208

Xu H, Jiang H, Wang J, Xie J (2010) Rg1 protects iron-induced neurotoxicity through antioxidant and iron regulatory proteins in 6-OHDA-treated MES23.5 cells. *Journal of Cell Biochemistry* **111 (6)**, 1537-1545

Yagi A, Akita K, Ueda T, Okamura N, Itoh H (1994) Effect of a peptide from *Panax ginseng* on the proliferation of baby hamster kidney-21 cells. *Planta Medica* **60**, 171-173

Yan Y, Chen J, Li J (2003) Overexpression of a small medicinal peptide from ginseng in the yeast *Pichia pastoris. Protein Expression and Purification* **29**, 161-166

Yoon JY, Ha BH, Woo JS, Lim YH, Kim KH (2002) Purification and characterization of a 28-kDa major protein from ginseng root. C*omparative Biochemistry and Physiology B Biochemistry Molecular Biology* **132**, 551-557

Yu JL, Dou DQ, Chen XH, Yang HZ, Guo N, Cheng GF (2004) Immunoenhancing activity of protopanaxatriol-type ginsenoside-F3 in murine spleen cells. *Acta Pharmacological Sinica* **25**, 1671-1676

Zhang C, Yu H, Bao Y, An L, Jin F (2001) Purification and characterization of ginsenoside-beta-glucosidase from ginseng. *Chemical and Pharmaceutical Bulletin (Tokyo)* **49**, 795-798