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Some Biochemical Effects of Saponin Fraction of *Panax Ginseng* C.A. Meyer

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It has been demonstrated that ginseng has a wide range of pharmacological properties including antifatigue and antistress actions, mild normalizing effects on blood pressure and carbohydrate metabolism.

Brekhman and Dardymor(1969) described in their review article that the basic effect of ginseng action is its capacity to increase nonspecific resistance of the organism to various untoward influences from their experimental results.

Oura and his coworkers(1974, 1975) found that aqueous extracts of ginseng stimulated the synthesis of rat liver nuclear RNA *in vitro*. They also found that ginseng could stimulated the carbohydrate metabolism in the liver and could increase the lipid content of adipose tissue. They have considered that the action of ginseng has some special feature in its mode of action and suggested ginseng sapogenin being a kind of metabolic regulator or hormone-like substance.

We(1973, 1977, 1978, 1980) invetigated the solubilizing effect of ginseng saponin. When the saponin was added to chicken intestinal lumen fluids, the lipids found dispersed effectively. Observations of the effect of ginseng saponin on pancreatic lipase and cholesterol esterase supproted the idea that saponin might act as excellent lipid solubilizer. It was demonstrated that water-insoluble vitamins sush as α-tocopherol are more easily absorbed when the vitamins were given orally with the saponins(Joo and Kim, 1984).

Saponins in nature are terpenoides with side chain occur naturally as glycosides in plants. These glycosides(saponins) were long known to lower the surface tension of water and therefore their aqueous solutions froth readily and the saponin causes hemolysis. It is easily understood from their structure that they are amphiphatic having both hydrophobic sapogenin aglycon part and hydrophilic sugar moiety in the molecule, and therefore, they disperse lipids in aqueous medium.

We examined the effect of either purified ginsenoside or the saponin fraction of *Panax ginseng* C.A. Meyer on various enzymes such as dehydrogenases, transaminases, lipases and found that moderate amount(10^{-6}~10^{-4}%) of the saponin stimulated the all enzyme catallyzed reaction so far tested *in vitro* but their higher concentration inhibited the enzyme reactions unexceptionally as shown in Fig. 1. Therefore we have considered that the biphasic and nonspecific action of the saponins might be due to their surface activity and suggested that the surface activity of the saponin might play a significant role on the enzyme catalyzed reactions(Joo *et al.*, 1976; Joo and Han, 1976).

Our ginseng saponin absorption experiment in rats using ^{14}C-labelled saponins prepared from ^{14}C-acetate using ginseng root slices as enzyme source showed that ginsenosides were absorbed partly in the undissociated form and the saponin level in the liver might be maintained at 10^{-6}~10^{-5}% in ginseng administered rats. The turnover rate of the saponins was relatively fast and the half life of ginsenoside Rb_1 was estimated to be about 5 hours(Joo *et al.*, 1986, Lee and Joo, 1983).

From the above considerations it can be expected that ginseng saponins might stimulates unfavoured metabolisms and/or detoxication of toxic substances by raising up the related enzyme activities *in vivo*.

Ethanol is one of the favorite mood-altering drug and its psychic effects, both pleasant and unpleasant, are well known enough but what is less

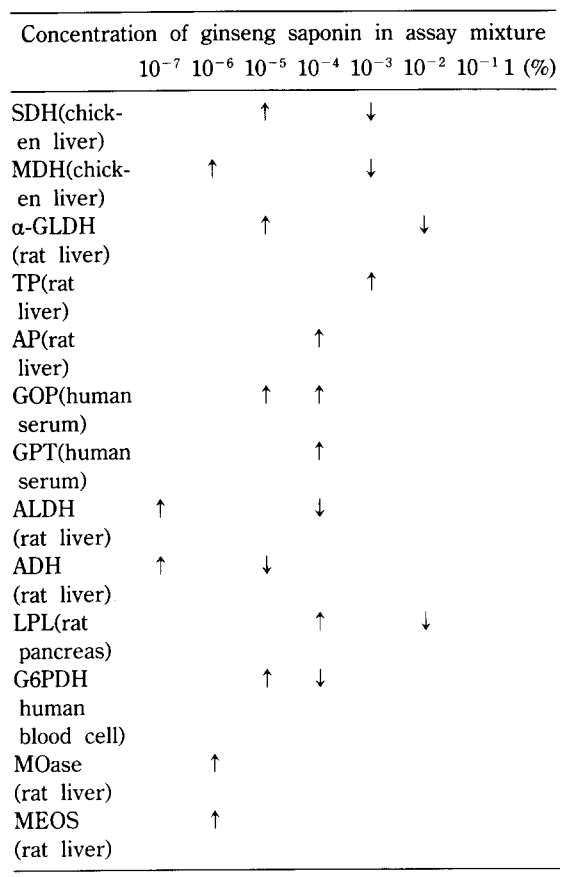

Fig. 1. The effect of ginseng saponin on enzyme catalyzed reactions. The corresponding enzyme reaction rate reached maximum at the concentrations shown by mark(↑) and inhibited when the saponin concentration was over those shown by mark(↓).
Abbreviation : Succinate dehydrogenase(SDH), malate dehydrogenase(MDH), α-Keto-glutarate dehydrogenase(α-KGDH), Isocitrate dehydrogenase (ICDH), L-Glutamate dehydrogenase (GLDH), Glutamate-oxaloacetate transminase (GOT), Glutamate-pyruvate dehydrogenase (GPT), Aldehyde dehydrogenase(ALDH), Alcohol dehydrogenase(ADH), Lipoprotein lipase (LPL), Alkaline phosphatase(AP), Tryptophan pyrolase(TP), Glucose 6-phosphate dehydrogenase (G6PDH), Monoamine oxidase(MOase), Microsomal ethanol oxidizing system (MEOS).

known is that alcohol is a toxic drug: its overconsumption taxes the body's economy, produced a number of pathological changes particularly in the liver and impairs biological functions.

Table 1. Subcellular distribution of aldehyde dehydrogenase (ALDH) in rat liver

Subcellular fraction	Relative activity(%)
Mitochondria	51.2
Cytosol	17.3
Microsome	34.0

From Joo, C.N. and Oh, E.S.(1989): *Korean Biochemical J.*, **22**(3), 312-320.

Table 2. The effect of the saponin fraction of *Panax ginseng* C.A. Meyer on alcohol dehydrogenase (ADH), cytosolic, mitochondrial and microsomal aldehyde dehydrogenase(ALDH) and microsomal ethanol oxidizing system(MEOS) of rat liver. Relative activity(mean of three determinations) was expressed assuming that of control being 100

Saponin conc.(%)	ADH	Mitochondrial ALDH	Cytoplasmic ALDH	Microsomal ALDH	MEOS
10^{-10}	–	–	–	109	104
10^{-9}	107	–	–	122	114
10^{-8}	101	108	142	109	113
10^{-7}	113	123	166	104	116
10^{-6}	103	113	166	105	140
10^{-5}	93	–	–	106	138
10^{-4}	89	–	–	94	102
10^{-3}	–	–	–	98	112

Unlike carbohydrate and fats, alcohol is essentially foreign to the body and it is known that the body get rid of it by oxidizing alcohol mainly in the liver. Present knowledge on alcohol metabolism showed that over-consumption of alcohol causes cirrhosis and death not only because alcoholism promotes malnutrition but also because alcohol and its metabolite effects are reported directly linked to the first two products of its oxidation, hydrogen and acetaldehyde.

Acetaldehyde is produced during ethanol metabolism and is known to be oxidized mainly by aldehyde dehydrogenase(ALDH) but is also oxidized by catalase and microsomal ethanol oxidizing system (MEOS). Table 1 showed that the ALDH activity was found mainly in the mitochondrial fraction but a significant ALDH activity was also present in microsomal fraction. A small ALDH activity was in

Table 3. The effect of ginseng saponin on alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), and microsomal ethanol oxidizing system (MEOS) in prolonged ehtanol fed rats *in vivo*. The rats were fed with 10% ethanol (Control) and/or 10% ethanol containing 0.1% ginseng saponin (Test) instead of water for 6 days

Group	ADH (unit[a]/mg protein	ALDH (unit[a]/mg protein	MEOS (unit[b]/mg protein
Normal	8.743± 0.159 (100)	3.076± 0.600 (100)	3.165± 0.472 (100)
Control	10.136± 0.221 (116)	2.303± 0.661 (75)	4.443± 0. 681 (140)
Test	9.242± 0.123 (106)	2.678± 0.015 (87)	7.028± 0.775 (222)

the cytosol fraction(Oh and Joo, 1989). As shown in Table 2, there were optimum concentration of the ginseng saponins for the maximum activity of enzymes such as ADH, ALDH, MEOS respectively.

Wistar rats(150~200 g, male) were given freely with 12% ethanol (Control) and/or 12% ethanol containing 0.1% ginseng saponins(Test) instead of water for 6 days and the liver was analyzed. Liver homogenate was used for ADH, ALDH, and MEOS assay. As shown in Table 3, ALDH activities of both control and test group were lower than that of normal group. Test ALDH was less inhibited than control. ADH activites of both control and test were slightly higher than that of normal group. We know that ADH activity is usually simulated by ethanol feeding at initial stage but our previous data showed that it became gradually steady after prolonged ethanol feeding. MEOS activites of both control and test group were much higher than that of normal group. MEOS enzymes are inducible but the activity of test group was greatly higher than that of control.

For the determination of $[NAD^+]/[NADH]$ ratio, the rats were killed by dislocation of the neck. The liver was rapidly removed and pressed between metal clamps previously cooled in liquid N_2. The average time between dislocation of the neck and deep-freezing the tissue was 10 seconds. The frozen liver was pulverized in a mortar to a fine powder,

Table 4. $[NAD^+]/[NADH]$ ratio and $[NADP^+]/[NADH]$ ratio of the liver of rat fed with ordinary diet and 12% ethanol along with(Test) and/or with(Control) 0.1% ginseng saponin instead of water(free access). Normal group was fed only ordinary diet and water. Calculation of $[NAD^+]/[NADH]$ ratio and $[NADP^+]/[NADPH]$ ratio was described in the text

	Normal	Control	Test
Cytoplasm	886	507	676
Mitochondria	8.58	4.68	6.12

with frequent addition of liquid N_2. The powder was transferred to a weighed plastic centrifuge tube containing 6 m*l* of ice-cold 0.6 N (w/v) $HClO_4$. After a rapid reweighing, the tissue was mixed with the $HClO_4$ and immediately homogenized in the centrifuge tube. Protein was removed by centrifugation in the cold at 30,000×g for 15 min and the supernatant fluid was adjusted to pH 5~6 with 2 N(w/v) KOH and, after standing for 30 min in the cold, the precipitate of $KClO_4$ was centrifuged off. The yellow supernatant fluid was used for the analysis, and lactate, pyruvate, α-ketoglutarate, isocitrate, ammonia and glutamate were determined and the $[NAD^+]/[NADH]$ ratio in cytoplasm was calculated using $K_{eq}(1.11\times10^{-4})$ for lactate dehydrogenase reaction(lactate + NAD^+ → pyruvate + NADH + H^+) and that in mitochondria was calculated using $K_{eq}(3.87\times10^{-6})$ for glutamate dehydrogenase reaction (glutamate + NAD^+ → a-ketoglutarate + NH_4^+ + NADH + H^+) at pH 7.0, 38℃. As shown in Table 4, $[NAD^+]/[NADH]$ value of test group was recovered close to the normal level.

Ethanol containing $[^{1\text{-}14}C]$ ethanol(5 μCi) was injected to the above three groups. 30 min later, the distribution of radioactivity of hepatic lipids was investigated. As shown in Table 5, radioactivity of hepatic lipids of both control and test group was higher than that of normal group, but that of test group was much lower than that of control. Analysis of individual lipids such as phospholipids, cholesterol, fatty acid and trigylcerides, showed that phospholipid biosynthesis was significantly impared and fatty acid and triglyceride biosynthesis were greatly stimulated. However, the saponin prevented the

Table 5. Distribution of radioactivity(DPM) of hepatic lipids of rat which received intraperitoneal injection of 1 ml of 10% ethanol (containing [1−^{14}C] ethanol, 5 Ci). The rats were killed 30 min. later. Rats were fed with 12% ethanol(control) or 12% ethanol containing 0.1% saponin(test) instead of water for 6 days prior to [1−^{14}C] ethanol injection

Lipid fraction	Radioactivity(DPM)			Relative C/N	Ratio T/N
	Normal	Control	Test		
Total lipid	114,089(100)	176,867(100)	142,637(100)	155.0	125.0
Phospholipid fraction	49,045(43.0)	12,407(7.0)	34,322(24.1)	25.3	70.0
Cholesterol fraction	10,528(9.2)	10,248(5.8)	10,267(7.2)	97.3	97.5
Fatty acid fraction	22,895(20.1)	49,820(28.2)	47,119(33.0)	217.6	205.8
Triglyceride fraction	35,817(31.4)	73,141(41.4)	59,326(41.6)	204.2	165.6

Table 6. Electron microscopic observation of the effect of ginseng saponin on hepatocytes of rats dosed with 12% ethanol instead of water for 6 days

Group	Sweling & disruption of mitochondria	Dilation & vesiculation of RER	Proliferation of SER	Pyknosis	Fat deposition
Control 1	+++	+++	+++	+	++
2	+++	+++	+++	+	+++
3	+++	++	++	+	++
4	++	++	++	+	++
5	++	+++	++	+	++
Test I 1	−	+	+	−	−
2	−	−	−	−	+
3	−	−	−	+	−
4	+	+	−	−	+
5	+	−	−	−	−

Control group was fed with 12% ethanol only instead of water for 6 days. Test I group was fed with 0.1% ginseng saponin in 12% ethanol instead of water.
− : normal, + : mild, ++ : moderate, +++ : severe.

phospholipid biosynthesis depression and triglyceride biosynthesis stimulation consisderably. It seems that the ginseng saponin might stimulate ADH, ALDH and MEOS in accelerating ethanol oxidation and acetaldehyde removal from the tissue and excess hydrogen can be shunt more quickly into lipid biosynthesis.

Electron microscopic observation showed that the hepatic cell of control group was significantly damaged. Mitochondrias were swollen and disrupted severely. The rough endoplasmic reticulum(RER) were dilated and vesiculated and smooth endoplasmic reticulum(SER) were proliferated. Peroxisomes were increased in number and prominant golgi apparatus were seen, and pyknosis occurred and large fat droplets were seen. However, hepatocytes of test group showed that swollen or disrupted mitochondrias were not seen, and dilated or vesiculated RER were very few(Table 6).

It was demonstrated in this laboratory that the ginseng saponin has some preventive effect against hypercholesterolemia induced by prolonged high cholesterol diet administration in rabbits(Joo, 1980) and rats(Joo *et al.*, 1987).

It has been reported that more than 93% of all cholesterol of the animal body are in cell membranes, where it performs vital structural functions, while only about 7% circulates in plasma. It is the plasma cholesterol level, however, which is strongly implicated as a cause of atherosclerosis. Consequently, factors regulating the plasma cholesterol level are the subject of intense study. Cholesterol is tra-

Table 7. Lipid composition of liver of rats under different feeding conditions

Group	Cholesterol	Triglyceride (mg/g wet weight of liver)	Phospholipid	Chol./PL	TG/PL
Normal	3.8± 0.2	4.9± 0.3	18.3± 0.2	0.21	0.27
Test I	3.7± 0.7	4.4± 0.8	16.7± 0.9	0.22	0.26
Control	13.8± 1.7	8.4± 0.7	24.0± 1.8	0.58	0.35
Test II	7.3± 1.2	6.2± 0.4	23.8± 0.8	0.31	0.26

Normal : normal diet fed rats.
Test I : normal diet and ginseng saponin fed rats.
Control : high cholesterol diet fed rats.
Test II : high cholesterol diet and ginseng saponin fed rats.

nsported in the plasma in macromolecules called lipoproteins, which consist of varying amounts of specific proteins, cholesterol, cholesterol ester, triglycerides and phospholipids.

The importance of the Low Density Lipoprotein (LDL) receptor is highlighted by studies of familial hypercholesteremia. The total concentration of cholesterol and LDL in the plasma is markedly elevated in this genetic disorder, which results from a mutation at a autosomal locus. Cholesterol is deposited in various tissues because of the high concentration of LDL-cholesterol in the plasma. Nodules of cholesterol called xanthomas are prominant in skin and tendons. More harmful is the deposition of cholesterol in arterial plaques, which produce atherosclerosis. The molecular defect in most cases of familial hypercholesterolemia is an absence or deficiency of the functional receptor for LDL(Brown and Goldstein, 1986).

Table 7 showed that the level of liver lipids such as cholesterol and triglycerides of ginseng fed rats (Test II) was greatly lower than that of nonfed group (Control) in high cholesterol administered rats while in ordinary fed rat, no significant difference in the above lipid level occurred between ginseng fed group(Test I) and nonfed group(Normal) ^{125}I-LDL was injected intravenously to rats which had been fed with high cholesterol diet with and/or without ginseng saponin for 15 days and the radioactivity disappearance from test group was faster than from control group as shown in Fig. 2.

Table 8 showed that the binding activity of ^{125}I-LDL to rat liver plasma membrane. It was found that the activity was higher in test group than cont-

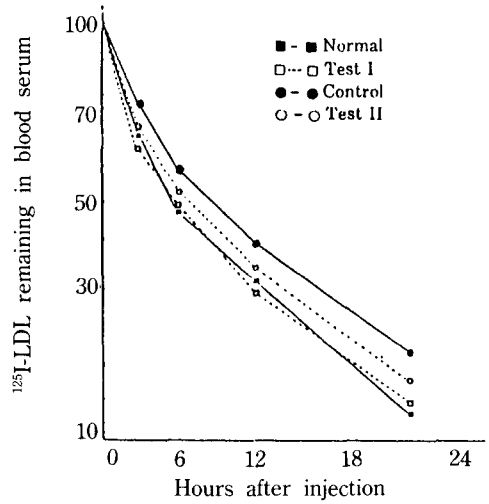

Fig. 2. Effect of ginseng saponin of ^{125}I-LDL removal from the blood serum of normal diet fed rats (normal). Normal diet and ginseng saponin fed rats(Test I). High cholesterol diet fed rats(control) and high cholesterol diet and ginseng saponin fed rats(Test II) from 15 days.

rol. It was also observed that LDL receptor activities of other organs such as kidney, adrenal cortex and testis of ginseng saponin fed rats were higher than that of control group. It was reported(Kita *et al.*, 1980) that there are EDTA-sensitive binding site and EDTA-resistant binding site for LDL in rabbit liver and the hypocholesteremic action of cholestryamin and mevinolin is due to increase of the number of EDTA-sensitive binding site. The total LDL receptor activity was lowered in control group but the saponin prevented LDL receptor activity loss due to high cholesterol administration. However the activity of EDTA-resistant binding site did not alte-

Table 8. Binding of ^{125}I-LDL to the liver plasma membrane of rabbits fed under different feeding conditions

Group	Total (ng/mg)	EDTA-resistant (ng/mg)	EDTA-sensitive (ng/mg)
Normal	52.6	26.1	26.5
Test I	55.3	27.0	28.3
Control	38.4	25.8	12.6
Test II	50.8	26.4	24.4

Normal : Normal diet fed rabbit(12 days).
Test I : Normal diet fed and ginseng saponin fed rabbits(12 days).
Control : High cholesterol diet fed rabbits(12 days).
Test II : High cholesterol diet and ginseng saponin fed rabbits(12 days).
Assay mixture(150 *l*) contained 100 g of membrane protein and 25 g/m*l* of ^{125}I-LDL(50 cpm/ng) in the absence or presence of 30 mM EDTA.

Table 9. Binding of ^{125}I-LDL to liver membrane of normal rabbit in the presence and/or absence of ginseng(*in vitro*)

Conc. of saponin(%)	Total (ng/mg)	EDTA-resistant (ng/mg)	EDTA-sensitive (ng/mg)
Control	50.2	24.1	26.1
10^{-5}	49.7	23.3	26.4
10^{-4}	50.8	25.2	25.0
10^{-3}	50.5	24.6	25.9
10^{-2}	52.4	25.4	27.1

Assay mixtures(150 *l*) contained 100 g of membrane protein, 5 g/m*l* of ^{125}I-LDL(120 cpm/ng) and various concentrations of saponin in the absence of presence of 30 mM EDTA.

red under different feeding conditions but that of EDTA-sensitive binding site did. No significant activity change of both the EDTA-sensitive and resistant binding sites of normal rabbits in the presence of ginseng saponin(Table 9). This indicates that ginseng saponin might not stimulate binding affinity but the population of hepatic LDL receptor.

It is easily expected from the nonspecific enzyme stimulation effect of ginseng saponin that the saponins might stimulate the cholesterol conversion to bile acids and *in vitro* experiment showed this was the case as shown in Table 10. Furthermore, the secretion of bile acids by ginseng saponin fed group was faster than nonsaponin fed group(Fig. 3).

Effect of total saponin extract and some purified ginsenoside $Rb_1$and Rb_2 on LDL receptor biosynthesis of chinese hamster ovary(CHO) cells cultured in a high cholesterol medium was investigated. Cholesterol uptake by CHO cell cultured in a medium containing various amounts of cholesterol was traced and found that the cholesterol uptake was proportional to the concentration of cholesterol in

Table 10. The effect of ginsenosides on the bile acid biosynthesis from($^{4\text{-}14}$C)-cholesterol by rat liver. The values are mean value of three determinations

Con. of ginsenosides in the reac. mixture(%)	0	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Ginsenoside mixture	115,509± 12,761 (100)	126,558± 27,855 (110)	147,889± 5,330 (128)*	136,856± 25,931 (118)	128,977± 10,222 (112)
Ginsenoside Rb1	115,509± 12,761 (100)	150,869± 9,316 (131)*	152,448± 57,243 (132)	145,796± 5,332 (126)*	131,368± 40,428 (114)
−Rb2	115,509± 12,761 (100)	115,323± 15,461 (100)	135,233± 36,526 (117)	153,808± 34,398 (133)	151,571± 10,109 (131)*
−Re	115,509± 12,761 (100)	142,564± 12,616 (123)	143,201± 10,644 (124)	156,015± 4,439 (135)*	137,205± 20,444 (119)
−Rg1	115,509± 12,761 (100)	144,016± 12,061 (125)	150,837± 14,327 (131)*	126,744± 35,509 (110)	136,024± 8,747 (118)

*$p<0.05$.
Reaction mixture(2 m*l*) contained 137 mM NaCl, 5.2 mM $NaHCO_3$, 1% glucose, 0.2% BSA, 10 mM phosphate buffer (pH 7.4), 10 μg cholesterol containing($^{4\text{-}14}$C)-cholesterol(0.09 μCi), ginsenoside mixture and purified ginsenoside Rb1, −Rb2, −Re, −Rg1 fractions(10^{-2}~10^{-5}%), and 20% rat liver homogenate.
The figures in brackets are relative percentage assuming that of control group(0% saponin) being 100.

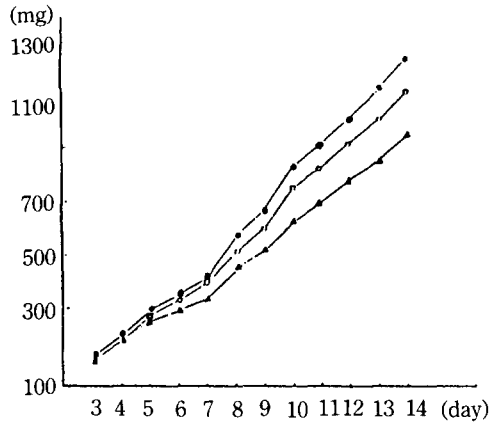

Fig. 3. The accumulative amount of bile acids from feeds of rats fed under various conditions two weeks course. The values are mean value of liver determinations.
Control group(▲—▲) were administered normal diet with high cholesterol diet(cholesterol 100 mg olive oil 0.5 m*l*. Na-cholate 10 mg/rat/day). Test I group(○—○) were administered under similar diet conditions of control group but either with ginsenoside mixture(1 mg/rat/day). Test II group(●—●) were administered under similar diet conditions of control group but either with ginsenoside Rb_1(1 mg/rat/day).

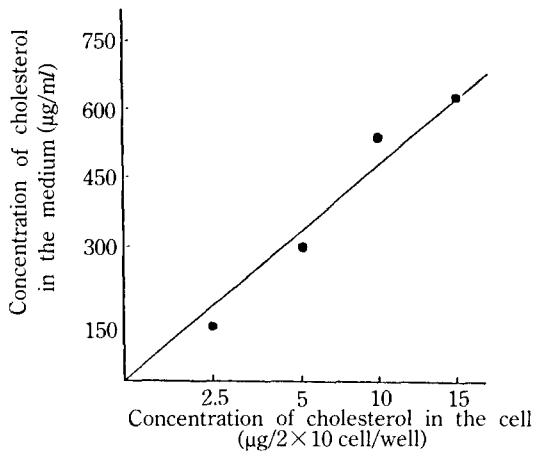

Fig. 4. Uptake of cholesterol by CHO cell cultured in medium containing both cholesterol and($^{4\text{-}14}$C)-cholesterol.

the medium(Fig. 4), and the population of LDL receptors were gradually decreased as the cholesterol concentration in the medium increased(Table 11). However, when the CHO cells were cultured in

Table 11. LDL receptor activity of CHO cells cultured in medium containing various amounts of cholesterol. The values are mean value of three determinations

Concentration of cholesterol (g/m*l*)	LDL receptor activity ng bound LDL/mg protein	Relative activity (%)
0(control)	1,158± 113	100
2.5	1,026± 186	87
5.0	977± 106	84
10.0	816± 81	70*
15.0	793± 103	68*

Relative activity of LDL receptor was expressed assuming that of control being 100.
*$p<0.05$.

Table 12. Cholesterol concentration of CHD cells cultured in medium containing various amounts of cholesterol. The values are mean value of three determinations

Group	Cholesterol concentration(μg)	Relative (%)
Normal	37± 1.51	100
Control	56± 2.16	150*
Ginsenoside mixture(10^{-5}%)	34± 3.92	91
Ginsenoside Rb^1(10^{-6}%)	32± 2.39	86*
$-Rb_2$(10^{-4}%)	23± 1.41	61*
$-Re$(10^{-7}%)	40± 7.32	107
$-Rg_1$(10^{-6}%)	36± 0.82	96

*$p<0.05$.

the medium containing ginsenosides, cholesterol concentration was lowered resulting in less loss of LDL receptor activity(Table 12).

It is now known that an excess of cholesterol in the liver reduces transcription of the LDL-receptor gene into messenger RNA, the nucleic acid that is subsequently translated by the cell's protein-synthesizing machinary to make the LDL-receptor. The biosynthesis of protein and RNA of the above cells was higher than that of CHO cells cultured in the absence of the ginsenosides(Table 13), suggesting that the ginsenosides might stimulate LDL receptor biosynthesis by lowering cholesterol level. It was also observed that the ginsenosides stimulate the biosynthesis of estradiol and progesterone from cholesterol in the CHO cells as shown in Table 14.

Table 13. The biosynthesis of RNA and protein of CHO cells cultured under various conditions*

	Radioactivity of ^{3}H-Uridine incorporated into RNA(cpm)	Radioactivity of ^{14}C-Leucine incorporated into protein(cpm)
Normal	8,239± 1,461 (100)	18,383± 4,628 (100)
Control	2,730± 194 (33)**	10,473± 1,445 (57)
Ginsenoside mixture	4,103± 125 (50)**	12,774± 1,862 (69)
Ginsenoside		
Rb_1	4,099± 1,296 (50)**	11,438± 969 (62)
$-Rb_2$	4,096± 1,391 (50)**	11,274± 996 (61)
$-Re0$	2,821± 53 (34)**	11,391± 216 (62)
$-Rg_1$	3,799± 1,140 (46)**	11,439± 614 (62)

Normal group was cultured in standard medium. Control group was cultured in standard medium containing cholesterol(10 g/m*l*). Test groups were cultured under similar conditions of control group but either with ginseng saponin mixture(10^{-5}%) or with ginsenoside Rb_1(10^{-6}%), $-Rb_2$(10^{-4}%), $-Re$(10^{-7}%), $-Rg_1$(10^{-6}%) fractions.

* The figures in brackets are relative percentage assuming that of normal group being 100.

** $p<0.05$.

It is well known that phospholipids play a significant role in the transport of lipids including cholesterol, subsequently, it is expected that the stimuallation of phospholipid biosynthesis by the saponin may facilitate the transport of lipids including cholesterol under such condition as prolonged feeding of high cholesterol diet to rabbits. Investigation of the ^{32}P incorporation to the hepatic phospholipids of ginseng saponin administered rats(Test Group) and control group showed that the incorporation was found much greater in the former than the latter suggesting phospholipid synthesis was stimulated by the saponin. The radioactivity of blood plasma lipid fraction of test group was also higher than that of the control group. It appeared that the saponin stimulated the phospholipid biosynthesis of both cytosolic and mitochondrial fractions. Observation of phospholipid biosynthesis *in vitro* using rat liver homogenate again showed that the adequate amount of ginseng saponin greatly stimulated the biosynthesis of phospholipid. It seemed that the increase of phospholipid biosynthesis might come from the sum of stimulation of several enzymes related to phospholipid biosynthesis by the saponin. Moreover, the *in vivo* stimulation of phospholipid biosynthesis by the saponin might result in the better transport of lipids including triglyceride and cholesterol in the animal body(Lee *et al.*, 1981).

Table 14. The effect of ginsenoside on the biosynthesis of progesterone and esteradiol from [$^{4\text{-}14}C$]-cholesterol in cultured CHO cells

	Radioactivity(dpm)					
	Cell			Medium		
	Progesterone	Estradiol	Cholesterol	Progesterone	Estradiol	Cholesterol
Control	1,168± 421 (1.5)	885± 231 (1.2)	75,472± 25,128 (100)	412,212 (0.4)	885± 340 (0.8)	109,355± 21,269 (100)
Ginsenoside mixture	2,691± 968 (4.0)	962± 57 (1.4)	67,659± 19,457 (100)	414± 98 (0.6)	855± 32 (1.3)	64,818± 4,659 (100)
Ginsenoside Rb1	1,690± 279 (3.6)	1,064± 144 (2.2)	47,033± 15,854 (100)	1,016± 203 (1.6)	2,349± 48 (3.6)	64,866± 15,717 (100)
$-Rb2$	1,546± 211	1,242± 1,089	70,892± 7,398	1,115± 585	1,612± 361	84,396± 19,145
$-Re$	2,265± 719 (3.3)	915± 147 (1.3)	68,704± 14,487 (100)	1,005± 722 (1.6)	1,083± 561 (1.7)	64,052± 12,635 (100)
$-Rg1$	1,813± 113 (3.2)	938± 81 (1.6)	57,544± 6,244 (100)	514± 121 (0.6)	2,472± 510 (2.8)	88,197± 21,216 (100)

*The figures in brackets are relative percentage assuming that of cholesterol being 100.

Table 15. The effect of ginseng saponin fraction(10^{-4} %) on the early growth of rice seed. The values are mean value of the length of 100 seedlings

Group day	Control (cm)	Test (cm)	Relativity** (%)
2	0.39± 0.14	0.40± 0.18	103
3	0.93± 0.28	1.06± 0.54	114*
4	2.34± 0.57	2.32± 0.64	99
5	4.61± 0.73	4.65± 0.81	101
6	6.80± 0.73	6.94± 0.72	102
7	8.16± 0.80	8.58± 1.01	105*
8	9.53± 1.29	10.59± 1.43	111*
9	11.47± 1.63	12.59± 1.68	110*
10	13.40± 1.76	14.56± 1.77	109*

The relative percentage expressed assuming that of control group being 100.
*$p<0.05$.

Table 16. Effect of ginseng saponin fraction on amylase activity *in vitro*. The values are mean values of three determinations

Added saponin concentration(%)	Enzyme activity(unit)*	Relativity** (%)
0	0.585	100
10^{-1}	0.320	55
10^{-2}	0.480	82
10^{-3}	0.490	84
10^{-4}	0.510	87
10^{-5}	0.780	113
10^{-6}	0.650	111
10^{-7}	0.625	107

* One units of enzyme was defined as mg maltose liberated in 50 min at 25℃ by 1 ml enzyme solution.
** The relative percentage is expressed assuming the activity of control is 100.

Ginseng saponins stimulated plant enzymes too. It was found that ascorbate synthesized during the germination of Glycine max was always much higher than that of control when the soybeans were either rinsed in 10^{-4}% ginseng saponin solution at first for 24 hours or poured afterward. Using the homogenate of germinating soybean(2nd day) as enzyme source and glucose containing [U-^{14}C]-glucose as substrate, it was confirmed that the saponin fraction stimulated the ascorbate biosynthesis during germination as shown in Table 8(Bae *et al.*, 1986).

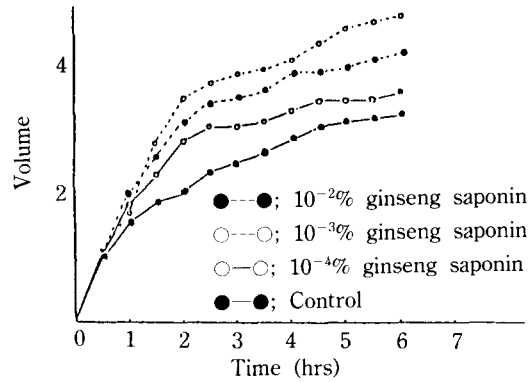

Fig. 5. Generation of CO_2 from yeast cells incubated (37℃) in glucose medium containing 0.1 M phosphate buffer(pH 6.8), 3% glucose, 1 g of yeast cake and ginseng saponin fraction($0\sim10^{-3}$ %).

Table 17. Distribution of radioactivities of glycolytic intermediates of yeast cells incubated in glucose medium containing [U-^{14}C]-glucose

	Control (cpm)	Test (cpm)	Test/ control(%)
Initial spotting amount	30,000	30,000	
Glucose	4,440	7,914	178
Glucose 6-phosphate	1,574	1,669	106
Fructose 6-phosphate	3,630	5,767	158
Phosphoenol pyruvate	2,304	2,248	98
Pyruvate	4,564	2,910	63
3-Phosphoglycerate	914	910	100

*The extract was spotted on wattman No. 1 paper and chromatographed by developing solvent(n-Buthanol : Acetate : Water, 74 : 19 : 50) for 24 hrs.

The effects of ginseng saponin on the germination and early growth of rice seeds(*Oryza sativa* L.) were investigated. The early growth(length) of test rice seeds which were rinsed for 60 hrs in 10^{-4} % saponin solution prior to transplantation to water agarbed was about 20% faster than that of control seeds as shown in Table 19. It was also found that the amylase activity of the seeds was most active when the seeds were rinsed in 10^{-4}% saponin soulution for 48 hrs(Table 15). *In vitro* investigation showed that the amylase activities were stimulated about 30% by the treatment of 10^{-5}% saponin solution compared with control group as shown in Ta-

Table 18. The effect of ginseng sapon fraction on yeast glycolytic enzymes *in vitro*. The values are mean value of three determinations

Saponin conc.(%) in reaction mixture	Relative activity			Puruvate decarboxylase
	Hexokinase	Phosphoglucoisomerase	Pyruvate kinase	
0	100	100	100	100
10^{-7}	102	101	108	99.5
10^{-6}	108	114	113	105.3
10^{-5}	119	170	115	119.2
10^{-4}	115	100	113	127.3
10^{-3}	118	93	92	108.0
10^{-2}	118	96	72	75.3
10^{-1}	54	90	90	45.0

ble 16. From the above results, it seems that the ginseng saponin might activate amylase of rice seed during germination, resulting in rapid growth of rice(Lee and Joo, 1987).

The effect of ginseng saponin fraction on several glycolytic enzymes of yeast cell was examined. The amount of CO_2 formed during the incubation of yeast cells in medium containng saponin fractin of *Panax ginseng* C.A. Meyer was greater than that of control cells and found that the CO_2 formation was greatest when the uptake of inorganic phosphate and glucose consumption were increased(Fig. 5). Radioactivity study of several glycolytic intermediates of yeast cells cultured in the medium containing [U-^{14}C]-glucose showed that the radioactivity of fructose 6-phosphate of test cells was as much as 1.6 times that of control group. On the other hand, the radioactivity of pyruvate of test cells was considerably decreased compared to control (Table 17). Investigation of the effect of ginseng saponin on yeast hexokinase, phosphoglucose isomerase, pyruvate kinase and pyruvate decarboxylase *in vitro* showed that the maximum activites of the above enzymes were observed when the concentration of ginseng saponin was 10^{-5}% in the reaction mixture as shown in Table 18.

From the above considerations, I would again suggest that nonspecific enzyme stimulatory effect of the saponin might play a significant role in biochemical and physiological system in the living body.

References

1. Brown, M.S. and Goldstein, J.L.: *Science,* **232**, 34 (1966).
2. Bae, S.K., Kwak, H.S. and Joo, C.N.: *Korean Biochem. J.,* **19**, 1 (1986).
3. Brekman, I.I. and Dadymov.: *Annu. Rev. Pharmacol.,* **187**, 419 (1969).
4. Joo, C.N., Choe, R.S., Lee, J.J., Cho, S.H. and Son, M.H.: *Korean Biochem. J.,* **6**, 185 (1973).
5. Joo, C.N. and Han, J.H.: *ibid.,* **9**, 43 (1976).
6. Joo, C.N., Oh, J.H. and No. S.J.: *ibid.,* **9**, 53 (1976).
7. Joo, C.N. and Han, J.H.: *ibid.,* **9**, 235 (1976).
8. Joo, C.N.: *Proceedings of 2nd Int. Ginseng Symp.,* Seoul, pp. 115-134 (1978).
9. Joo, C.N. and Lee, S.J.: *Korean Biochem. J.,* **60**, 59 (1977).
10. Joo, C.N.: *Proceedings of 3rd Int. Ginseng Symp.,* Seoul, pp. 27-36(1980).
11. Joo, C.N.: Surfactant in solution(Proceedings of Int. Sym. on Surfactant in solution, held June 27-July 2, in Lund, Sweden) ed. K.L. Mittal and M. Lindoman Vol. 3, pp. 2093-2112 (1984). Plenum press, New York and London.
12. Joo, C.N. and Kim, J.W.: *Kor. J. Ginseng Sci.,* **8**, 75 (1984).
13. Joo, C.N.: The Proceedings of 4th Int. Ginseng Sym.(Seoul), pp. 47-54, 63-74 (1984).
14. Joo, C.N., Lee, H.B. and Kim, J.W.: *Kor. J. Ginseng Science,* **10**, 108 (1986).
15. Joo, C.N., Kang, I.C. and Lee, H.B.: *Korean J. Ginseng Sci.,* **12**, 104 (1988).
16. Joo, C.N.: The Proceedings of 5th Int. Ginseng Symp.(Seoul), pp. 47-54 (1988).

17. Kang, C.H. and Joo, C.N.: *Korean J. Ginseng Sci.*, **10**, 2 (1986).
18. Kita, T., Brown, M.S., Watanabe, Y. and Goldstein, J.L.: *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 2268 (1980).
19. Kim, K.Y., Lee, H.B. and Joo, C.N.: *Korean Biochem. J.*, **17**, 312 (1984).
20. Kim, J.W. and Joo, C.N.: *Korean Biochem. J.*, **18**, 270 (1985).
21. Kim, J.W., Lee, H.B. and Joo, C.N.: *Korean Biochem. J.*, **18**, 453 (1985).
22. Lee, H.B. and Joo, C.N.: *Korean Biochem. J.*, **16**, 136 (1983).
23. Lee, S.O., Koo, J.H. and Joo, C.N.: *Korean Biochem. J.*, **14**, 161 (1981).
24. Lee, Y.W. and Joo, C.N.: *Korean J. Ginseng Sci.*, **11**. 77 (1987).
25. Namba, T., Yoshizaki, M., Tominori, T., Kobachi, K. and Hase, J.: 6th Symp. of oriental drugs, Toyama University (1972).
26. Oh, E.S. and Joo, C.N.: *Korean Biochem. J.*, **22**, 312 (1989).
27. Oh, K.K. and Joo, C.N.: *Korean Biochem. J.*, **19**, 356 (1986).
28. Oura, H. and Hiai, S.: Proceedings of Int. Ginseng Symp. Seoul (1974).
29. Oura, H., Hiai, S., Nabetani, S., Kurata, Y. and Sasaki, N.: *Planta Medica*, **28**, 76 (1975).