Journal of Biochemistry and Molecular Biology, Vol. 31, No. 3, May 1998, pp. 233–239

JOURNAL OF
Biochemistry
and
Molecular Biology

Stimulatory Effects of Ginsenosides on Bovine Brain Glutamate Decarboxylase

Soo Young Choi,* Jae Hoon Bahn, Seong Gyu Jeon, Young Mee Chung, Joung Woo Hong, Jee-Yin Ahn, Eunjoo Hwang Lee, Sung-Woo Cho,† Jinkyu Park,‡ and Nam-In Baek§

Department of Genetic Engineering, Division of Life Sciences, Hallym University, Chunchon 200-702, Korea

† Department of Biochemistry, College of Medicine, University of Ulsan, Seoul 138-746, Korea

‡ Korea Ginseng & Tobacco Research Institute, Taejon 305-345, Korea

§ Department of Life Resources Science, Kyung Hee University, Suwon 449-701, Korea

Received 6 January 1998

A GABA synthesizing enzyme, glutamate decarboxylase, has been purified from bovine brain by several chromatographic procedures. The preparation appeared homogeneous on SDS-PAGE. The enzyme is a homodimeric protein with a molecular mass of 120 kDa.

The activation of glutamate decarboxylase by ginesenosides from *Panax ginseng* C.A. Meyer has been studied. Preincubation of the enzyme with total ginsenoside, Rb_2 and Rc ginsenosides, increased glutamate decarboxylase activities in a dose-dependent manner. There was a reproducible decrease in K_m, in addition to a increase in V_{max}, in response to increasing concentrations of the Rc ginsenoside fraction. Upon addition of the ginsenoside to the enzyme, a decrease in flurorescence intensity was discernible, together with an increase in emission anisotropy. Judging from the anisotropy values, the ginsenoside is rapidly trapped by the protein matrix.

Total ginsenoside was administered to rats and the rat brains were removed for the measurement of the changes of GABA shunt regulating enzyme activities. Among the GABA shunt regulating enzymes, only the glutamate decarboxylase activities were increased after ginsenoside treatment.

Therefore, it is suggested that the ginsenosides may elevate the GABA level in brain by activation of glutamate decarboxylase and the enzymatic activation might be due to the conformational change induced by binding of ginsenoside to the enzyme.

* To whom correspondence should be addressed.
Tel: 82-361-240-1463; Fax: 82-361-241-1463
E-mail: sychoi@sun.hallym.ac.kr

Keywords: Activation, Brain, GABA shunt, Ginsenosides, Glutamate decarboxylase.

Introduction

GABA (γ-aminobutyric acid) is present in many tissues of mammals and is believed to be a major inhibitory chemical neurotransmitter in the Central Nervous System (CNS), in which it is present in competitive amounts (Fletcher and Fowler, 1986). The release of GABA by nerve terminals and its subsequent binding to its receptor must be followed by a rapid inactivation of the neurotransmitter. When the concentration of GABA in brain diminishes to below a threshold level, various neurological disorders including epilepsy, seizures, convulsions, Huntington's disease, and Parkinsonism may occur (Perry *et al.*, 1973; De Biase *et al.*, 1991; Lloyd *et al.*, 1997).

The concentration of GABA in the brain is controlled by two pyridoxal-5′-phosphate (PLP) dependent enzymes, i.e., glutamate decarboxylase (GAD) and GABA transaminase (GABA-T). The first enzyme catalyzes the synthesis of GABA, whereas the second enzyme catalyzes the conversion of GABA to succinic semialdehyde in a transamination reaction. Succinic semialdehyde is oxidized to succinate by a succinic semialdehyde dehydrogenase (SSADH) and can also be reduced to γ-hydroxybutyrate (GHB) by succinic semialdehyde reductase (SSAR). The observation that activation of GAD or the inactivation of the GABA-T, SSADH, SSAR in brain tissues increases the concentration of GABA supports the contention that these enzymes exert a controlling influence on GABA levels. It is reported that the irreversible inhibition of GABA-T by chemical analogues of GABA is the basic mechanism of action of drugs used in the treatment of convulsive disorders (Lippert *et al.*, 1977).

It is well known that total ginsenoside, a mixture of saponins from *Panax ginseng* C.A. Meyer, have been used as a preventive drug for thousands of years in oriental countries (Kim *et al.*, 1992). The studies on the pharmacology and clinical application of ginsenosides on the decrease in blood pressure (Takagi *et al.*, 1972), a supression of conditioned avoidance response (Nabata *et al.*, 1973), inhibition of gastric ulceration (Chang and Kim, 1974), and facilitation of sexual behavior (Lim and Kim, 1982) have been done.

The saponin fraction Rb has been shown to possess CNS depressant, anticonvulsant, antipsychotics, improvement of learning and memory, analgesic, and antifatigue actions (Petkov, 1978). Very recently, effects of Korean red ginseng on the central dopaminergic transmission system in CNS were investigated (Lee *et al*, 1995c). The detailed mechanisms of actions of ginsenosides on brain, however, are still unknown.

In this study, we examined the effects of ginsenosides on brain GABA shunt regulating enzymes and showed that some ginsenosides activate GAD activity which catalyzes the synthesis of GABA from glutamate. To our knowledge, this is the first report on the stimulatory effects of ginsenosides on brain GAD activity and on it usefulness as an anticonvulsant drug in clinical applications.

Materials and Methods

Materials GABA, succinic semialdehyde, NAD^+, PLP, 2-oxoglutarate, L-glutamate, 2-mercaptoethanol were purchased from Sigma Chemical Co. (St. Louis, USA). CM-Sephadex, DEAE-Sephadex, DEAE-Sephacel, AH-Sepharose-4B, CM-Sepharose, Blue-Sepharose, 5′-AMP-Sepharose, Mono-Q, Hydroxyapatite were purchased from Pharmacia/LKB, Ltd. (Uppsala, Sweden). L-[1-^{14}C]glutamate was purchased from New England Nuclear Corp. (specific activity 52.6 mCi/mmol) and Iodoactamidefluoreceine (IAF) from Molecular Probes (Eugene, USA).

Bovine brains were obtained from Majang Slaughter House, Seoul, Korea. Total ginseng saponin mixtures containing 26 ginsenosides such as Rb_1 18.26%, Rb_2 9.7%, Rc 9.65%, Rd 8.24%, Re 9.28%, Rf 3.48%, Rg_1 6.42%, Rg_2 3.62%, Rg_3 4.7% and purified Rb_1, Rb_2, Rc, Re, Rg_1 fractions were obtained from Korea Ginseng & Tobacco Research Institute, Taejon, Korea.

Purification of GAD The enzyme preparation procedures were carried out at 2–5°C unless otherwise indicated. Bovine brains were placed in ice as quickly as possible after slaughter and preparation was started within 1 h. A Waring blender was used to prepare a 15% (w/v) homogenate in a solution of 5 mM potassium phosphate buffer (pH 7), containing 1 mM 2-mercaptoethanol, 2.5 mM EDTA (buffer I). The homogenate was adjusted to pH 5.7 by the addition of acetic acid (7%), heated to 50°C for 5 min and, after cooling to 4°C, centrifuged at 10,000 × *g* for 30 min. The precipitate was discarded and the supernatant treated with $(NH_4)_2SO_4$. The precipitate obtained at 30–70% saturation was dissolved in buffer I and dialyzed overnight against several changes of the same buffer. It was then applied to a column (2.6 × 30 cm) of DEAE-Sephacel previously equilibrated with buffer I. The column was washed with 200 ml of equilibration buffer I and eluted by a linear gradient of the equilibration buffer (200 ml) and the same volume of buffer I containing 0.8 M KCl.

The active fractions were combined and concentrated to a final volume of 50 ml using an Amicon concentrator (membrane PM 30). The protein was dialyzed against 5 mM potassium phosphate buffer (pH 7) containing 1 mM 2-mercaptoethanol (buffer II) and applied to a hydroxyapatite column (1.5 × 20 cm) previously equilibrated with buffer II. The enzyme was eluted by using a linear gradient made with buffer II (50 ml) and the same volume of 0.35 M potassum phosphate (pH 7).

The active fractions were combined, dialyzed against buffer II and applied to a column (0.9 × 15 cm) of L-glutamate-Sepharose equilibrated with buffer II. The enzyme was eluted with a linear gradient of buffer II containing 0.01 M NaCl and buffer II containing 0.6 M NaCl. The enzyme was eluted at NaCl concentration of 0.25 M.

The active fractions were combined and dialyzed against buffer II. The specific activity of purified enzyme is defined as that amount of protein which catalyzes the production of 1 nmol/min of CO_2 at 37°C.

Enzymatic assay of GAD Glutamate decarboxylase activity was measured by trapping and counting $^{14}CO_2$ from L-[1-^{14}C]glutamate (Choi *et al.*, 1994a). In a typical assay, the incubation vessel contained 4 mM L-glutamate in 10 mM potassium phosphate buffer (pH 7.2). The reaction was started by injecting enzyme solution (80 μl) into the vessel and allowed to proceed at 37°C for 30 min. The reaction was terminated by the addition of 100 μl of 2.5 M H_2SO_4. The reaction mixture was incubated for another 60 min to ensure the complete release of CO_2 and its absorption into the hyamine base. $^{14}CO_2$ absorbed in hyamine was then counted in a liquid scintillation counter. The protein concentration was determined by the colorimetric method of Bradford (1976).

Preparation of L-glutamate coupled with AH-Sepharose-4B AH-Sepharose-4B (5 g) was dissolved in 100 ml 0.5 M NaCl. Sodium glutamate (8.5 g) was dissolved in 100 ml of water and adjusted to pH 4.5; the carbodiimide reagent [1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide HCl] (500 mg) was also dissolved separately in 100 ml water at pH 4.5.

The ligand and coupling solutions were mixed with the gel suspension of AH-Sepharose-4B, placed in a rotary shaker and incubated at 37°C for 25 h. L-Glutamate coupled to AH-Sepharose-4B was washed with 0.5 M NaCl, water, and 0.1 M NaCl.

Purification of other GABA shunt regulating enzymes GABA-T was purified according to a procedure of Choi *et al.* (1993) and SSADH and SSAR were prepared by the method of Lee *et al.* (1995a) and Cho *et al.* (1993), respectively.

Polyacrylamide gel eletrophoresis The enzyme preparations were examined by polyacrylamide gel eletrophoresis according to the original procedure of Davis (1964). Protein bands were detected by staining with Coomassie blue dye for 1 h and subsequently destained overnight in a solution containing 10% methanol and 7% acetic acid in water.

Labeling of GAD with fluorescent probe IAF GAD, at a concentration of 2 mg/ml, was allowed to react with 1 mM IAF in 0.05 M potassium phosphate (pH 7) at 4°C. The reaction was allowed to proceed for 5 h at 4°C. The degree of labeling of the enzyme was determined spectrophotometrically using an extinction coefficient of $3.4 \times 10^4\ M^{-1} cm^{-1}$ at 490 nm. The incorporation of 1.1 mol of dye/mol of enzyme does not affect the catalytic activity.

Spectroscopy UV-Vis spectrophotometric measurements were carried out with a Kontron UVIKON 930 double beam spectrophotometer. Fluorescence spectra were recorded on a Kontron SFM 25 spectrofluorometer.

Effects of ginsenosides on GAD enzyme activity Total ginsenoside and other ginsenoside fractions were dissolved in water at a high concentration and stored at −20°C. These stock solutions were diluted with distilled water. Preincubation of GAD with the ginsenosides was performed in the 10 mM potassium phosphate buffer, pH 7.0, at 25°C. An equal concentration of ethanol was added to the control experiments. Aliquots were withdrawn and assayed for GAD activities by adding the standard assay mixtures as described above.

Ginsenoside treatment to animals Five male Sprague-Dawley rats weighing 150–200 g were used in each group. The animals were housed in a controlled environment of 23 ± 1°C temperature and given regular food throughout the experimental period.

For *in vivo* assay of GAD, the total ginsenosides were suspended in distilled water and administated intraperitoneally in doses of 25 and 50 mg/kg/day for one week, respectively. The control animals received an equivalent volume of saline (10 ml/kg) for 1 week.

Results

Purification and properties of bovine brain GAD Glutamate decarboxylase from bovine brain was purified for the first time to homogeneity by following ammonium sulfate fractionation, DEAE-Sephacel, Hydroxyapatite and Affinity column chromatographic steps. The results of a typical purification procedure are shown in Table 1.

The enzyme purified through the Sepharose derivatized affinity step exhibited one protein band on SDS-PAGE (Fig. 1) and native PAGE (data not shown). The result

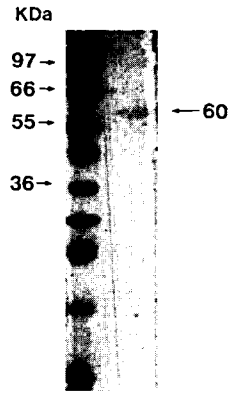

Fig. 1. SDS-PAGE of bovine brain GAD. The gel electrophoresis was performed on 12.5% separating gel and 4% stacking gel in a Tris-glycine buffer system. The gel was stained with Coomassie blue. Lane 1, molecular weight standard (Bio-Rad); Lane 2, purified bovine brain GAD.

from the FPLC Superose-12 gel filtration gave an estimated molecular mass for the native enzyme of 120 kDa, which dissociates into subunits of 60 kDa under reducing conditions on SDS-PAGE. These results indicate that the enzyme is a dimer with identical subunits. The enzyme contains 1.1 mol of bound cofactor PLP per mole of dimeric protein when determined by the method of Wada and Snell (1961). Upon addition of exogenous PLP, the purified enzyme acquires maximum catalytic activity, suggesting that weakly bound molecules of cofactor are released from the protein during the purification steps.

The activation of GAD by ginsenosides As shown in Fig. 2, preincubation of the enzyme with total ginsenoside activated the enzymatic activity of GAD which is involved in the synthesis of GABA. The GAD activities were also enhanced significantly by the ginsenoside-Rb_2 and ginsenoside-Rc fractions in a dose-dependent manner. The effects of ginsenosides on GAD were further analyzed by the fundamental kinetic parameters for the enzymatic activity. As shown in Table 2, it was observed that there was a reproducible decrease (up to 3.5 fold) in K_m for the glutamate, in addition to a substantial increase (up to 1.5 fold) in V_{max}, in response to increasing concentrations of the ginsenosides-Rc fraction of up to 15 μg/ml.

Table 1. Purification of GAD from bovine brain. Specific activity was determined after addition of excess PLP (50 μM) to the enzyme solution.

Treatment	Total Protein (mg)	Total Activity (unit)	Specific Activity (unit/mg)	Yield (%)
Homogenate	45,250	1854	0.041	100
Dialysed 30–70% $(NH_4)_2SO_4$ Fraction	3120	768	0.246	41
DEAE-Sephacel	323	520	1.61	28
Hydroxyapatite	52	409	7.87	22
Affinity Chromatography (derivatized Sepharose)	2	95.6	47.8	3

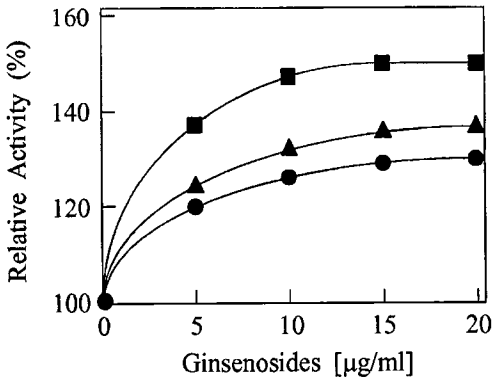

Fig. 2. Activation of GAD by total ginsenoside (●), Rb_2-ginsenoside (▲), and Rc-ginsenoside fractions (■), respectively.

Table 2. Effect of ginsenoside Rc fraction on kinetic parameters of bovine brain GAD

[Rc] (μg/ml)	K_m (for glutamate) (mM)	V_{max} (mol/min)
0	1.8	0.045
1	1.1	0.051
5	0.8	0.059
15	0.5	0.067

Compared to the total, Rb_2, and Rc fractions, the other ginsenoside fractions (i.e. Rb_1, Re, Rg) did not show any effect on GAD activity (Fig. 3). These results suggest that Rb_2 and Rc activates the activity of the GABA synthesizing enzyme GAD and they may also be involved in increasing the GABA level *in vivo*.

We also investigated the effects of ginsenosides on enzymes involved in GABA degradative enzymes (GABA-T, SSADH, and SSAR). As shown in Fig. 4, there were no changes of those enzyme activities, respectively.

Conformational changes of GAD by ginsenoside

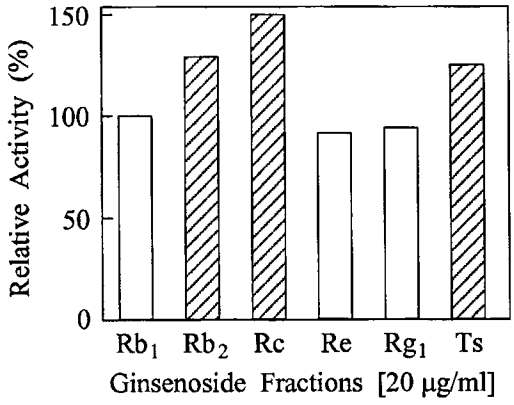

Fig. 3. Effect of several ginsenoside fractions on GAD enzyme activity.

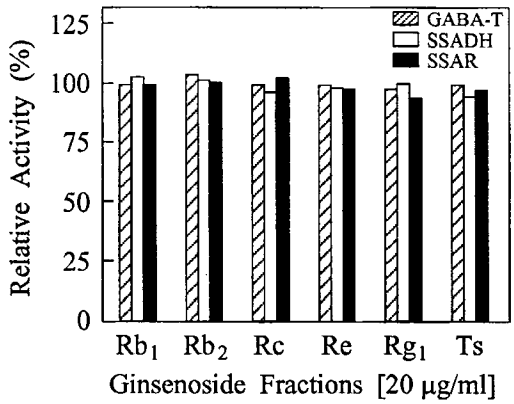

Fig. 4. Effect of ginsenosides on neurotransmitter GABA degradative enzymes: GABA transaminase (GABA-T), succinic semialdehyde dehydrogenase (SSADH), and succinic semialdehyde reductase (SSAR).

Protein conformational changes elicited by ginsenoside binding were examined using a fluorescent probe covalently linked to the protein. IAF was selected for these studies because it displays absorption and emission properties distinct from the cofactor PLP.

The enzyme was reacted with IAF under the condition described in the Materials and Methods. The labeled enzyme exhibited an absorption at 490 nm. Using an extinction coefficient of $3.4 \times 10^4\ M^{-1}cm^{-1}$ for bound fluoreceine, a degree of labeling of 1.1 chromophores/dimer was determined for the reacted enzyme. The fluorescence spectroscopic properties and a steady emission anisotropy of bound fluoreceine can be used conveniently to detect structural fluctuations induced by binding with ginsenoside.

When IAF-GAD was incubated with the ginsenoside Rc fraction at pH 7.0, a decrease of fluorecence intensity was observed (Fig. 5). The polarization of IAF-GAD ($p = 0.25$) increased in the presence of the Rc fraction ($p = 0.34$).

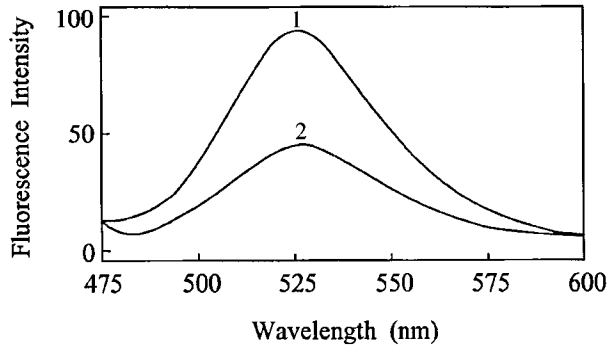

Fig. 5. Fluorescence emission spectra of native and modified IAF-GAD. The enzyme (2 μM) was incubated without Rc ginsenoside (1) or with Rc ginsenoside fraction (2) in 0.05 M potassium phosphate buffer, pH 7.0 at 25°C.

Figure 6 shows the steady emission anisotropy values of IAF-GAD with the ginsenoside Rc fraction. The emission anisotropy values were obtained at different wavelengths by Eq. (1).

$$A = \frac{2P}{3 - P} \quad (1)$$

where A is the emission anisotropy and P is the measured polarization.

Judging from the changes of fluorescence intensity and anisotropy values, it appears that the ginsenoside is rigidly trapped by the protein matrix and conformational changes are induced in the enzyme.

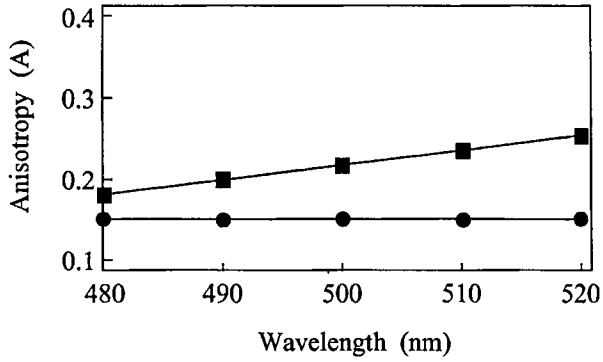

Fig. 6. Steady anisotropy values of IAF-GAD (●) and of IAF-GAD in the presence of the ginsenoside Rc fraction (■).

Changes of the GAD enzymatic activities by the administration of total ginsenoside Changes on the enzyme specific activity of GABA shunt regulating enzymes after pretreatment of total ginsenosides are shown in Table 3. The GAD activity after total ginsenoside administration increased two folds compared to that of the control. However, the enzyme activities of the GABA degradative enzymes were not affected at all.

Discussion

GABA is a major inhibitory neurotransmitter in the mammalian brain. Its biosynthesis from glutamate is effected by the cytoplasmic enzyme GAD, which has proved to be a valuable immunocytochemical marker for GABA neurons (Wu, 1983).

Since abnormal levels of neurotransmitter GABA in brain have been associated with a variety of neurological disorders including epilepsy, seizure, and convulsant disorders, a specific activator of the GABA synthesizing enzyme (i.e. GAD) or inhibitor of the GABA degradative enzyme (i.e. GABA-T, SSADH, SSAR) would be useful in attempts to elevate GABA levels in certain pathological conditions. It is well known that the GAD activity seems to be a crucial factor in regulating the function of GABA, the latter being the most widely distributed inhibitory neurotransmitter in the mammalian brain.

In this study, we have purified and characterized GAD from bovine brain for the first time. It contains 1.1 mol of a tightly bound cofactor PLP–enzyme dimer. Upon addition of exogenous PLP, the enzyme acquires its maximum catalytic activity.

Incubation of GAD with increasing concentrations of ginsenosides resulted in a progressive increase in enzyme activity (Fig. 2). Among the ginsenosides fractions, the Rb_2 and Rc fractions stimulated the GAD enzymatic activity in a dose-dependent manner. The reactions between GAD and the ginsenosides were investigated by UV-Vis difference spectroscopy (data not shown). With increasing ginsenoside concentrations, a differential absorbance maximum emerged at 220 nm and a minimum at 275 nm with an isosbestic point at 250 nm. The UV-Vis difference spectroscopic studies suggest the possibility that the activation of GAD comes about by binding of the ginsenoside to the enzyme. The results are similar to the activating effects of ginsenoside on yeast alcohol dehydrogenase, where the activation is due to the conformational changes of the protein caused by the ginsenoside (Kim *et al.*, 1985).

The results obtained using chemical and biophysical methods indicated that the conformational changes have taken place in GAD as a result of ginsenoside binding to the enzyme. The spectroscopic properties of a fluorescent

Table 3. The changes of enzyme activities of rat brain GABA shunt regulating enzymes after administration of total ginsenoside

Treatment of total ginsenoside (mg/kg/day)	Enzyme Specific Activity (unit/mg)			
	GAD	GABA-T	SSADH	SSAR
Control	0.47 ± 0.02	4.02 ± 0.24	5.32 ± 0.42	1.10 ± 0.07
25 mg	0.69 ± 0.03	4.06 ± 0.24	5.06 ± 0.56	1.06 ± 0.08
50 mg	0.94 ± 0.03	3.96 ± 0.31	5.02 ± 0.47	1.07 ± 0.07

Each value represents the mean ± SD of 5 rats.

probe (IAF) covalently linked to sulfhydryl groups are influenced by local conformational changes in the protein. The quenching of fluorescence intensity and increase of the fluorescence polarization values reveal that the GAD remains relatively immobilized upon binding of ginsenoside and thus binding of ginsenoside to GAD undergoes conformational changes.

The hypothesis of ginsenoside binding to GAD was supported by the results of the interaction between phospholipid and GAD. In the previous report, we have shown that purified porcine brain GAD interacts with derivatized fluorescent phospholipid *in vitro* (Choi *et al.*, 1994a) and the synthetic lipid vesicle was selected to monitor the effect and interaction on the GAD purified from porcine brain (Choi *et al.*, 1994b). Like lipid vesicles, the similar characteristics of a hydrophobic structure in ginsenosides might play an important role in the actions of binding to the enzyme.

Unlike the action of ginsenoside to GAD, most of the ginsenosides have no effect on GABA degradative enzymes, GABA-T, SSADH, and SSAR. These results suggest that the ginsenosides could be involved in the elevation of GABA levels *in vivo*.

As shown in Table 3, the only GAD activity of rat brain was activated after administration of total ginsenoside. These results are consistent with the results of *in vitro* experiments. According to the stimulatory effects of ginsenoside on GAD *in vivo* and *in vitro*, we have assumed that the activation of GAD caused the elevation of GABA levels in brain. It has been demonstrated that the total saponin diminishes the PTZ (pentylene tetrazole)-induced seizure of rat (Lee *et al.*, 1995b; Park *et al.*, 1995). Therefore, to our knowledge, the present article is the first report which showed the activation of the GABA synthesizing enzyme GAD by a ginsenoside. Further studies to elucidate the elevation of GABA levels by ginsenosides in brain and interaction of ginsenoside with GAD may provide insights into approaches for the clinical application of ginsenoside as an anticonvulsant or antiepileptic therapeutic drug.

Acknowledgments This study was supported by a grant (HMP-97-D-4-0024) of the 1997 Good Health RNP Project, Ministry of Health and Welfare, R.O.K and partially supported by the grant from the Society for Korean Ginseng.

References

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.

Chang, W. A. and Kim, C. J. (1974) Influence of panax ginseng upon gastric ulceration produced by Restraint. *J. Cath. Med. Coll.* **27**, 249–255.

Cho, S. W., Song, M. S., Kim, G. Y, Kang, W. D., Choi, E. Y., and Choi, S. Y. (1993) Kinetics and mechanism of an NADPH-dependent succinic semialdehyde reductase from bovine brain. *Eur. J. Biochem.* **211**, 757–762.

Choi, S. Y., Han, K., Jang, S. H., Song, M. S., Kim, Y. K., Choe, J. H., and Cho, S. W. (1994a) Interaction of phospholipid with brain glutamate decarboxylase. *Kor. Biochem. J.* **27**, 249–253.

Choi, S. Y., Kim, I., Jang, S. H., Lee, S. J., Song, M. S., Lee, Y. S., and Cho, S. W. (1993) Purification and properties of GABA transaminase from bovine brain. *Mol. Cells* **3**, 397–401.

Choi, S. Y., Lee, B. R., Jang, S. H., Song, M. S., Wee, S., Choi, E. Y., Lee, K. S., and Lee, H. B. (1994b) Reaction of phospholipid with brain glutamate decarboxylase. *Exp. Neurobiol.* **3**, 39–45.

Davis, B. J. (1964) Disc gel electrophoresis II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* **121**, 404–427.

De Biase, D., Barra, D., Bossa, F., Pucci, P., and John, R. H. (1991) Chemistry of the inactivation of 4-aminobutyrate aminotransferase by the antiepileptic drug vigabatrin. *J. Biol. Chem.* **266**, 20056–20061.

Fletcher, A. and Fowler, L. J. (1980) 4-aminobutyric acid metabolism in rat brain following chronic oral administration of ethanolamine-O-sulfate. *Biochem. Pharmacol.* **29**, 1451–1454.

Kim, H., Chen, X., and Gillis, N. (1992) Ginsenosides protect pulmonary vascular endothelium against free radical-induced injury. *Biochem. Biophys. Res. Commun.* **189**, 670–676.

Kim, J. W., Kim, J. W., Lee, H. B., and Joo, C. N. (1985) Biochemical studies on ginseng saponin(XXV): Ginseng saponin-protein interaction. *Kor. Biochem. J.* **18,** 453–458.

Lee, B. R., Hong, J. W., Yoo, B. K., Lee, S. J., Cho, S. W., and Choi, S. Y. (1995a) Bovine brain succinic semialdehyde dehydrogenase: Purification, kinetics and reactivity of lysyl residues connected with catalytic activity. *Mol. Cells* **5**, 611–617.

Lee, S. C., Lee, S. J., Chung, K. S., You, K. H., and Kim, H. S. (1995b) The central effects of red ginseng total saponin component. *Korean J. Ginseng Sci.* **19,** 22–26.

Lee, S. C., Yoo, K. H., and Yamamoto, T. (1995c) Behavioral pharmacological studies of nootropic candidates on the central dopaminergic activity in rats. *Korean J. Ginseng Sci.* **19**, 197–201.

Lim, S. Y. and Kim, C. C. (1982) Influence upon male rats' sexual behavior of ginseng administered before and after mature state. *J. Cath. Med. Coll.* **35**, 9–14.

Lippert, B., Metcalf, B. Y., Jung, M. J., and Casara, P. (1977) 4-Aminohex-5-enoic acids: a selective catalytic inhibitor of 4-aminobutyrate aminotransferase in mammalian brain. *Eur. J. Biochem.* **74**, 441–445.

Lloyd, K. G., Sherman, L., and Hornykiewicz, O. (1977) Distribution of high affinity sodium independent [^{3}H]-γ-aminobutyrate binding in the human brain. Alteration in Parkinson's disease. *Brain, Res.* **127**, 269–275.

Nabata, H., Saito, H., and Takagi, K. (1973) Pharmacological studies of neutral saponins (GNS) of panax ginseng root. *Jpn. J. Pharmacol.* **23,** 29–41.

Park, J. K., Chepurnov, S. A., Chepurnova, N. E., and Nam, K. Y. (1995) Effects of Korean red ginseng on memory deficit and seizure susceptibility following hyperthermia induced in the rat pups, in *Proceedings of '95 Korea-Japan Ginseng*

Symposium. pp. 89–102.

Perry, T. L., Hansen, S., and Kloster, M. (1973) Huntington's chorea: Deficiency of 4-aminobutyric acid in brain. *New. Engl. J. Med.* **288**, 337–342.

Petkov, V. D. (1978) Effects of ginseng on the brain biogenic monoamines and 3′,5′-AMP system: Experiments on rat. *Arzeim. Forsch.* **28,** 388–393.

Takagi, K., Saito, H., and Nabata, H. (1972) Pharmacological studies of panax ginseng root: Estimation of pharmacological action of panax ginseng root. *Jpn. J. Pharmacol.* **22**, 245–259.

Wada, H. and Snell, E. E. (1961) The enzymatic oxidation of pyridoxine and pyridoxamine phosphates. *J. Biol. Chem.* **236**, 2089–2095.

Wu, J. Y. (1983) Purification, characterization and kinetic studies of GAD and GABA-T from mouse brain; in *Glutamine Glutamate and GABA in the Central Nervous System*, Hertz, L., Kvamme, E., McGeer, E.G., and Schousboe, A. (eds.), pp. 161–176, Alan Liss, New York.