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Ginsentology II: Chemical Structure-Biological Activity Relationship of Ginsenoside

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Abstract : Since chemical structures of ginsenoside as active ingredient of *Panax* ginseng are known, accumulating evidences have shown that ginsenoside is one of bio-active ligands through the diverse physiological and pharmacological evaluations. Chemical structure of ginsenoside could be divided into three parts depending on diol or triol ginsenoside: Steroid- or cholesterol-like backbone structure, carbohydrate portions, which are attached at the carbon-3, -6 or -20, and aliphatic side chain coupled to the backbone structure at the carbon-20. Ginsenosides also exist as stereoisomer at the carbon-20. Bioactive ligands usually exhibit the their structure-function relationships. In ginsenosides, there is little known about the relationship of chemical structure and biological activity. Recent reports have shown that ginsenoside Rg_3, one of active ginsenosides, exhibits its differential physiological or pharmacological actions depending on its chemical structure. This review will show how ginsenoside Rg_3, as a model compound, is functionally coupled to voltage-gated ion channel or ligand-gated ion channel regulations in related with its chemical structure.

Keywords : *Panax* ginseng; Ginsenoside Rg_3; Bioactive ligand; Ion channels; Receptors; Structure-activity relationships

INTRODUCTION

Ginseng, the root of *Panax ginseng* C.A. Meyer, has been used as a representative tonic for two thousand years in the Far East countries like Korea, China, and Japan. Now, ginseng is one of the most famous and precious herbal medicines consumed in around the world.[1] Although ginseng exhibits multiple pharmacological actions *in vitro* or *in vivo* studies such as antistress, antihypertension, antioxidant, or neuroprotection, its mechanisms on various efficacies are still elusive. Recent accumulating evidences show that ginsenosides are one of the main active ingredients of *Panax* ginseng (Fig. 1). Ginsenoside is one of the derivatives of triterpenoid dammarane consisting of thirty carbon atoms. Each ginsenoside has a common hydrophobic four ring steroid-like structure with sugar moieties attached. Therefore, ginsenosides have amphiphatic property. About 30 different types of ginsenosides have been isolated and identified from the root of *Panax ginseng*. They are mainly classified into protopanaxadiol (PD) and protopanaxatriol (PT) ginsenosides according to the position of different carbohydrate moieties at the carbon-3 and carbon-6 position.[2] Thus, each type of ginsenoside consists of three portions. First, carbohydrate portions, as mentioned above, are attached to carbon-3, carbon-6 or carbon-20. These side chains with sugar are monomer, dimer, or trimer. These sugar components might provide an amphiphatic property of ginsenoside. Second, the backbone of ginsenosides is hydrophobic four ring steroid-like structure. Third is the aliphatic side or alkene chain $CH_2CH_2CH=C(CH_3)_2$ at the carbon-20 of backbone structure (Fig. 1). As mentioned above, ginsenoside produces diverse pharmacological effects *in vivo* or *in vitro*. However, little is known about the relationship of chemical structure and biological activity of ginsenosides, although the previous report demonstrated that ginsenosides regulate voltage-gated ion channels outside of cell.[3] This review will mainly focus on voltage-gated ion channel regulations by ginsenosides, since recent reports show that ginsenosides regulate various types of ion channels, which of them are activated or inhibited in neurons or in non-neuronal cells in the presence of ginsenosides and ginsenoside-induced voltage-gated ion channel regulation might be a molecular basis of ginsenoside-induced neuroprotections caused by various neurostimulatory agents/

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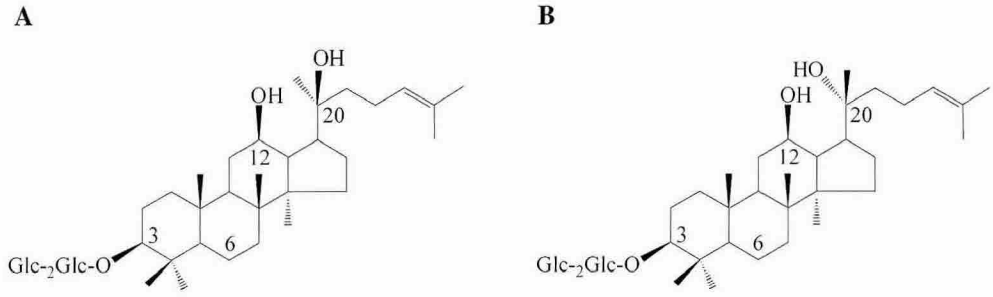


20(*S*)-Ginsenoside Rg_3 **20(*R*)-Ginsenoside Rg_3**

Fig. 1. Ginsenoside Rg_3 exists as epimers at the carbon-20. They differ at the position of hydroxyl group. Abbreviations for carbohydrates are as follows: Glc, glucopyranoside. Superscripts indicate the carbon in the glucose ring that links the two carbohydrates.

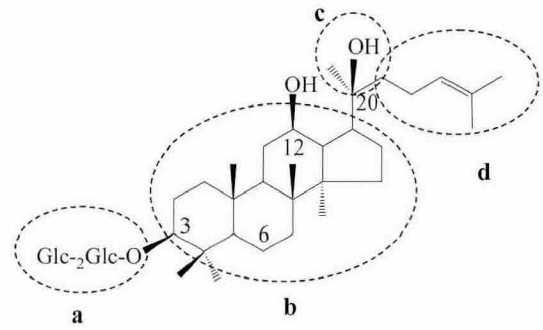
neurotoxins. Therefore, this review article will cover some recent findings on the chemical structure-activity relationship of ginsenosides in ginsenoside-induced ion channel regulations.

Ginsenoside Rg_3 as a model compound for structure-activity relationship study

20(*S*)-ginsenoside Rg_3, 20-*S*-protopanaxadiol-3-[O-β-D-glucopyranosyl (1→2)-β-glucopyranoside]), is one of protopanaxadiol ginsenosides. The chemical structure of ginsenoside Rg_3 is shown in Figure 1. Ginsenoside Rg_3 has two glucoses at the carbon-3 position and has no sugar at the carbon-20. Its chemical structure is quiet contrast to ginsenoside Rf, which has two glucoses at the carbon-6 and also has no sugar at the carbon-20. Ginsenoside Rg_3 exists as a pair of stereoisomers, with a change in the position of the hydroxyl group at the carbon-20 differentiating between the epimers, 20(*R*)-ginsenoside Rg_3 and 20(*S*)-ginsenoside Rg_3 (Fig. 1A and B).[4] The main reasons that ginsenoside Rg_3 are chosen as a model compound as follows: First, ginsenoside Rg_3 among other ginsenosides is the most potent regulator of various types of ion channels such as voltage-dependent Ca^{2+}, K^+ and Na^+ channels and ligand-gated ion channels such as 5-HT_3, NMDA and muscle- and neuronal types of nicotinic acetylcholine receptors.[5-9] Second, ginsenoside Rg_3 exists as stereoisomers as mentioned above and it is relatively easy to differential purification between 20(*R*)-ginsenoside Rg_3 and 20(*S*)-ginsenoside Rg_3 without contamination of the other form.

Role of carbohydrate portion of 20(*S*)-ginsenoside Rg_3 in the regulation of ion channels

Ginsenoside is one of kinds of saponins, which are characterized by glycosides with sugars at specific position of backbone structure, aglycone. Thus, 20(*S*)-ginsenoside Rg_3 is *O*-glycosylated saponin at the carbon 3 position of ginsenoside backbone (Fig. 2a). Despite of many reports on 20(*S*)-ginsenoside Rg_3-induced regulations on various types of voltage-gated ion channel activities, the previous studies did not explain the functional roles of carbohydrate portion of 20(*S*)-ginsenoside Rg_3 in ion channel regulations. Recently, Kim et al, (2004) demonstrated that 20(*S*)-ginsenoside Rg_3 regulates brain Na^+ channel activity, which was heterologously expressed in *Xenopus* oocytes.[10] To know the role of carbohydrate portion in Na^+ channel regulation, they prepared a derivative of 20(*S*)-ginsenoside Rg_3 by conjugation process of second glucose at the carbon-3 with other small hydrophobic compound and examined the effect of the derivative of 20(*S*)-ginsenoside Rg_3 on the Na^+ channel regulation and found that this derivative had no effect on Na^+ channel activity. Furthermore, simple opening of second glucose by oxidation also did not exhibit any effect



20(*S*)-Ginsenoside Rg_3

Fig. 2. 20(*S*)-Ginsenoside Rg_3 consists of three main portions. Carbohydrate portion at the carbon-3; The main backbone; aliphatic side chain. The possible functions of these portions in voltage-gated ion channel or ligand-gated ion channel regulations are in detail described in text.

on Na^+ channel activity. Interestingly, even though treatment of cells with the protopanaxadiol (PPD) alone (which is only backbone structure of 20(*S*)-ginsenoside Rg_3), sophorose alone (which is only dimmer of two glucoses attached to the carbon-3), or combination of PPD and sophorose did not affect Na^+ currents.[10] These results indicate that the carbohydrate portion 20(*S*)-ginsenoside Rg_3 should be intact and that the presence of carbohydrate portion of 20(*S*)-ginsenoside Rg_3 is necessary for 20(*S*)-ginsenoside Rg_3-induced Na^+ channel regulation. It is then questioned what is the role of carbohydrate portion in Na^+ channel regulation. Until now it is not clear on the role of carbohydrate portion of 20(*S*)-ginsenoside Rg_3. However, it is likely that the hydroxyl groups of carbohydrate portion of 20(*S*)-ginsenoside Rg_3 might be required in the interactions with amino acids of channel or receptor proteins by forming hydrogen bonds. Thus, the formation of hydrogen bonds of sugar with amino acids near ion channel pore or receptor channel pore might provide primary basis for the interaction of ginsenosides with ion channels or ligand-gated ion channels. In addition, as secondary role of hydrogen bonds between channel or receptor and carbohydrate portions of ginsenosides are to provide proper orientations of backbone structure or aliphatic chain to channel or receptor proteins. Supporting this speculation is that in site-directed mutagenesis study mutation of ginsenoside interaction caused changes of hydrogen bonds between ginsenoside Rg_3 and ion channels (in preparation). In addition, as mentioned above, modifications of carbohydrate portion also abolished ginsenoside Rg_3-induced regulation of ion channel activity.[10]

Role of backbone structure of ginsenoside Rg_3 in ion channel regulations

The backbone structure of 20(*S*)-ginsenoside Rg_3 is the main skeleton portion. The pathway of ginsenoside backbone synthesis in *Panax* ginseng is almost same with that of mammalian sterol synthesis (Fig. 2b). The initial steps for backbone structure synthesis of ginsenosides begin with squalene as sterols but are divided into two pathways. One is for sterols, the other one is for triterpenoid dammarane. Thus, the backbone structure of ginsenoside is not much different from plant or mammalian sterols except several methyl and hydroxyl groups at several positions. Although the backbone structure of ginsenoside is main portion of ginsenoside, it is not clear what is the role of backbone structure, i.e. non-polar hydrophobic portion of 20(*S*)-ginsenoside Rg_3, in regulation of 20(*S*)-ginsenoside Rg_3-induced voltage-gated ion channels or ligand-gated ion channel activity. One speculation is that the hydrophobic steroid-like component could act as a physical wedge into channel pore regions and affect ion permeability elicited by depolarization or receptor activations. Although the hydrophobic non-polar backbone of 20(*S*)-ginsenoside Rg_3 inserts as a wedge into ion channel or ligand-gated ion channel pore region, the degree of plugging of 20(*S*)-ginsenoside Rg_3 into pore region might be dependent on the pore sizes as well as open or resting state of voltage-gated ion or ligand-gated ion channels. It is known that the pore sizes of voltage-dependent ion channels or ligand-gated ion channels are not homogenous, depending on types of ion channels.[13] For example, muscular nicotinic acetylcholine receptor has larger pore size than other Na^+ or K^+ channels in order of sequence of muscular nicotinic acetylcholine receptor > Na^+ channel > K^+ channel.[11] However, it is also likely that the degree of hydrogen bond formations between carbohydrate portion of ginsenoside Rg_3 and channel proteins might determine how much 20(*S*)-ginsenoside Rg_3 interacts with channel pore amino acid residues. In addition, if the pore size of ion channels or ligand-gated ion channels is not enough large for 20(*S*)-ginsenoside Rg_3 to go into deep pore site, 20(*S*)-ginsenoside Rg_3 might interact with outer pore entrance. Again, supporting this notion is that site-directed mutations of ion channel or ligand-gated ion channels abolished or greatly attenuated 20(*S*)-ginsenoside Rg_3-induced inhibitions at channel pore or outer entryway channel pore.[5]

The role of hydroxyl group at the carbon-20: involvement of 20 (*R*)- and 20(*S*)-ginsenoside Rg_3 epimers

The previous reports have shown that if ginsenoside has no carbohydrates at the carbon-20 and instead have hydroxyl group at that position, some of ginsenoside exist as stereoisomers. Thus, ginsenoside epimers are produced by the position of hydroxyl group at the carbon-20 of backbone structure of ginsenoside (Fig. 2c). It is known that most of ginsenoside epimers are made during the heat process for the preparation of Red ginseng from White ginseng. Until now we do not know exactly what is role of ginsenoside epimers in biological systems. For example, in chemo-physical properties three dimensional structure of ginsenoside Rg_3 epimer is different from each other, depending on the position of the hydroxyl group at the carbon-20. Thus, 20(*R*)-ginsenoside Rg_3 maintains as more straight form than, whereas 20(*S*)-ginsenoside Rg_3 maintains as a little bent form to the direction of backbone of ginsenoside Rg_3. In structure and biological activity

relationships, 20(*S*)- but not 20(*R*)-ginsenoside Rg_3 inhibited voltage dependent Ca^{2+}, K^+, and Na^+ channels, indicating that these channels respond with stereoselective manner of ginsenoside Rg_3, whereas both epimers inhibited ligand-gated ion channel activities such as $5\text{-}HT_{3A}$ and nicotinic acetylcholine receptors, although the inhibitory efficacy on these ligand-gated ion channel activities by 20(*R*)-ginsenoside Rg_3 was less than 20(*S*)-ginsenoside Rg_3.[6] A slight difference in chemical structure between the ginsenoside Rg_3 epimers produces a large difference in ion channel regulation. Thus, it appears that 20(*S*)-ginsenoside Rg_3 has more tight hydrophobic packing near the chiral center than 20(*R*)-ginsenoside Rg_3. Tertiary structures and activities of 20(*S*)-ginsenoside Rg_3 has more tight hydrophobic packing near the chiral center than 20(*R*)-ginsenoside Rg_3 and indicate that 20(*S*)-ginsenoside Rg_3 may have stronger interactions with the receptor region in ion channels than 20(*R*)-ginsenoside Rg_3.[12] These results indicate that voltage-dependent ion channels and ligand-gated ion channels respond to ginsenoside Rg_3 epimers with differential manners and that the position of hydroxyl group at carbon-20 in 20(*S*)- rather than 20(*R*)-form might play a key role in providing a favorable environment for 20(*S*)-ginsenoside Rg_3 to access the interaction sites of pore when voltage-dependent ion channels are in the resting or open state following the voltage steps for channel activation. In *ex vivo* experiment using swine coronary artery, we further demonstrated that treatment with 20(*S*)- but not 20(*R*)-ginsenoside Rg_3 caused a potent concentration-dependent, endothelium-independent relaxation of coronary artery contraction induced by high K^+. However, treatment with both 20(*S*)- and 20(*R*)-ginsenoside Rg_3 induced a significant, concentration-dependent relaxation of 5-HT-induced coronary artery contractions in intact samples, while only 20(*S*)-ginsenoside Rg_3 inhibited coronary artery contraction in endothelium-denuded arterie.[13] Thus, in addition to ion channel regulations in single cell, ginsenoside Rg_3 epimers also exhibit differential regulations in smooth muscle contractions. These results also show further possibility that ginsenoside Rg_3 epimers might differ from each other in their *in vivo* actions depending on the position of hydroxyl group of the carbon-20.

Role of aliphatic or alkene side chain at the carbon-20 of 20(*S*)-ginsenoside Rg_3

As shown in Figure 1d, all ginsenosides have aliphatic side chain $CH_2CH_2CH{=}C(CH_3)_2$ at the carbon-20 of backbone structure (Fig. 2c). This aliphatic side chain is similar to that of cholesterol except the double bonds at the carbon-24 and -25. In different way from cholesterol, this aliphatic side chain of 20(*S*)-ginsenoside Rg_3 was oxygenated when it was orally administered to experimental animals. In feces the oxygenated 20(*S*)-ginsenoside Rg_3 metabolites and backbone metabolites without the moiety of the $CH_2CH_2CH{=}C(CH_3)_2$ were found.[14] Although we now know that this aliphatic side chain of 20(*S*)-ginsenoside Rg_3 undergoes metabolic processes *in vivo*, we do not know exactly what is the functional role of this aliphatic side chain in ion channel regulations. It will be possible to speculate the role of aliphatic side chain if we analyze the effect of the oxygenated metabolites or the backbone structure with carbohydrate portion but without $CH_2CH_2CH{=}C(CH_3)_2$. The experiments using these metabolites shows a possibility that the aliphatic side chain 20(*S*)-ginsenoside Rg_3 could be one of contributors in 20(*S*)-ginsenoside Rg_3-mediated ion channel regulations. Supporting this hypothesis, there are several possibilities that this aliphatic side chain might play as a representative functional group. First, the aliphatic side chain is hydrophobic and could directly interact with hydrophobic domain(s) of voltage-gated ion channels or ligand-gated ion channel proteins when hydrophobic backbone portion of ginsenoside is inserted into lipid bilayer as a wedge as mentioned above. Second, we could observe that the modification of dimethyl group of aliphatic side chain coupled to the carbon-25 induced changes of 20(*S*)-ginsenoside Rg_3-mediated ion channel inhibitions (in preparation). Third, the deletion of aliphatic side chain, remaining with backbone structure and two glucoses intact, also caused a loss of voltage-gated ion channel regulation (in preparation). Thus, these results suggest that the aliphatic side chain might also participate in voltage-gated ion channel regulations and that modifications of aliphatic side chain by addition of hydroxyl group, deletion of aliphatic side chain, or reduction of double bonds at carbon-24 and -25 could greatly affect 20(*S*)-ginsenoside Rg_3-mediated ion channel regulation.

Conclusion and future perspectives

Ginsenoside, an unique saponin in only *Panax* ginseng, exerts its effect in various pharmacological and physiological systems from diverse cells to living animals, although its efficient concentrations in most of *in vitro* and *in vivo* experiments appear much higher than those of well-known agents. However, until now we could not clearly explain what is principle functional group of ginsenoside in its action as other biological active agents

have. To explain its basic action of ginsenoside, first it should need to analyze the role of structural part that consists of ginsenoside. As described above, ginsenoides consists of four main parts such as aliphatic side chain, backbone portion, carbohydrate portion, and epimer forms at the carbon-20. We could obtain several evidences that ginsenoside-induced voltage-gated ion channel regulations *in vivo* and *in vitro* systems require the presence of carbohydrate portions for their hydrogen bond formations with ion channel proteins. In addition, ginsenoside epimers also regulate voltage-gated ion channel and ligand-gated ion channel with differential manners. Thus, it seems that all portions of ginsenoside Rg_3 are involved in voltage-gated ion channels or ligand-gated ion channel regulations. In future, using further chemically modified ginsenoside derivatives or ginsenoside metabolites that have already been modified in animal or human body, it is possible to elucidate what are really necessary portions of ginsenoside in its pharmacological and physiological actions. Furthermore, through these works related with structure-activity of ginsenoside Rg_3 if we are lucky it is possible to find out more active or inactive forms than the natural ginsenoside isolated from ginseng. Second, it needs to have or develop suitable tools for the analysis of action of ginsenoside at single cell level with minimum interferences from other factors. For example, in my laboratory we have utilized *Xenopus* oocytes gene expression systems. *Xenopus* oocyte is a single cell and bigger than other cells. It is easy for management. Moreover, this cell is dull and does have few ion channels itself. The other merit is that oocytes do express receptors and ion channels well that were exogenous. Many researchers used this heterologous gene expression system after injection of cDNA or cRNA for a specific ion channel or receptor signal transduction pathway investigation without inferences of other factors. In future, with well-modified ginsenoside analogs and experimental tools, the elucidations on how ginsenoside interacts with voltage-gated ion channel or ligand-gated ion channel proteins and which portion of ginsenoside plays a key role in regulations of ion channels or ligand-gated ion channels will give us chances to explain the physiological and pharmacological mechanisms of ginsenoside actions on membrane protein of cells.

REFERENCES

1. Tyler, V. E.: Herbal remedies. *J Pharm Technol* **11**, 214-220 (1995).
2. Nah, S. Y.: Ginseng; Recent advances and Trends. *J. Ginseng Res.* **21**, 1-12 (1997).
3. Lee, B. H., Jeong, S. M., Ha, T. S., Park, C. S., Lee, J. H., Kim, J. H., Kim, D. H., Han, J. S., Kim, H. C., Ko, S. R. and Nah, S.Y.: Ginsenosides regulate ligand-gated ion channels from the outside. *Mol Cells.* **18,** 115-121 (2004)
4. Shoji, J. *Recent Advances in Ginseng Studies.*, pp. 11-31, Hirokawa Publishing, Tokyo (1990).
5. Lee, B. H., Lee, J. H., Lee, S. M., Jeong, S. M., Yoon, I. S., Lee, J. H., Choi, S. H., Pyo, M. K., Rhim, H, Kim, H. C., Jang, C. G., Lee, B. C., Park, C. S. and Nah, S. Y.: Identification of ginsenoside interaction sites in 5-HT_{3A} receptors. *Neuropharmacology.*, **52**, 1139-1150 (2007).
6. Jeong, S. M., Lee, J. H., Kim, J. H., Lee, B. H., Yoon, I. S., Lee, J. H., Kim, D. H., Rhim, H., Kim Y. and Nah, S. Y.: Stereospecificity of ginsenoside Rg_3 action on ion channels. *Mol Cells.* **18**, 383-389 (2004).
7. Lee, J. H., Jeong, S. M., Kim, J. H., Lee, B. H., Yoon, I. S., Lee, J. H., Choi, S. H., Kim, D. H., Rhim, H., Kim, S. S., Kim, J. I., Jang, C. G., Song, J. H. and Nah S. Y. : Characteristics of Ginsenoside Rg_3-Mediated Brain Na^+ Current Inhibition. *Mol Pharmacol.* 68, 1114-1126 (2005).
8. Rhim, H., Kim, H., Lee, D. Y., Oh, T. H. and Nah, S. Y.: Ginseng and ginsenoside Rg_3, a newly identified active ingredient of ginseng, modulate Ca^{2+} channel currents in rat sensory neurons. *Eur. J. Pharmacol.* **436**, 151-158 (2002).
9. Lee, B. H., Jeong, S. M., Lee, J. H., Kim, D. H., Kim, J. H., Kim, J. I., Shin, H. C., Lee, S. M. and Nah, S. Y.: Differential effect of ginsenoside metabolites on the 5-HT_{3A} receptor-mediated ion current in *Xenopus* oocytes. *Mol. Cells* **17**, 51-56 (2004).
10. Kim, J. H., Hong, Y. H., Lee, J. H., Kim, D. H., Nam, G., Jeong, S. M., Lee, B. H., Lee, S. M. and Nah, S. Y.: A role for the carbohydrate portion of ginsenoside Rg_3 in Na^+ channel inhibition. *Mol Cells.* **19**, 137-142 (2005).
11. Hille, B.: Ion channels of excitable membranes. *Sinauer Associates* **Chapter 14**, 462-470 (2001).
12. Kang, D. I., Lee, J. Y., Yang, J. Y., Jeong, S. M., Lee, J. H., Nah, S. Y. and Kim, Y.: Evidence that the tertiary structure of 20(S)-ginsenoside Rg_3 with tight hydrophobic packing near the chiral center is important for Na^+ channel regulation. *Biochem Biophys Res Commun.*, **333**, 1194-1201 (2005).
13. Kim, J. H., Lee, J. H., Jeong, S. M., Lee, B. H., Yoon, I. S., Lee, J. H., Choi, S. H. and Nah, S. Y.: Stereospecific effects of ginsenoside Rg_3 epimers on swine coronary artery contractions. *Biol Pharm Bull.* **29**, 365-370 (2006).
14. Qian, T., Cai, Z., Wong, R. N. S., Mak, N. K. and Jiang, Z. H.; In vivo rat metabolism and pharmacokinetic studies of ginsenoside Rg_3. *J. Chromatograph. B.*, **816**, 223-232 (2005).