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Action of Dammarane-Type Triterpenoidal Glycosides and Their Aglycones on Lipid Membranes

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Abstact : We investigated the effects of ginseng glycosides and their aglycones on processes of single ion channel formation and channel properties. The glycosides, Rg_1 and Rb_1, and their aglycones, 20-(S)-protopanaxatriol (PT) and 20-(S)-protopanaxadiol (PD) increased the membrane permeability for ions. PT, PD, Rg_1, and Rb_1; at concentrations of 0.5, 3.0, 10.0 and 30.0 μg/m*l* respectively; induced single ion channel fluctuations with the life times in the range of 0.1~100s in open states and conductances from 5 to 30 pS in 1 M KCl. At high concentrations of these substances, rapid fluctuations of transmembrane ion current with amplitude from hundred pS to dozen nS were observed. Against other substances, ginsenoside Rb_1 began to increase the membrane conductance at concentration of about 60 μg/m*l* without fluctuation of single ion channel. Membranes treated with PT, PD, Rg_1 and Rb_1 are more permeable to K^+, than to Cl while zero current membrane potentials with 10 gradients of KCl were 12, 16, 8, 25 mV respectively.

Key words : Membrane conductance, single ion channel, ginsenosides.

Introduction

Saponins are the major components of extracts from root of *Panax ginseng*.[1] Preparations obtained from extracts of ginseng root are used as practical medicines in various countries. Ginseng saponins are classified into two groups. They are triterpenoidal glycosides of 20-(S)-protopanaxadiol and triterpenoidal glycosides of 20-(S)-protopanaxatriol, which show different biological activities.[1] The properties observed are associated with their actions on cellular membranes.[2-4] The mechanism of interactions of these substances with membranes, however, remains unknown.

Investigation of molecular mechanism of effects of these substances on membranes is particularly important for the application of therapeutic uses.

We describe the experimental results of action of total saponin, 20-(S)-protopanaxadiol, 20-(S)-protopanaxatriol, and glycosides Rg_1 and Rb_1 from *Panax ginseng* C.A. Meyer on lipid membranes. The technique of planar lipid bilayer allows to reveal their mechanism in molecular level, as well as to establish the details of relation between structures of substances and their membrane activities.

Materials and Methods

Planar lipid bilayers were formed according to Montal[5] from two monolayers obtained at the air/

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water interface. A thin teflon (tetrafluoroethylene) film (25 μm thick) with a small aperture was clamped between two halves of a trough and kept stationary. Membrane was formed by following steps. The two compartments was first filled with water or saline to below the aperture. Lipid monolayers are secondarily spread on each side. For the last, water levels are slowly raised above the aperture by gravity flow one after the other. When the aperture descent below the water level, the two monolayers came in contact and began to form a bilayer by joining their hydrocarbon chains.

The measuring chamber and the electrical arrangement were also similar to those of Montal.[5] A constant voltage pulse was applied to the membrane at the same time as a capacitative current was displayed on a storage oscilloscope. The capacity was calculated from the current records by $C=(\int_0^\infty Idt)/\Delta V$, where I is the current and ΔV is the amplitude of the constant voltage pulse. The specific capacity of membranes was equal to 9 ± 1 mF/m^2. Because the capacity current is small, the feedback resistance was kept about 100 kΩ.

In order to elucidate the selectivity of membranes for K^+ and Cl ions, we measured zero current potential with 10 gradients of KCl. 1 M KCl solution was contained on one side of membrane, and 0.1 M KCl on the other side. In experiments with single channel, 80% egg lecithin (Fluka, Germany) and 20% cholesterol (Serva, Germany) were contained in lipid bilayer. The solution was consisted of 1 M KCl, 5 mM Tris-HCl (pH 7.4, 23℃).

The total saponins, 20-(S)-protopanaxatriol, 20-(S)-protopanaxadiol, Rg_1, and Rb_1 were offered from analytic center of Korea Ginseng & Tobacco Research Institute (Taejon, Korea).

Results and Discussion

Action of total saponin on membrane was registered adding the total saponin into aqueous phase of one side of the membrane. It was similar to jumps of current through a transmembrane channel (Fig. 1). Current-voltage curves of single channel as well as multiple channels of membrane were linear in the range from −100 mV to +100 mV. The membrane with an ion channel formed by total saponin was more permeable to cations. Zero current potential of membrane with total saponin was 20 mV.

The current fluctuation of large amplitude was registered when increasing the concentration of total saponin subsequently. We have assumed that the appearance of this large current fluctuation can be connected with the formation of ion-conducting structures such as transmembrane pores in the membrane. The conductance of these structures was in the range from hundred pSm to dozen nSm. Such fluctuations of membrane current were registered on the membranes without cholesterol at given concentration of substances. Under these conditions, the membrane was not selective to cations and anions.

1. Action of 20-(S)-protopanaxatriol, 20-(S)-protopanaxadiol, Rg_1 and Rb_1

Our experiment demonstrates that the studied

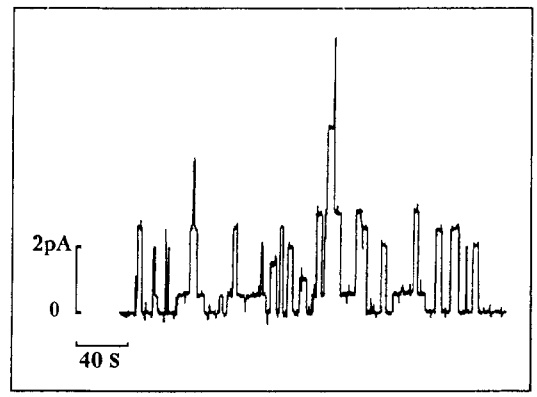

Fig. 1. Current fluctuation of a single channel formed by total saponin at concentration of 1 μg/ml. Applied voltage was 100 mV.

substances increase the permeability for ions when they are added into one side of the aqueous phase of the membranes. Depending on the concentration of substances and lipid composition of membrane; protopanaxatriol, protopanaxadiol and Rg_1 form two types of ion-conducting structures: one is a single channel possessing low conductance and the other is an ion-conducting structure having large conductance.

Protopanaxatriol, protopanaxadiol and ginsenoside Rg_1 with the concentrations of 0.5 μg/m*l*, 10 μg/m*l* and 3 μg/m*l* respectively, formed the ion channels having low conductances (Fig. 2). At high concentration of these substances, rapid fluctuations of ion current with amplitudes from dozen pA to hundred nA were registered with the membrane potential of 100 mV. This process did not depend on the cholesterol contents in membranes. The life-time of open state was in the range of 0.1~100 s.

Ginsenoside Rb_1 began to increase the membrane conductance at the concentration of about 60 μg/m*l*. Against other substances, we could not observe the single channel fluctuation but observe a chaotic noise of membrane current. At high concentration of Rb_1, the current fluctuation with large amplitude was registered identically to the cases of protopanaxatriol, protopanaxadiol and Rg_1.

The membranes whose conductances were induced by protopanaxatriol, Rg_1 and Rb_1 were more permeable to K^+ than to Cl^-. Zero current potentials with 10 gradients of KCl on membrane were 12 mV, 8 mV, 16 mV, respectively.

It is reported that 20-(S)-protopanaxadiol is able to increase the conductance of membranes formed by α-monoolein.[7] However, the increase of membrane conductance by 20-(S)-protopanaxadiol is not clearly explained on the basis of our present results. Our experiments show that this substance forms ion channels in lipid membranes at low concentration. One of the conditions of registration of ion channels is the cholesterol presence in the membrane.

The membranes with 20-(S)-protopanaxadiol were more permeable for K^+ ions than for Cl^- ions. Zero current potential was more than 30 mV. In Fig. 3, the experimental record of single channel current fluctuation of 20-(S)-protopanaxadiol is adduced, the polarity of which corresponds to the cation selectivity.

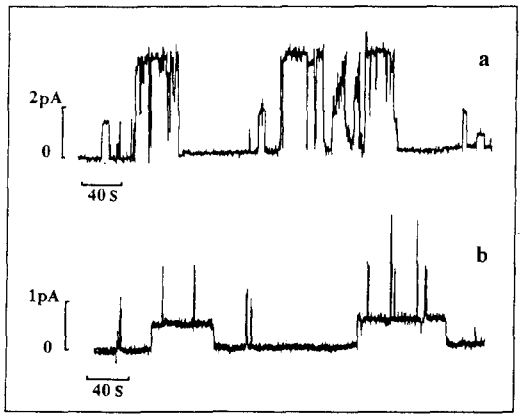

Fig. 2. Current fluctuations through transmembrane channels formed by a) 20-(S)-protopanaxatriol and b) ginsenoside Rg_1. Applied voltage was 100 mV.

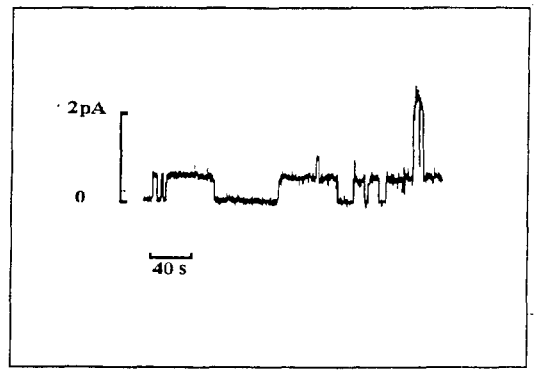

Fig. 3. The transmembrane current of short circuit through channels induced by 20-(S)-protopanaxadiol. 1 M KCl solution was contained on one side of membrane, and 0.1 M KCl solution was on the other side. The polarity of the current jumps corresponds to cation selectivity.

It was shown that the holostan-type triterpenoidal glycosides from Holothuria mexicana form ion channels only on adding to the both sides of lipid membrane.[8] With the addition on one side of membrane, single channels were not registered.

The results of our studies show that dammarane-type triterpenoidal glycosides from the root of *Panax ginseng* C.A. Meyer differ from glycosides from Holothuria mexicana in the channel-forming properties. First of all, ginsenosides form ion channels in membranes when added to one side of the membranes. Moreover, it is found that their interactions with lipid membranes do not depend on the cholesterol content.

Ginsenosides differ in single channel conductances (single channel conductances of glycoside from Holothuria mexicana and of ginsenoside Rg_1 were 28 pSm and 5 pSm, respectively, in 1 M KCl solution). This difference can be mainly connected with the difference in chemical structures of these glycosides.

The discrete changes of the current with large amplitude were observed in the studies of action of glycoside from Holothuria mexicana[8] and commercial saponins[9] on lipid membranes. The discrete fluctuations of current with large amplitude are registered at electrical breaks of membranes and phase transitions. Such fluctuations of current are explained to structural defects, pore-forming in membranes,[10] micellar structures of different sizes[9] and fusion of single channels.[8]

Depending on their molecular structures, ginsenosides exhibit the different biological and pharmacological activities.[1] The technique used in our study establishes that the characteristic of ion channel and value of threshold concentration at which membrane conductance begins to increase depend on the molecular structures of ginsenosides. It is assumed that the distinction in membranotropic activity of preparations underlie the variety of their biological and pharmacological actions.

For the study of more details of interrelations between structure and membrane activity, the experiments with other ginsenosides and their synthetic analogues are required.

요 약

Glycoside인 Rg_1과 Rb_1 및 그것들의 aglycones인 20-(S)-protopanaxatriol(PT)과 20-(S)-protopanaxadiol(PD)은 막의 투과성을 높였다. PT, PD, Rg_1 및 Rb_1은 2종류의 막에서 이온을 통과시키는 구조를 형성했다. PT, PD, Rg_1 및 Rb_1은 각기 0.5, 3.0, 10.0, 30.0 μg/m*l*의 농도에서 1 M KCl용액조건에서 5 pS부터 30 pS에 이르는 전도성을 보이며 0.1초부터 100초의 시간동안 열린채 있는 single ion channel을 형성했다. 고농도에서 PT, PD, Rg_1 및 Rb_1은 수백 pS에서 수십 nS에 이르는 크기를 가진 막을 통과하는 빠른 ion current를 일으켰다. Rb_1은 약 60 μg/m*l*의 농도에서 막의 전도성을 높였으며 다른 물질들에게서 관측되어지는 single ion channel current는 없었다. PT, PD, Rg_1 및 Rb_1으로 처리된 막은 Cl^- 이온 보다는 K^+ 이온에 더 높은 투과성을 보였으며, 10배의 KCl농도 차에서 측정한 영전류에서의 막전압은 각각 12, 16, 8, 25 mV였다.

References

1. Shibata, S. : *3rd Int. Conf. on Chem. and Biotechnol. of Biologically Active Natural Products*, Sofia, p. 148 (1985).
2. Popov, A. M., Atopkina, L. N., Semoshina, N. F. and Uvarova, N. I. : *Antibiotici Chimioterapya*, **39**, 19 (1994).
3. Nakamura, T., Inoue, K., Nogima, S., Sankawa, U., Shoji, J., Kawasaki, T. and Shibata, S. : *J. Pharm. Dyn.*, **2**, 374 (1979).
4. Zhang, W. J., Zhong, G. G., Jiang, Y., Wang, X. M. and Wang, Z. F. : *Acta Pharm. Sinica.*, **15**, 173 (1994).
5. Montal, M. and Mueller, P. : *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 3561 (1972).

6. Kolomytkin, O. V. and Abdrasilov, B. S. : *Biol. Membrane*, **2**, 519 (1985).
7. Likhatskaya, G. N., Popov, A. M., Odinokova, L. E., Atopkina, L. I., Uvarova, N. I., Kuznetsova, T. A. and Anisimov, M. M. : *Ser. Biol.*, **6**, 942 (1990).
8. Popov, A. M, Rovin, Yu. G., Likhatskaya, G. N., Anisimov, M. M. and Rudnev, V. S. : *Dokl. AN SSSR.*, **262**, 987 (1982).
9. Gogelein, H. and Huby, A. : *Biochim. Biophys. Acta*, **773**, 32 (1984).
10. Antonov, V. F., Smirnova, E. Yu. and Shevchenko, E. V. : *The Lipid Membranes at Phasic Conversions*, M. Nauka (1992).