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Effect of Ginseng Saponin on the Integrity of Lysosomes

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인삼사포닌이 Lysosome의 안정성에 미치는 영향

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Abstract

The effect of ginseng saponin on acid phosphatase (AP) activity in liver lysosomes was investigated and the mechanism by which ginseng saponin may function on the integrity of lysosomes was discussed.

The experimental results obtained are summarized as follows;

1. A very marked increase in the AP activity was observed in the supernatant of hypotonic medium, as compared with that of isotonic medium, indicating that the hypoosmotic shock *per se* results in activation through osmotic lysis of particles.

2. Ginseng saponin had no effect on the activity of AP if once released from lysosomes when lysed in the hypotonic medium, suggesting that ginseng saponin has no effect on the enzyme molecules *per se*.

3. The AP activity in isotonic medium suspensions was decreased at the concentrations of 10^{-6}, 10^{-5} and 10^{-4}% of ginseng saponin, but increased at 10^{-2} and 10^{-1}%. It's suggested that ginseng saponin enhances the integrity of lysosomes at 10^{-6}, 10^{-5} and 10^{-4}%, but decreases it at 10^{-2} and 10^{-1}%.

4. Suspending particles in distilled water resulted in no correlation of AP activity with treatment with ginseng saponin.

5. The AP activity was decreased in the presence of ATP, showing the possible significance of ATP as a lysosomal stabilizer and the possibility that ginseng saponin may affect a membrane bound ATPase system by which lysosomal AP release may be controlled.

Introduction

Since lysosomes have many kinds of hydrolytic enzymes and mediate the digestive and lytic process of the cell, their stability in the living cell is of fundamental importance. Damage to lysosomes, with release of the enzymes they contain, might play a part in carcinogenesis.[1] Some of the agents that stabilize the membrane of lysosomes and other labilizing agents on the lysosomal membrane have been found.[1] Some of the agents that stabilize the membrane of lysosomes and other labilizing agents on the lysosomal membrane have been found.[2-9]

A number of papers have been published on the chemical components and on the pharmacological and physiological effects of ginseng. Despite these studies, there are few reports regarding to the integrity of lysosomes.

Therefore, the present experiment was carried out to investigate the effect of ginseng saponin on the acid phosphatase activity in liver lysosomes and to pursue the mechanism by which ginseng saponin may function on the integrity of lysosomes.

Materials and Methods

Ginseng saponin was prepared from powdered Korean white ginseng roots (Keumsan, 4 years, 50pcs/300g) according to the procedure described in Fig. 1.

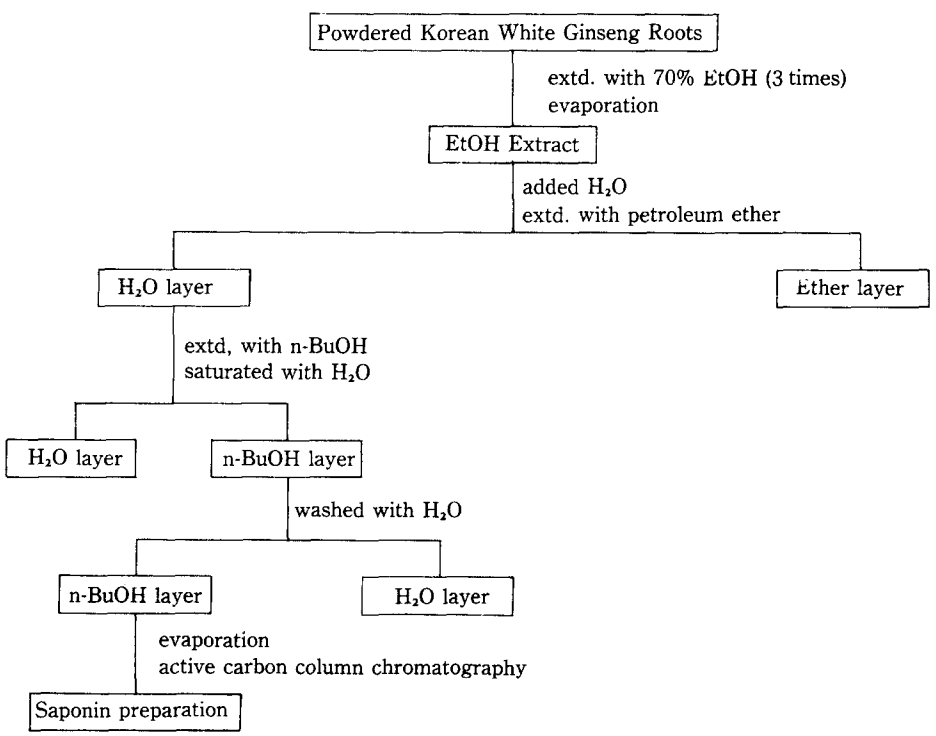

Fig. 1. Extraction procedure of total saponin from the ginseng.

Succinate, DCIP (Dichloro indo phenol), PMS (Phenazine methosulfate), NADPH, cytochrome c, NADH, pyruvate, Fiske & Subbarrow Reducer, 4-nitrophenyl phosphate, ATP and BSA (Bovine serum albumin) were obtained from Sigma Chemical Co.; Na-β-glycerophosphate from Daiichi; and other reagentts from Wako Chem. Co..

Sprague Dawley rats (150-250g,) were killed by decapitation after 48 hours of fasting, and livers were removed. A lysosomal fraction was prepared by the differential centrifugation method which is based upon the schemes of de Duve *et al.*[10] and Stahn *et al.*[11] (Fig. 2).

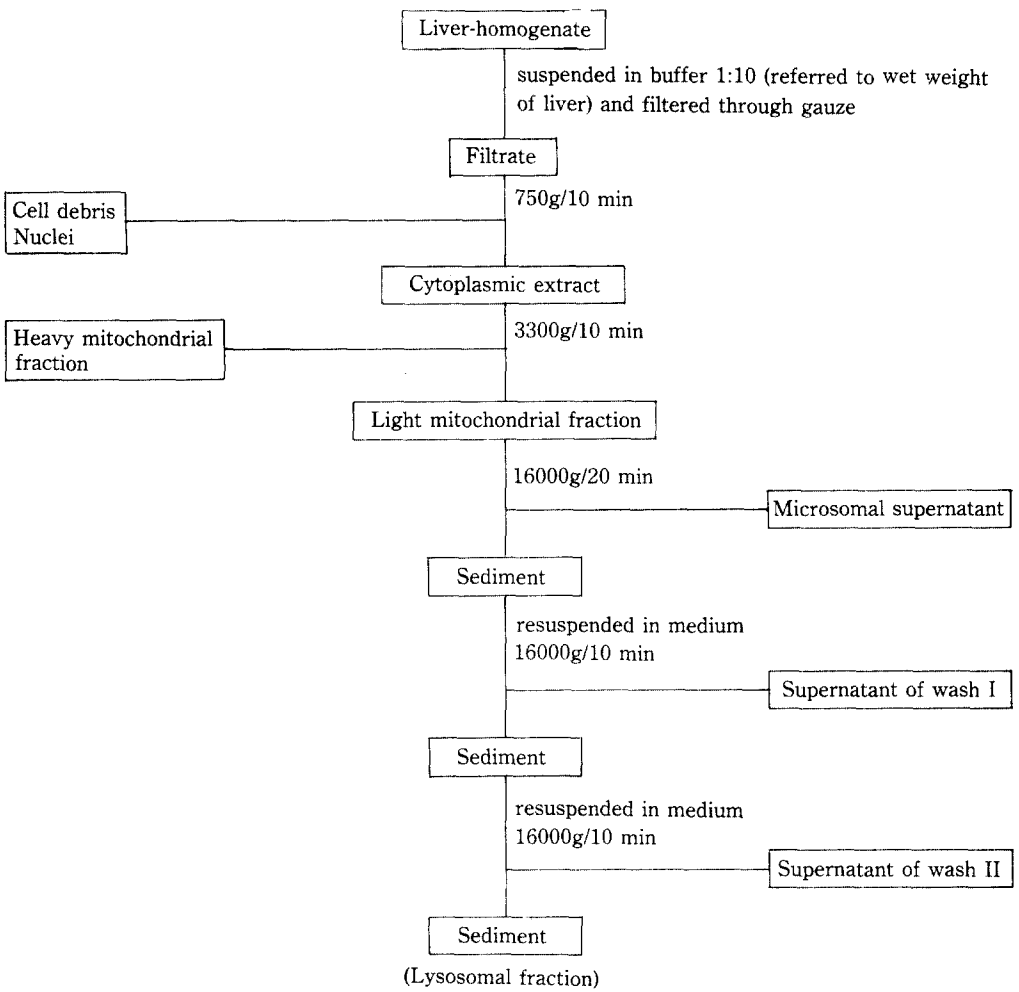

Fig. 2. Scheme of the lysosomal preparation with differential centrifugation.

Succinate dehydrogenase was assayed as a marker for the mitochondrial fraction according to Singer[12]; acid phosphatase as a marker for the lysosomal fraction according to Chung *et al.*[13] and Walter and Schutt[14]; and NADPH-cytochrome c reductase and lactate dehydrogenase as a marker for the microsome-cytosol fraction according to Pederson *et al.*[15] and Neiland[16] respectively. Protein was measured by the method of Biuret.[17]

In order to observe the effect of ginseng saponin on the acid phosphatase (AP) activity, lysosomes were suspended in isotonic medium and hypotonic medium, followed by centrifugation at 16,000×g for 30 min. In the experiment to investigate the effect of ATP on lysosomal fraction, 3×10^{-3}M ATP (disodium salt) and 5×10^{-3}M $MgCl_2$ were added to the reaction mixture and the AP activity was measured.[18]

Results and Discussion

Table 1. shows that lysosomal fraction used for this study was little contaminated with other organelles.

Table 1. Intracellular distribution of enzymes for identification of cell fractions. The relative activity is given in parenthesis.

Cell Enzyme fraction	Protein	Succinate dehydrogenase	Acid phosphatase	NADPH-cytochrome c reductase	Lactate dehydrogenase
	mg	Total activity △ O.D./min			
Homogenate	742 (100)	148.4 (100)	24.3 (100)	88.0 (100)	2819.6 (100)
Mitochondria	176 (23.7)	70.4 (47.4)	6.1 (25.1)	6.0 (6.8)	211.2 (7.5)
Lysosome	25 (3.4)	N.D.*	13.9 (57.2)	N.D.*	27.5 (1.0)
Supernatant**	265 (35.7)	N.D.*	3.0 (12.3)	22.3 (25.3)	318.0 (11.3)

*N.D.: none detected
**Microsome + cytosol fraction

The release from particle suspensions of hydrolases in a soluble form is a very popular parameter used to evaluate the integrity of lysosomes.[19] Therefore the AP activity was measured in the supernatant after removal by centrifugation of the sedimented fraction.

A very marked increase in the AP activity was observed in the supernatant of hypotonic medium, as compared with that of isotonic medium ($P<0.001$) (Fig. 3), indicating that the hyposmotic shock *per se* results in activation through osmotic lysis of particles.

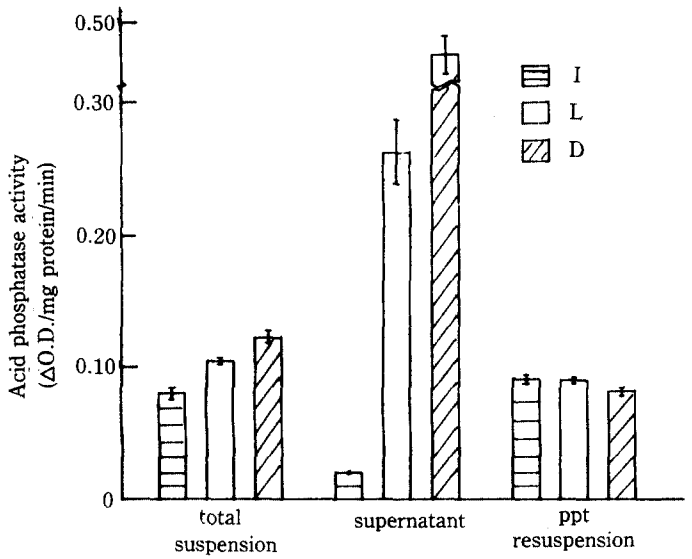

Fig. 3. Release of acid phosphatase in hypotonic medium. The data are presented as mean ±SD. (sucrose): I, 0.33M; L, 0.025M; D, OM

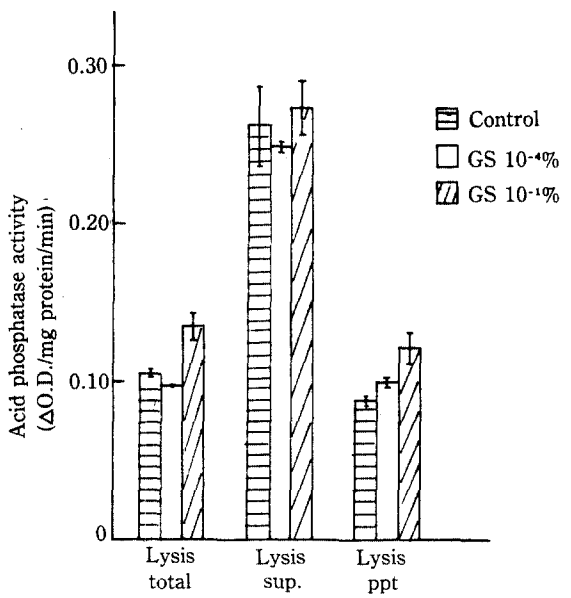

Fig. 4. Effect of ginseng saponin on the acid phosphatase activity after released from lysosome into hypotonic medium. Results are the mean of three experiments ±SD.

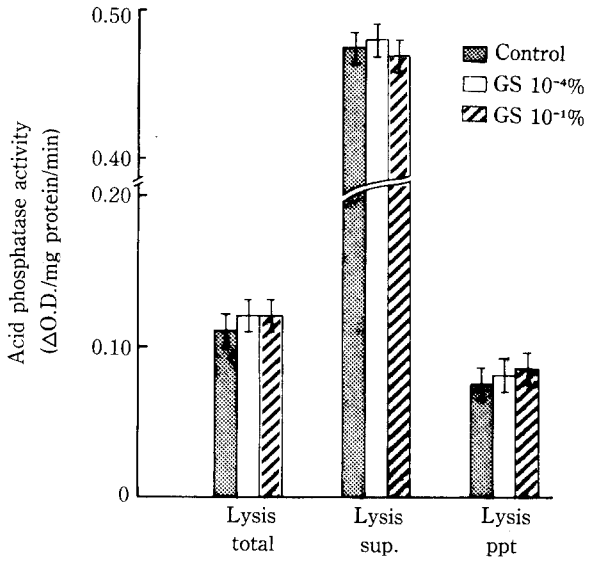

Fig. 5. Effect of ginseng saponin on the acid phosphatase activity after treated with extensive hypoosmotic shock. Results are the mean of three experiments ±SD.

Figs. 4 and 5 show that there was no significant change in the AP activity in the supernatant of hypotonic medium which contained ginseng saponin, as compared with control.

β-glycerophosphate as a substrate for AP, in spite of its relatively small molecular weight, cannot penetrate lysosomes.[20] Therefore the present results indicate that ginseng saponin does not have an effect on the enzyme molecules *per se* already freed from lysolemma, but has an effect on the integrity of lysosomes, so as to free the lysosomal enzymes.

As shown in Fig. 6, 10^{-6}, 10^{-5} and 10^{-4}% of ginseng saponin decreased significantly the AP activity in isotonic medium suspensions, suggesting that those concentrations of ginseng saponin enhance the integrity of lysosomes, followed by inhibition of the release of AP from particle suspensions. However, 10^{-2} and 10^{-1}% of ginseng saponin increased significantly the AP activity, suggesting that those concentrations of ginseng saponin decrease the integrity of lysosomes. In the experiment using radioactive ginseng saponin, Lee[22] showed that the concentration of saponin in the rat liver was about 10^{-5}% 2 hours after oral administration. It is easily understood therefore that the ginseng saponin may not labilize the lysolemma *in vivo* since in the present experiment the AP activity was decreased in the presence of 10^{-5}% of ginseng saponin.

When suspended in 0.025M sucrose medium, ginseng saponin decreased the AP aceivity at the concentration of 10^{-4}%, but increased at 10^{-1}%, in agreement with its

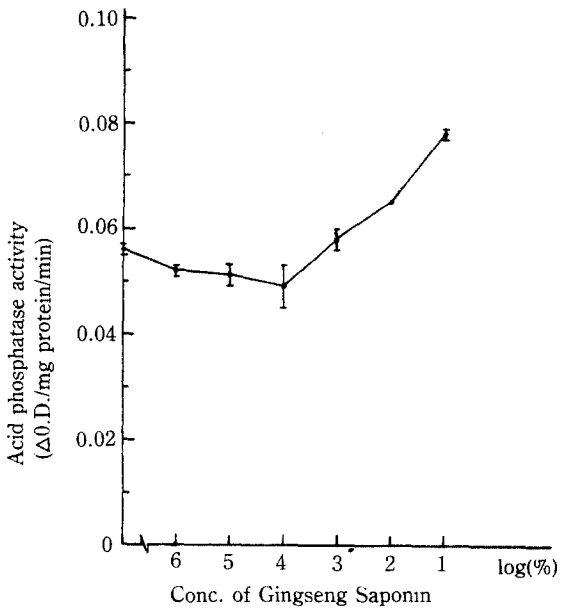

Fig. 6. Effect of ginseng saponin on the release of acid phosphase from the lysosomes suspended in isotonic medium. Results are expressed as mean ±SD.

effect when suspended in isotonic medium (Fig. 4). However, suspending particles in distilled water resulted in no correlation of AP activity with treatment with ginseng saponin (Fig. 5). Considering that RLA (Residual Latent Activity) remains latent even after incubation in distilled water for 30 min at 0°C (extensive hypoosmotic shock)[22, 23], present results suggest that most of all acid phosphatases except RLA are released from lysosome, causing condition of total suspension similar to one of supernatant. Whether ginseng saponin has an effect on RLA or not, however, is not clarified by the present findings.

The stabilizing effect of ATP on lysosomal fractions exposed to cysteine and other thiols and the possible involvement of a lysosomal membrane bound ATPase in controlling AP release were reported.[18, 24] On the other hand, it was suggested that ginseng saponin enhanced mitochondrial ATPase activity through the direct action upon the ATPase system rather than the alteration of mitochondrial membrane structure.[25] As shown in Table 2, the AP activity was decreased in the presence of ATP by 53±2, 49±3 and 48±1% at the concentration of 0, 10^{-4} and 10^{-1}% of ginseng saponin respectively. Therefore the present data show the possible significance of ATP as a lysosomal stabilizer and the possibility that ginseng saponin may affect a membrane bound ATPase system by which lysosomal AP release may be controlled. However, the data are insufficient to allow firm conclusion to be drawn concerning the exact mechanism by which ginseng saponin has an effect on the integrity of lysosome. In order to deter-

Table 2. Effect of ATP on Ginseng Saponin (GS)-Induced Lysosomal Labilization.[a]

Additions	Acid phosphatase (% of control[c])[b]
ATP, 3×10^{-3} (M)	53±2
GS, 10^{-4} (%)	88±4
+ATP, 3×10^{-3} (M)	43±2
GS, 10^{-1} (%)	141±3
+ATP, 3×10^{-3} (M)	67±3

[a]: ±SD, 3 rats
[b]: The percentage values are based on the control value (0.056±0.001ΔO.D./mg protein/min).
[c]: absence of ATP and GS

mine it, it seems necessary to evaluate the effect on the lysosomal ATPase system of the ginseng saponin.

要 約

Lysosome의 안정성에 미치는 인삼사포닌의 영향을 관찰하기 위하여 AP 활성을 조사하고 이의 기작을 규명하고자 하였다.

저장성 용액의 상층액에서 AP 활성이 등장성에 비해 크게 증가되었으며 인삼사포닌 처리에 무관하였다.

등장성 용액에서 AP 활성이 10^{-6}, 10^{-5}와 10^{-4}의 인삼사포닌 농도에서는 감소되었으나 10^{-2}와 10^{-1}%에서는 증가되었으며, 강력한 저삼투성 충격을 가했을 AP 활성은 인삼사포닌 처리에 무관하였다.

ATP를 첨가하면 AP 활성이 감소되었다.

이상의 결과로 보아 인삼사포닌은 일단 유리된 AP에는 영향을 주지 않고 10^{-6}, 10^{-5}와 10^{-4}의 농도에서는 lysosome의 안정성을 증가시킨다고 사료된다.

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