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Effect of Ginseng Saponin on the Protein Biosynthesis of *E. coli* Cells

Ji Hun Ko, Hee Bong Lee* and Chung No Joo

Department of Biochemistry, College of Science, Yonsei University, Seoul 120
*Department of Chemistry, Kangreung National College**

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인삼사포닌 분획이 *E. coli* 세포의 단백질 합성에 미치는 영향

고지훈, 이희봉*, 주충노
연세대학교 이과대학 생화학과, 강릉대학 화학과*

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Abstract

E. coli (K-12 W1485) was grown in M9 minimal medium containing ginseng saponin (10^{-3}%—2%) and found that the cells grew most rapidly in the presence of 10^{-1}% saponin. The cells, harvested at the early exponential phase, were transferred to the minimal medium containing 10^{-1}% saponin plus ^{14}C-labelled saponin (0.03 μCi) and the incubation was continued at 37°C for 20 minutes and the cells were fractionated into the outermembrane, innermembrane and cytosol fraction. Radioactivity data showed that the most radioactivity was detected in the innermembrane.

The activity of succinate dehydrogenase of the cells grown in the above saponin medium was significantly higher than that of the cells grown in ordinary minimal medium. No significant difference of the glucose 6-phosphate dehydrogenase activity was observed between the two groups.

It was also found that the saponin stimulated glucose uptake and biosynthesis of lipids and proteins of the cells. Incorporation of ^{14}C-leucine into the protein fraction of the cell was also accelerated.

I. Introduction

It has been demonstrated that the growth of *E. coli* was stimulated in the presence of moderate amounts of saponins extracted from the roots of *Panax ginseng* C.A. Mayer by several workers.[1-3]

It was found in this laboratory that when *E·coli* (AD01) cells were grown in nutrient rich medium such as BHI broth, no effect of ginseng saponin was observed. However, when *E. coli* cells were grown in a minimal medium containing saponin (10^{-2} %-10^{-3}%), the growth rate was accelerated, suggesting that the saponin stimulated the metabolism particularly under unfavorable conditions.

Analysis of *E. coli* cells grown in the minimal medium containing ginseng saponin showed that the synthesis of lipids and proteins of the cells was stimulated. A similar effect of ginseng saponin on the growth of wild type of *E. coli* was also observed. Choi[3] also reported that when *E. coli* (k-12) cells were grown in the minimal medium containing ginseng saponin (10^{-4}%-10^{-1}%), the growth rate of *E. coli* cells was steadily increased proportionally to the concentration of the saponin in the medium.

It has been demonstrated that moderate amounts of the saponin stimulate the reactions catalyzed by several enzymes so far tested in this laboratory such as mitochondrial dehydrogenase,[4] and Joo[1] suggested that the synthesis of carbohydrates, lipids, proteins and nucleic acids of the bacterial cells might be stimulated in the presence of the saponin resulting in a rapid growth of the organism.

It was therefore attempted in the present study to know the uptake of the saponin by *E. coli* cells and to investigate the distribution of the saponin in the cells by assaying some enzymes such as succinate dehydrogenase and glucose 6-phosphate dehydrogenase, which were known to present in the innermembrane and cytosol fraction of the cell, respectively.

It was also investigated the effect of ginseng saponin on glucose uptake and its turnover to the lipid and protein of the organism. The degree of incorporation of ^{14}C-leucine to protein fraction of the *E. coli* cells in the presence and/or absence of ginseng saponin was examined.

II. Materials and Methods

E. coli (K-12 w1485) cells were grown in nutrient broth up to early exponential phase and cells were transferred to M 9 minimal medium (composition: Na_2HPO_4 6g, KH_2PO_4 3g, NH_4Cl 2g, NaCl 1g, glucose 3g, $CaCl_2$ 0.01g and $MgSO_4$ 0.015g in 1000ml of water), and cultivated.

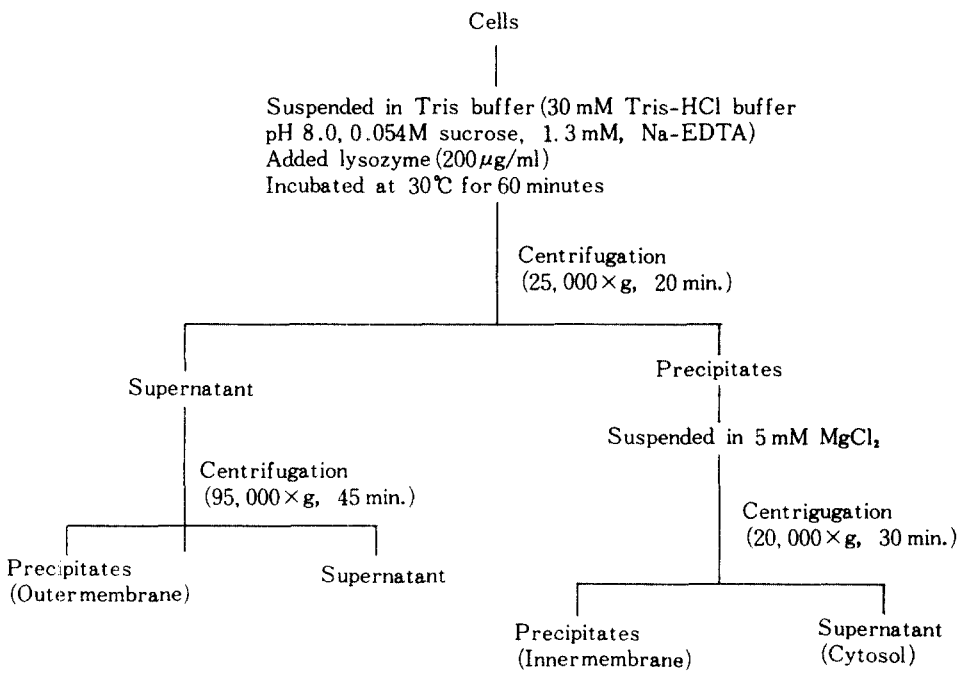

Figure 1. Cell fractionation scheme

The growth rate at the exponential phase of the cells in M9 minimal medium containing various concentration of ginseng saponin was monitored by measuring the turbidity at 600mm using Coleman Junior Spectrophotometer.

The ginseng saponin was extracted from the roots of *Panax ginseng* C.A. Mayer (Keumsan, 4 years, 50 pieces/300g) and ^{14}C-ginseng saponins were prepared from 1,2-^{14}C-acetate as described elsewhere.[5]

The outermembrane, innermembrane and cytosol fraction of *E. coli* cells were fractionated according to Miura[6] as shown in Figure 1. The harvested cells were suspended in ice-cold *Tris* buffer (30mM Tris-HCl buffer (pH8.0), 0.54M sucrose, 1.3mM Na-EDTA) and 200 μg of lysozyme/ml were added and incubated at 30°C for 60 minutes followed by centrifugation to obtain the supernatant (innermembrane and cytosol fraction)and precipitates (outermembrane).

Succinate dehydrogenase was assayed according to Earl *et al.*[7] and glucose 6-phosphate dehydrogenase activities were determined according to Tanahashi.[8]

Protein was determined according to Bradford[9] using coomassive brilliant blue.

Lipids and proteins of the *E. coli* cells were isolated by Bligh-Dyer method[10] after the cells were denatured by the addition of 10% TCA.

Radioactivity of the protein fractions was assayed using Packard Tri-Carb Scintillation spectrometer. [U-^{14}C]-glucose, [1,2-^{14}C]-accetate, [U-^{14}C]-leucine were purchased from the New England Nuclear Corporation and the medium materials were purchased from the Difco.

III. Results and Discussion

The growth rate at the exponential phase of *E. coli* K-12 in M9 minimal medium containing ginseng saponins (10^{-3}%-2%) was observed and found that the cells grew most rapidly in the presence of 10^{-1}% saponin (Fig. 2). Therefore, the minimal medium containing 10^{-1}% saponin and radioactive ^{14}C-saponin prepared from 1,2-^{14}C acetate (0.05 μCi) was used in this experiment.

The cells harvested at the early exponential phase were transferred to the minimal medium containing saponins and the incubation was continued at 37°C for 20 min., and the cells were fractionated according to the scheme shown in Fig. 1.

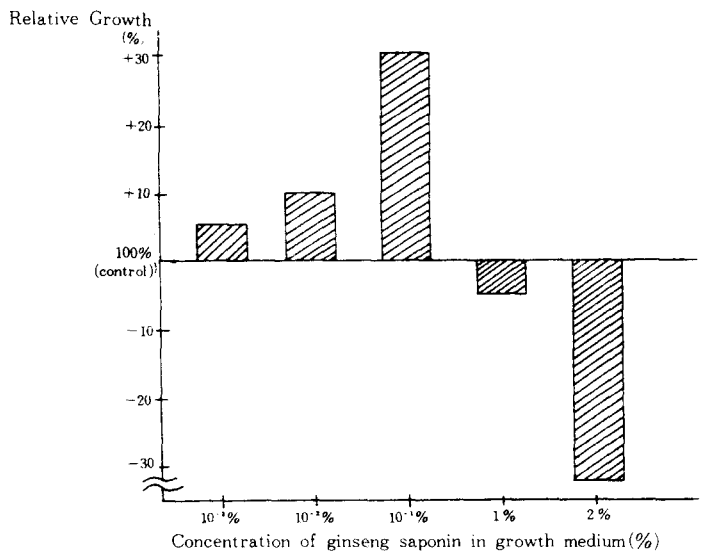

Figure 2. Relative growth of *E. coli* K-12 in the presence of ginseng saponin

Radioactivity data showed that the most radioactivity recovered from the cells were found in innermembrane and only trace was in outermembrane but no radioactivity was detected in cytosol fraction. This suggested that the saponin taken up by the cells were bound in innermembrane of the cells (Table 1).

Table 1. Radioactivity distribution in *E. coil* cells, incubated in the medium containing 10^{-1}% ginseng saponin and ^{14}C-saponins(0.05 μCi) at 37°C for 20 minutes.

	Radioactivity (CPM)	% Recovered
Total radioactivity in the medium	85, 435	100
Innermembrane	1. 071	1.2
Outermembrane	Trace	—
Cytoplasm	not detected	—

The innermembrane and cytosol fraction of the cells grown in the minimal medium containing saponin were used as enzyme sources to determine the activities of succinate dehydrogenase and glucose-6-phoshate dehydrogenase respectively, and compared with those of control cells.

It was found that the succinate dehydrogenase of the cells, incubated with the medium containing saponin, was significantly stimulated, while the Glucose 6-phosphate dehydrogenase was not as shown in Table 2.

Table 2. Enzyme activities of cytoplasm(G-6-PDH) and innermembrane(SDH) of the cells.

Group	Enzyme activity*		Relative activity Test/Control
	G-6-PDH	SDH	
Control	34. 8±2.0	32. 8±1.6	0.94
Test	30. 6±1.5	50. 0±4.2	1.63

*The enzyme activity was expressed in international unit.

**Incubated in the minimal medium containing 10^{-1}% saponin, and control cells. The reaction mixture (3. 0ml) for SDH contained 0. 1M phosphate buffer (pH7.6), 20mM Na-succinate, 1 mM KCN, 0.04 mM DICPIP and 0. 3ml of the innermembrane preparation. The reaction mixture (3.0ml) for G-6-PDH contained 50 mM Tris-HCl buffer (pH 8. 3), 10. 67 mM Glucose-6-phosphate, 0. 5 mM $NADP^+$ and 0. 3ml of the cytosol preparation.

From the data of Table 1, it was expected the concentration of the saponin in the cells being around 10^{-4}%-10^{-3}% level, which seemed to be adequate amounts of the saponin for the enzyme stimulation.

It was also found that the saponin stimulated considerably the glucose uptake by the *E. coli* cells. When the cells, harvested at the early exponential phase, were transferred to the minimal medium containing saponin and ^{14}C-glucose (0.03 μCi) and incubated for 15 minutes at 37°C, the radioactivity of the cells became much higher than the control cells. Furthermore, analysis of the lipid and

protein of the cells showed that the saponin stimulated greatly both lipid and protein biosynthesis of the cells as shown in table 3.

Table 3. The effect of ginseng saponin in the glucose uptake and biosynthesis of lipids and proteins.

	Control(DPM)	Test(DPM)	Relative* activity
Radioactivity recovered in cells	2300±22	6987±22	300
Proteins	403±31	1210±25	300
Lipids	440±12	690±26	160

*Radioactivities of the control cells were assumed being 100.

**The *E. coli* cells were incubated in M9 minimal medium containing U-^{14}C-glucose in the presence (test) and absence (control) of ginseng saponin (10^{-1}%). The values are mean± S.D.

This suggested that the saponin stimulated glucose uptake and its turnover to lipids and proteins, leading the *E.coli* cells to grow rapidly.

Incorporation of ^{14}C-leucine to protein fraction of *E. coli* cells, grown in the minimal medium containing ginseng saponin was found much higher than control cells as shown in table 4, suggesting again that the saponin stimulated the protein biosynthesis.

Table 4. Incorporation of[U-^{14}C]-leucine into the protein of *E. coli* in the presence of 10^{-1}% ginseng saponin.

	Control	Test
Radioactivity recovered (DPM)	2485−205	3340±150
% Recovered	1.12	1.51
Relative radioactivity recovered*	100	134

*The relative radioactivity of the control was assumed being 100.

**The cells, harvested at the early exponential phase, were transfered to minimal medium. After 15 minutes' incubation, U-^{14}C-leucine (0.01μCi) was added to the medium and the incubation was continued for another 20 minutes. The control cells were cultivated in the same medium but no saponin.

요 약

인삼뿌리에서 추출된 사포닌분획(10^{-3}% − 2%)을 함유한 기초배지액(M9)에서의 *E. coli* (K−12 W1485)의 증식상을 관찰한 결과 배지액의 인삼사포닌의 농도가 10^{-1}%일때 증식률이 가장 컸음을 확인하였다.

대수기까지 증식된 세포를 ^{14}C으로 표지된 인삼사포닌을 포함한 10^{-1}% 사포닌배지액에 옮기고 20분간 배양을 계속한 후 세포의 외막, 내막, 그리고 원형질 분획을 분리하고 각 분획의 방사능을 측정한 결과 세포에서 회수된 대부분의 방사능이 세포 내막에서 탐지되었다. 또한 내막에 존재하는 succinate dehydrogenase의 활성도 사포닌함유 배지액에서 증식한 세포에서 크게 촉진되었으나 원형질에 존재하는 Glucose 6-phosphate dehydrogenase의 활성은 시험군이나 대조군사이에 큰 차이가 없었다.

또한 인삼사포닌은 세포의 glucose 섭취를 촉진하고 glucose의 지질, 단백질로의 변화대사를 촉진하며 ^{14}C-leucine의 단백질로의 편입도 인삼사포닌이 촉진하는 것으로 관찰되었다.

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