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Effect of ginseng saponins on the induction of β-galactosidase in yeast

Hee Bong Lee, Kyunghoon Kim and Byoung-Don Han[1]

Division of Life Sciences, Kangwon National University, Chunchon 200-701, Korea
[1]Research Center of BMS, Kimpo-Kun Kyunggi-Do 415-850, Korea

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Abstract : The effect of red ginseng saponins (total saponins, Rb1- and Rg1- fraction of saponins) on the induction of β-galactosidase in yeast, *Saccharomyces cerevisiae*, was investigated to see that ginseng saponins would penetrate the cell membrane and have a function in a nucleus as steroid hormones do. To attain such a kind of purpose, a DNA fragment (685bp) containing GAL1 promoter was inserted into the sites of EcoR1 and BamH1 of polylinker region, upstream of *lacZ* gene of the plasmid YEp356 (7.966 Kb), and thus the resulting plasmid p*GAL1-lacZ* is supposed to express β-galactosidase only in the presence of galactose. The plasmid p*GAL1-lacZ* was introduced into yeast, KY106 (*a leu2 ura3 his3 trp1 lys2*), and the growth of the transformed cells was much slower in the presence of galactose than glucose. The effects of saponins on the specific activity of β-galactosidase from transformed yeast cells were detected. No significant increase was observed in case of total saponins, but the Rb1- or Rg1- fraction of saponins gave much higher increase in the activity. Maximum increase was observed as 35% in 10^{-3}% of Rb1 and as 75% in 10^{-1}% of Rg1. These data suggest that ginseng saponins might be able to enter the nucleus and stimulate transcription. However, further studies to find out the putative saponin receptor are needed to confirm this possibility.

Key words : Red ginseng saponin, β-galactosidase induction, Saccharomyces cerevisiae.

Introduction

Korea ginseng has been known as one of the important herbal medicines. It has a broad efficacy as it has been used in the Orient as an elixir for hundreds of years. Although the biochemical evidences for such a broad efficacy was not sufficiently provided, its effective compound has been reported as an adaptogen[1] stimulating nonspecifically the resisting force to the changed circumstances or as a prostisol,[2] a metabolic stimulator like a hormone stimulating protein biosynthesis. Later, many scientists paid attention to the saponin(s) as an effective compound in ginseng. Particularly, Joo[3] suggested the action of saponins as a surface active agent activating nonspecifically enzyme activities in appropriate concentrations of ginseng saponins. Since the structures of ginseng saponins were determined by Shibata[4] to be ginsenosides which were composed of a sugar (glycon) portion and a nonsugar (aglycon) portion called as sapogenin which was tetracyclic terpenes of the dammarane series, researches on ginseng saponins were proceeded actively and thus a significant progess has been made with the isolation of individual ginsenosides. All the pharmacological effects of ginseng saponins identified were come out after absorption. It means that they can penetrate the membrane of the cell and organelles. Author *et al.*[5] had prepared ^{14}C-labelled ginseng saponins using a biosynthesis system with natural ginseng root slices as an enzyme source, and had investigated the absorption of the saponins into the cell and the distribution in the cell with this ^{14}C-labelled ginseng saponins. About 10^{-5}% of the saponin con-

centration was identified in the liver, which was known to be the optimum concentration for most enzyme reac- tions to be stimulated, and also the radioactivity was observed in cell organelles such as mitochondria, microsomal fractions and nucleus. Entrance into the cell can be expected from the backbone, tetracyclic terpene, structure of saponins, which then suggest that it can pass through the cell membrane and bind to its receptor and then control the transcription in the nucleus as steroid hormones do.

In this study, we tried to investigate the effect of ginseng saponins (total saponins, Rb1- and Rg1-fraction of saponins) on the induction of β-galactosidase in yeast.

Materials and Methods

1. Materials

All restriction enzymes used and T4 DNA ligase were purchased from Poscochem or Bethesda Research Laboratories, Inc., and calf intestinal alkaline phosphatase (CIP) was from Pharmacia Molecular Division. Other special reagents were from Sigma Chemical Co.

2. Strains and growth conditions

E. coli strain HB101 was grown at 37℃ in LB medium and the transformed cells containing recombinant DNA were selected in LB medium supplemented with ampicillin. Yeast strain, *Saccharomyces cerevisiae*, KY106[6] (*a leu2 ura3 his3 trp1 lys2*) was grown at 30℃ in YEPD medium and its transformed cells were selected and cultured in uracil-minus minimal medium containing 0.7% yeast nitrogen base, 2% glucose or galactose.

3. General recombinant DNA techniques

Plasmids were isolated using alkaline lysis procedure according to Ish-Horowics *et al.*[7] Transformations were achieved by the $CaCl_2$ procedure for *E. coli* and by Li salts method for yeast. Newly constructed plasmids were isolated from transformants according to the procedure described by Garger *et al.*[8]

4. Construction of a recombinant DNA

As shown in Fig. 1, for the insertion of yeast

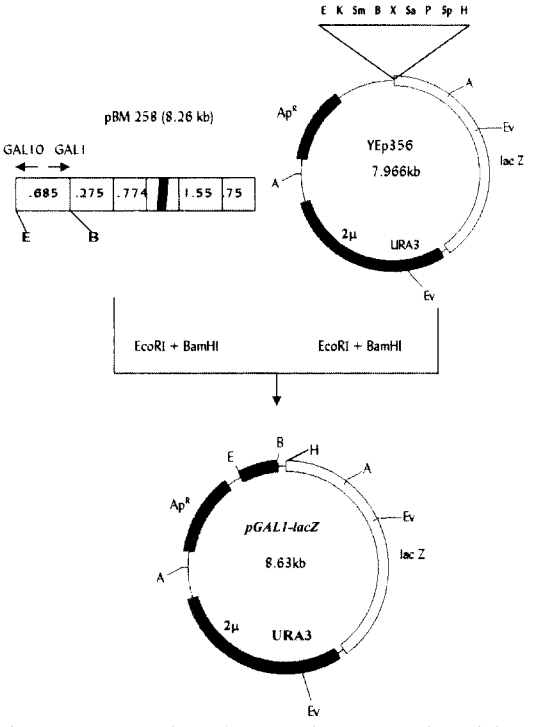

Fig. 1. Construction of recombinant plasmid, *pGAL1-lacZ*. Restriction istes are indicated for AatII (A), BamHI (B), EcoRI (E), EcoRV (Ev), HindIII (H), KpnI (K), PstI (P), SaaII (S), SmaI (Sp), and XbaI (X).

GAL1 promoter contained in plasmid pBM258[9] into the polylinker region, which has multiple unique restriction sites including EcoRI, KpnI, SmaI, BamHI, XbaI, SalI, PstI, SphI and HindIII, upstream of the *lacZ* gene of YEp356[10] vector, the EcoRI-BamHI DNA fragment (685 bp) of plasmid pBM258 including *GAL1* promoter was isolated and ligated into the same EcoRI-BamHI site (22 bp) in the polylinker region of YEp356 (7,966 bp) to produce a recombinant DNA contai- ning plasmid *pGAL1-lacZ* (Fig. 2).

5. Examination of the induction of β-galactosidase and the effect of ginseng saponins

The transformed yeasts which the recombinant DNA, *pGAL1-lacZ*, was introduced into were cultured in a minimal medium containing galactose to see whether β-galactosidase will be expressed in this transformed cells or not. To investigate the effect of ginseng saponins on the induction of β-

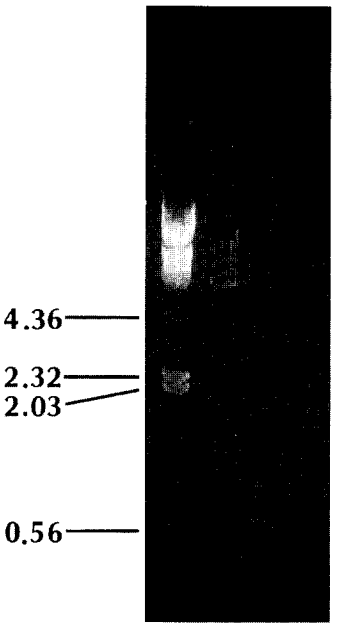

Fig. 2. Restriction patterns of vector, YEp356 and insert. 1; λDNA+HindIII, 2; YEp356+EcoRI+HindIII (7.944 kb), 3; Insert (*GAL1*, 685 bp).

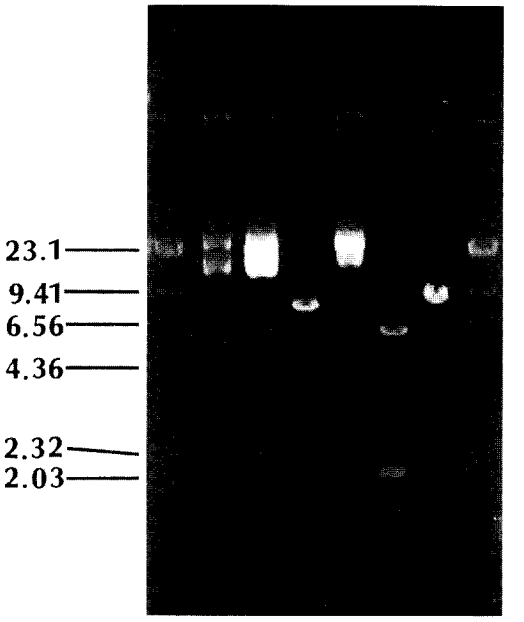

Fig. 3. Restriction patterns of vector, YEp356 and recombinant DNA. 1; λDNA+HindIII, 2; YEp 356, 3; Recombinant DNA (*pGAL1-lacZ*), 4; YEp356+KpnI, 5; Recombinant DNA+KpnI, 6; YEp356+SacI, 7; Recombinant DNA+SacI, 8; λDNA+HindIII.

galactosidase, the transformed cells were cultured in a galactose-medium supplemented with various concentrations (10^{-3}%~1%) of saponins (total saponins, ginsenoside Rb1 and Rg1) and then β-galactosidase activities of cells under different concentrations were measured and compared with the activity of cells cultured in a same medium without saponins.

6. β-galactosidase assay

β-galactosidase activity was measured as the amount of o-nitrophenol produced from substrate, ONPG (o-nitrophenyl β-D-galactoside) in one minute at 420nm according to the modified method of Rose and Botstein.[11] Protein content was determined as described by Lowry *et al.*[12]

Results and discussion

1. Construction of a recombinant DNA

Recombinant DNA, plasmid *pGAL1-lacZ*, was constructed as shown in Fig. 1. The 685bp (lane 3, Fig. 2) EcoRI-BamHI fragment of plasmid pBM258 containing *GAL1* promoter was ligated into YEp356 (7,944 bp, lane 2, Fig. 2) previously digested with EcoRI and BamHI (22 bp). Recom- binant plasmid, *pGAL1-lacZ*, introduced into *E. coli* strain HB101 and 20 colonies were selected on plates with ampicillinin. The plasmids isolated from transformants were characterized by digestion with various appropriate restriction enzymes such as BamHI, EcoRI, EcoRV, HindIII, KpnI and SacI to confirm the site shown in Fig. 1. Digestions of KpnI and SacI, which had single sites between EcoRI and BamHI in polylinker region of vector, YEp356, were tried to see whether their sites were removed from the recombinant DNA by the insertion of 685bp EcoRI-BamHI fragment of plasmid pBM258 containing GAL1 promoter into the same sites of the vector or not. Other enzymes such as BamHI, EcoRI, EcoRV, HindIII were used to compare the size of DNA fragments of vector to that of recombinant DNA after the treatment of each restriction enzyme. As shown in Fig. 3 , Fig. 4 and Fig. 5, it was identified that

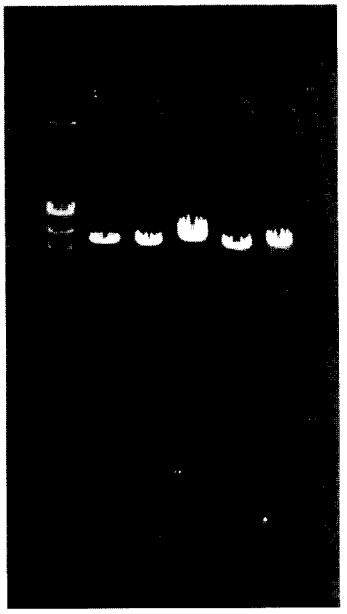

Fig. 4. Restriction patterns of vector, YEp356 and recombinant DNA. 1; λDNA+HindIII, 2; YEp 356+EcoRI, 3; YEp356+BamHI, 4; Recombinant DNA+BamHI, 5; YEp356+HindII, 5; Recombinant DNA+HindIII.

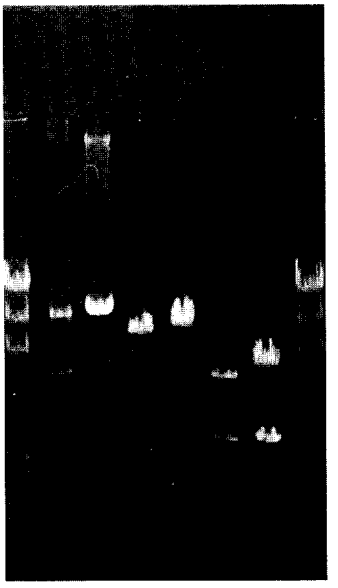

Fig. 5. Restriction patterns of vector, YEp356 and recombinant DNA. 1; λDNA+HindIII, 2; YEp356, 3; Recombinant DNA, 4; YEp356+HindIII, 5; recombinant DNA+HindIII, 6; YEp356+EcoRV, 7; Recombinant DNA+EcoRV, 8; λDNA+HindIII.

recombinant plasmid, *pGAL1-lacZ*, which contained *GAL1* promoter upstream of the *lacZ* in plasmid YEp356, was well constructed. The recombinant plasmid *pGAL1-lacZ* constucted as above was introduced into yeast uracil-minus mutant strain KY106 to observe the expression of the gene in yeast. Transformation into yeast was done according to lithium salt method, and six transformants were selected on the plate with C-ura minimal medium.

2. Effect of ginseng saponins on the induction of β-galactosidase in yeast

To know the effect of ginseng saponins on the induction of β-galactosidase, the transformed cells were cultured in a galactose-medium contai- ning various concentrations (10^{-3}%~1%) of saponins (total saponins, ginsenoside Rb1 and Rg1) and then β-galactosidase activities of cells under different concentrations were measured after cell disruption using glass beads and compared with that of cells cultured in a same medium without saponins (control group). The concentrations of saponins were chosen in this experiment according to the results obtained by the examination of the saponin effect on protein biosynthesis in *E. coli*.[13] The effect of

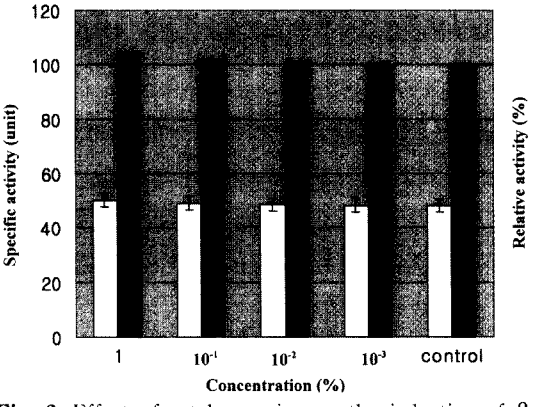

Fig. 6. Effect of total saponins on the induction of β-galactosidase in yeast. The induction of β-galactosidase was measured as β-galactosidase activity which was expressed as ΔOD_{420} per min per mg of protein. Control means β-galactosidase activity from the transformed cells cultured in a galactose-medium without saponins. Open square (□) means specific activity (unit) and closed square (■) means relative activity (%). $p<0.05$.

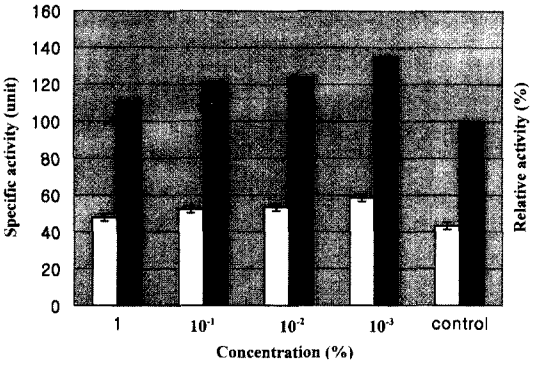

Fig. 7. Effect of ginsenoside Rb1 on the induction of β-galactosidase in yeast. The induction of β-galactosidase was measured as β-galactosidase activity which was expressed as ΔOD_{420} per min per mg of protein. Control means β-galactosidase activity from the transformed cells cultured in a galactose-medium without saponins. Open square (□) means specific activity (unit) and closed square (■) means relative activity (%). $p<0.05$.

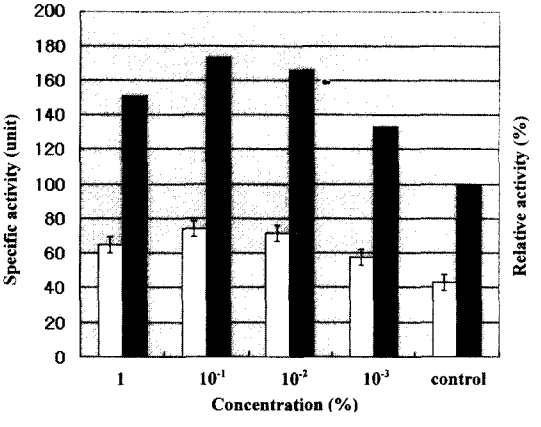

Fig. 8. Effect of ginsenoside Rg1 on the induction of β-galactosidase in yeast. The induction of β-galactosidase was measured as β-galactosidase activity which was expressed as ΔOD_{420} per min per mg of protein. Control means β-galactosidase activity from the transformed cells cultured in a galactose-medium without saponins. Open square (□) means specific activity (unit) and closed square (■) means relative activity (%). $p<0.05$.

total saponins on the induction of β-galactosidase was shown in Fig. 6. There was no significant increase in β-galactosidase activity under all detected ranges of concentration of total saponins compared to the control. However, the effect of ginsenoside Rb1 or ginsenoside Rg1 on it was much higher than the control. As shown in Fig. 7, ginsenoside Rb1 showed maximum 35% increase in 10^{-3}%, whereas, in case of ginsenoside Rg1 (Fig. 8), maximum 75% increase was observed in 10^{-1}%. These results were similar to the data of the effect of saponins on the induction of superoxide dismutase (SOD) reported by Rho *et al.*[14] Judging from these results, at least ginsenoside Rb1 and ginsenoside Rg1 of saponin fractions might be able to permeate the cell membrane and enter the nucleus and then stimulate transcription as steroid hormones do. However, further studies such as the investigation of increase in mRNA level, the identification of the putative saponin receptor using labelled saponins, the mechanism of membrane transport, and mechanism of gene regulation are needed to confirm this possibility.

요 약

인삼 사포닌이 steroid 호르몬처럼 세포막을 통과하여 핵 안에서 작용할 수 있는가를 조사하는 일환으로, 효모(Saccharomyces cerevisiae)에서의 β-galactosidase 효소 유도에 미치는 홍삼 사포닌(total saponins, ginsenoside Rb1, ginsenoside Rg1)의 영향을 조사하였다. 이를 위하여, 우선 plasmid YEp356(7.966Kb)의 *lacZ* 유전자 앞에 있는 polylinker 부위의 EcoRI과 BamHI에 GAL1 promoter를 함유한 DNA 절편(685 bp)을 삽입하여 재조합 DNA, *pGAL1-lacZ*를 제조, 확인하였고, 또한 이것을 효모, KY106(*a leu2 ura3 his3 trp1 lys2*)에 삽입하여 형질이 전환된 효모를 얻은 후, 사포닌을 함유한 galactose 배지에서 자란 형질전환 효모로부터의 β-galactosidase 효소 활성과 galactose만 존재하는 배지에서 자란 형질전환 효모(대조군)로부터의 β-galactosidase 효소 활성을 비교하여 β-galactosidase 유도에 미치는 사포닌 및 그의 분획들의 영향을 조사한 결과 total saponins 은 조사된 농도범위(1~10^{-3}%)에서 거의 영향이 없었으나 ginsenoside Rb1과 ginsenoside Rg1은 β-galactosidase 활성을 크게 증가시켰다. 특히 최대 활성증가가 ginsenoside Rb1 경우 10^{-3}% 그리고 ginsenoside Rg1 경우 10^{-1}%에서 관찰되었다. 이러한 결과는 사포닌이 핵으로의 들어가서 전사 단계에 작용할 수 있음을 보

여주고 있으나, 사포닌 수용체 확인 등의 직접적인 연구가 앞으로 더 진행되어야할 것으로 생각된다.

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