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Growth Of Cells And Defense Responses In *Panax Ginseng* Induced By Chitosans Of Different Molecular Weights

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ABSTRACT

The influence of the molecular weight of chitosan, which were prepared by enzyme hydrolysis and used to treat panax ginseng cells, on the growth and defense response of ginseng cells could promote the growth of the ginseng cells, the increase of the ginsenoside contents and the enhancement of the activities of phenylalaninine ammonialyase, peroxidase, and β-1,3 glucanase in panax ginseng cells apparently. The magnitude of the defense response of ginseng cells induced by chitosan is shown to be dominated by the molecular weight of chitosan. Comparatively the chitosan with the molecular weight of ca.2770 can induce the defense response of ginseng cells much more effectively. It is also found that the ginseonoside contents in ginseng may be associated with the anti-pathogen capability exhibited by the ginseng itself.

KEYWORDS

Panax ginseng;
Chitosan;
Defense response;
Saponin.

INTRODUCTION

Panax ginseng is a valuable traditional chinese medicine, which is widely planted in China and Southeast Asia. Nowadays, ginsenoside is believed to be the primary medical component of ginseng. As the secondary metabolization product of ginseng, saponin belongs to the secondary metabolization product of terperes. Raising the content of saponion can efficiently bring out a lot of benefits.

Chitosan is the deacetylated or partially deacetylated product of chitin. Chitin exists generally in microorganisms, yeasts, cell walls of mushrooms, insect cuticles, and shells of mollusks such as sepia. Chitosans of different molecular weights can be prepared by enzyme hydrolysis[1], acid hydrolysis[2], or chemical degradation[3]. The special biological functions of chitosans have recently drawn wide attention, especially in the agricultural area. There are already a large numbers of articles concerning chitosans which were used to improve the growth of corn[4], induce plant defense responses and enhance the capacity to tolerate stress in plants[5]. However, defense response of ginseng cells induced by chitosan is not well-understood so far. Herein, we would like to report on the preparation of chitosan with different molecular weight and treatment on ginseng.

EXPERIMENTAL

General

Chitosan, 91% deacetylation, was provided by Weikang Biology Co.(Shanghai, China); α-amylase (4000U.mg^{-1}) was purchased from Jienengke Biotechnologies, (Wuxi, China). Unless noted otherwise, all the analytically pure reagents were used as received.

The molecular weight of chitosans were analyzed by Gel permeation chromatography (GPC) using a Agilent 1100 chromatography unit using chitotriose as standard.

Preparation of chitosans of different molecular weights: In order to obtain the chitosans of different molecular weight, three of the 4%(w/v) chitosan solutions was prepared by dissovling chitosan in 1 L 0.5% acetic acid. The amylase was added into all solutions to hydrolysize. Enzymatic hydrolysis was carried out at 50°C for a given time (20min,2h,9.5h, respectiely). Consequenly, the chitosan with different molecular weight was precipitated from the obtained solution, washed to neutrality, and then freeze-dried.

As a result, the chitosans with the molecular weight of 32,600, 2770, 1335da were harvested and their molecular weight polydispersity index was 1.15,1.14 and 1.12, respectively.

Ginseng cells cultures

The *ginseng* (*Panax ginseng* C.A. Meyer) cells were maintained in fresh 67-V medium with various amount of all three chitosans used in this study at 25°C in darkness .All the cultures were routinely subcultured every 25 days into fresh 67-V medium. The ginseng cells cultured for 23 days were used as the experimental material.

Analysis of saponin in ginseng

The saponin was measured by the method reported previously[6]. Ginseng after treatment was extracted with 10ml anhydrous alcohol. After filtration, the filtrate was freeze-dried and the solid obtained was dissolved in distilled water. Saponin was extracted with the butanol and then dry to a constant weight.

Assay of PAL activity

PAL activity was determined as reported previously[7]. Upon chitosans treatments, ginseng cells obtained by centrifugation were homogenized in extraction buffer(0.2g/mL) containing 50 mM Tris-Cl (pH8.0), 10 mM 2-mercaptoet hanol, 40 mM EDTA and 10 μmol.L^{-1} leupeptin. Supernatant assayed for PAL activity were obtained by filtering the homogenate and then centrifuging at 15000g for 15 min. PAL activity was expressed as nM cinnamic acid mg^{-1} protein.

Assay of peroxidase activity

Peroxidase was determined based on the guaiacol method[8] in a Hitachi U-2000 spectrophotometer. Protein was assayed by the Bradford method[9]. By homogenizing *ginseng* cells in extraction buffer containing 0.02M potassium dihydrogen phosphate so-

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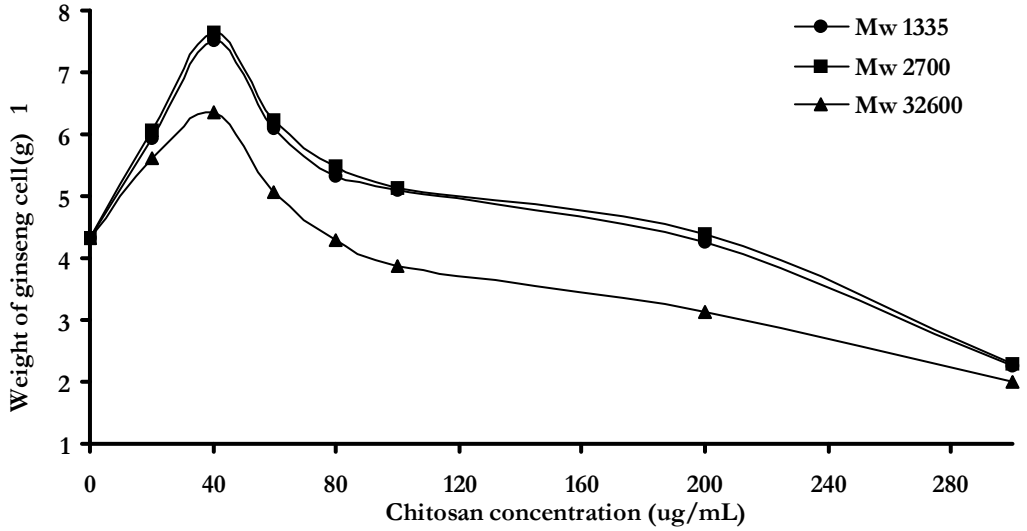

Figure 1. Effects of chitosan on the weights of Panax ginseng after growing for 23 days in 67-V medium. All experiments were carried out in triplicate and the average values are shown here.

lution, the homogenate was then centrifuged at 15000g for 15 min. The supatanant was measured at 470 nm for the measurement of peroxidase. Peroxidase activity was expressed as A470/mg protein per min.

Assay of β-1, 3 glucanase

Ginseng cells were homogenized in sodium acetate extraction buffer(0.2g.mL^{-1}) and the homogenate was centrifuged. Then the transparent solution in the superior layer was dialyzed with extract solution at 4°C overnight. *β*-1, 3 glucanase activity was determined as reported previously and calculated in μg reducing sugar mg^{-1} protein.min^{-1}.

RESULTS

Chitosan-induced growth of ginseng cells and accumulation of saponin

To investigate the effect of chitosans with different molecular weight on the growth of ginseng cells, the weight of ginseng cells was assayed. The experimental results show that chitosan treatment gives rise to the significant changes for the growth of ginseng cells and upon the treatment with chitosan with different molecular weight clear differences were observed in the weight of the ginseng cells(Figure 1). The growth of the ginseng cells reached the maximum when the chitosan concentration was 40mg.ml^{-1}. The growth wasn't inhibited (compared to the initial growth of ginseng cell) until the concentration of chitosan reached to 100mg.ml^{-1}. When comparing the weight of ginseng cells treated by chitosans with molecular weight of 1775, 2770, 13600 Da, the respective weight of ginseng cells treated by chitosans with molecular weight of 1775 and 2770 were significantly higher than the weight of ginseng cells treated by chitosans with molecular weight of 13600. At the same time, when comparing their saponin content (Figure 2), it can be seen that the most productive state for saponin content coincided on concentration 40mg.ml^{-1} ,being 4.12% upon the treatment of chitosan with the molecular weight of 2770 and lower for the treatment of chitosan with molecular weight of 1335 (4.12%) and 36200(3.47%).

The effect of chitosan-induced increase in defense related enzymes

The peroxidase, β-1, 3 glucanase and PAL are referred to as defense related enzymes in the ginseng cells. Figure 3, 4, 5 reveal that chitosans with different molecular weights had apparent effects on the activities of defense related enzymes. When the concentration of chitosan was 40mg.l^{-1}, the effect was most significant. The ginseng treated by chitosan with molecular weight of 2770 had a higher enzyme

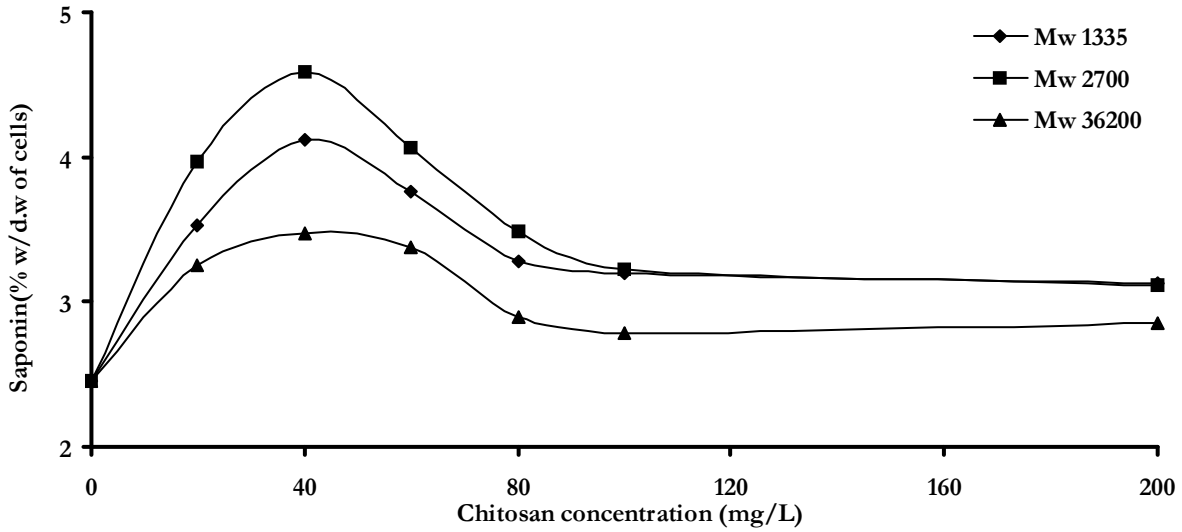

Figure 2: Effects of chitosan on accumulation of saponin in panax ginseng cells after growing for 23 days in 67-V medium. All experiments were carried out in triplicate and the average values are shown here.

activity(3.52, 6.4, 285, for activities of peroxidase, β-1,3 glucanase and PAL respectively) than the ginseng treated by chitosan with molecular weight of 1335 (4.12, 3.19, 247) and 36200 (3.47, 5.55, 236).

DISCUSSION

Chitosans of different molecular weights were prepared by non-specific enzyme hydrolysis and analysis showed that they had similar distribution index. It shows that Chitosans containing 6~9 aminoglucoses formed after 9-10h hydrolysis can be prepared by using α-amylase.

The effect of these two aqueous chitosans on growth of *panax ginseng* cells was similar. However, chitosan with molecular weight of 36200 has less capability compared to the others, because it became the cation aggregation in weakly acidic condition.

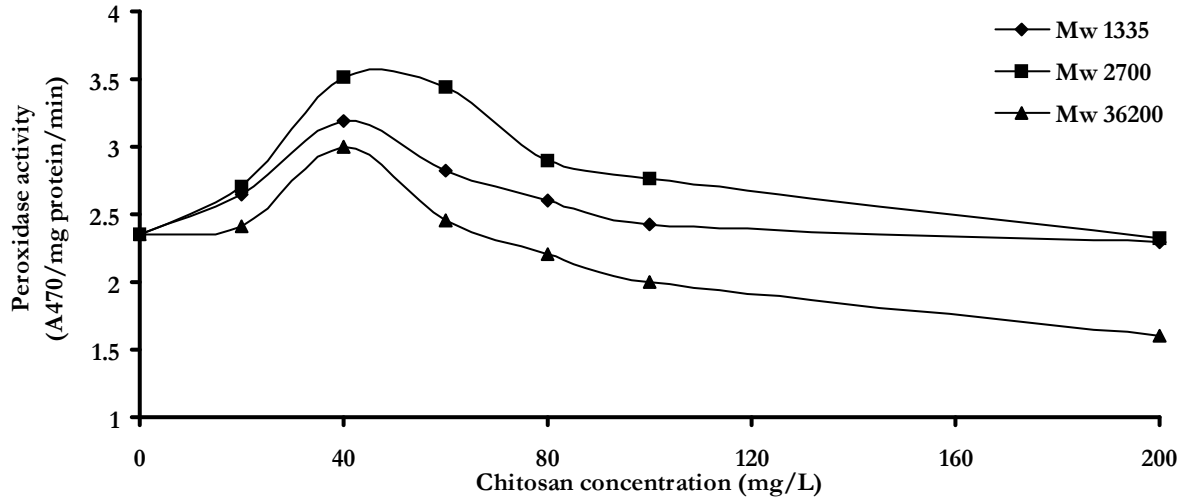

Figure 3: Effects of chitosan on activity of peroxidase in *panax ginseng* cells after growing for 23 days in 67-V medium. All experiments were carried out in triplicate and the average values are shown here.

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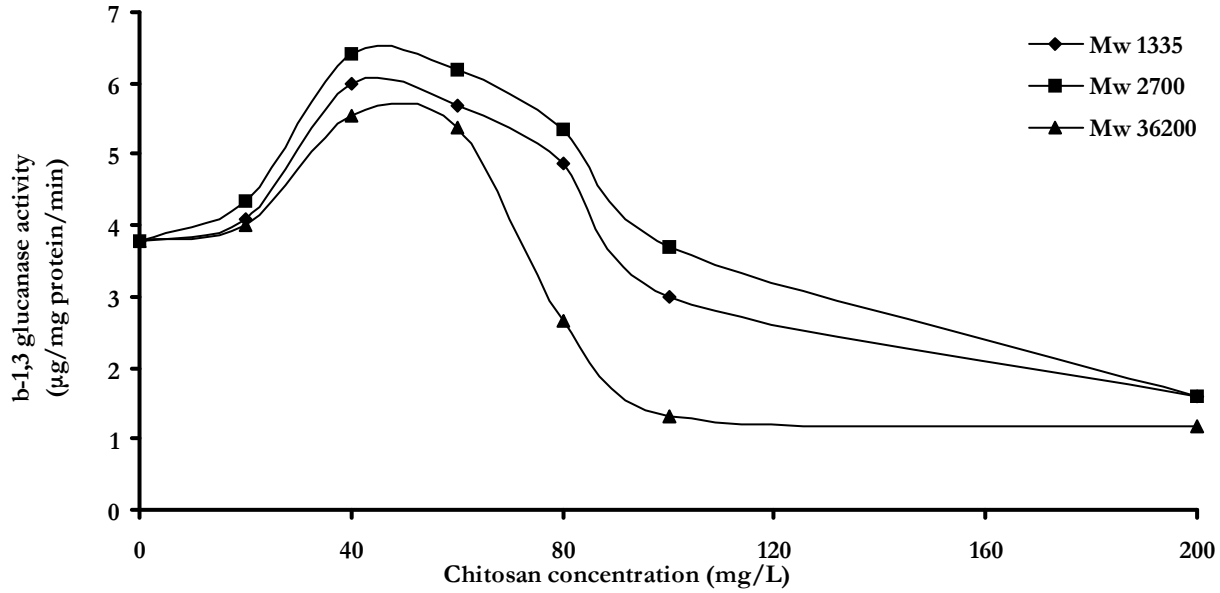

Figure 4: Effects of chitosan on activity of β-1,3 glucunase in *panax ginseng* cells grown in 67-V medium. All experiments were carried out in triplicate and the average values are shown here.

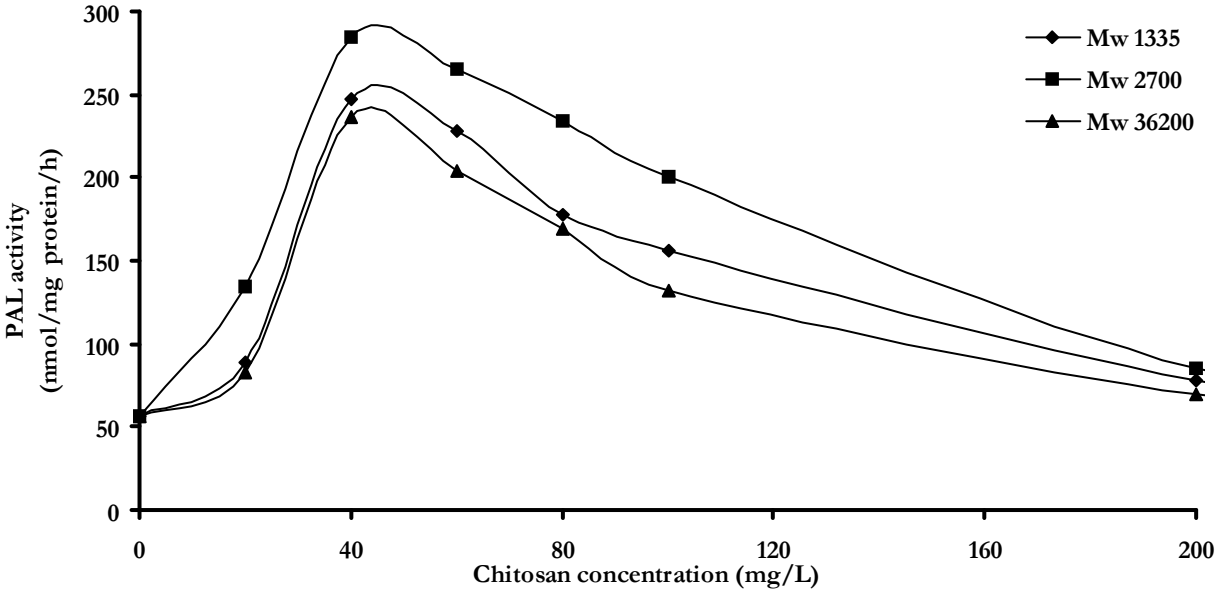

Figure 5: Effects of chitosan on activity of PAL in *panax ginseng* cells after growing for 23 days in 67-V medium. All experiments were carried out in triplicate and the average values are shown here.

Therefore, when the concentration reached 80 mg/L, it would react with the protein in the medium, generating flocculation and coagulation by electric neutralization and bridge framing effect, affected the growth of ginseng cells.

Various isoenzymes of peroxidase have effects on different process of the synthesis of xylogen, they can catalyze to produce hydrogen peroxide respectively and from the xylogen on the process of forming xylogen. β-1,3 glucanase can degrade cell walls of pathogenic fungi. Three enzymes investigated in this study are known to implicate in plant disease

resistance. Phenylalaninase is a key enzyme of the Phe pathway in plants that plays an important role in determining the content of phytoalexin and many secondary phenolic metabolites. Increasing the activities of these two enzymes would therefore be expected to enhance the plant's defence capacity. It has been suggested that chitosan can induce β-1,3 glucanase activity. Chitosan treatment can induce PAL activity in maize and grape. Our results shows that chitosans of different molecular weights have variable capacity to induce the activities of phenylalaninase, chitinase and β-1,3 glucanase, with the increase being inversely related to the CHN Mw. Furthermore, the effect of various chitosan on these enzymes depends upon the amount of chitosan used.

Now, it is believed that there are acceptors corresponding to the exiton on the membrane of plants. The process of distinguishing and affecting each other between exiton and acceptors is necessary for plants to respond for the stimulation of exiton. Therefore, there may be corresponding acceptors of chitosan in ginseng cells. After distinguishing and influencing each other between chitosan and acceptors, it could make the content of secondary metabolize products in plants increased. But when the acceptor was saturated by chitosan, it couldn't induce the synthesis of ginsenoside furthermore. Meanwhile, the process of distinguishing and affecting between chitosan and acceptor is related to the molecular weights of chitosan.

As a result, the activity of peroxidase, β-1,3-glucunase and PAL has a positive correlation with the capability of plants to resist diseases. These results indicate that the inductive effect of chitosan on all three of defense enzymes and saponin is similar. Some anti-bacteria materials in plants always live in the style of inactive glycosides in healthy ones. We suggest that the ginsenoside, as the secondary metabolization material in ginseng cells, are related to the capability to resist diseases.

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REFERENCES

[1] D.Pantaleone, M.Yalpani,M.Scollar; J.Carbohydr. Res., **237**, 325-332 **(1992)**.
[2] M.Yalpani, D.Pantaleone; J.Carbohydr.Res., **256**, 159-175 **(1994)**.
[3] E.Muraki, F.Yaku, H.Kojima; J.Carbohydr.Rec., **239**, 226-230 **(1993)**.
[4] M.M.Chang, L.A.Hadwiger, D.Horovitz; Plant Mol. Biol., **20**, 609-618 **(1992)**.
[5] S.Lee, H.Choi, S.Suh, I.S.Doo, K.Y.Oh, E.J.Choi, A.T.Schroeder Taylor, P.S.Low,Y.Lee; Plant Physiol., **121**, 147-152 **(1999)**.
[6] M.Tang, J.C.Smith; New Physiologist, **149**, 401-418 **(2001)**.
[7] P.Vander, K.M.Varum, A.Domard, N.E.El-Gueddari, B.W.Moerschbacher; Plant Physiol., **118**, 1353-9 **(1998)**.
[8] M.Bradford; Ann.Biochem., **72**, 248–254 **(1976)**.
[9] B.Chance, A.C.Maehly; Methods Enzymol., **2**, 764–775 **(1955)**.