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BRIEF COMMUNICATION

Importance of glucose-6-phosphate dehydrogenase in taxol biosynthesis in *Taxus chinensis* cultures

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Abstract

The roles of glucose-6-phosphate dehydrogenase (G6PDH) in paclitaxel production were investigated in cell suspension cultures of *Taxus chinensis.* In the normal cultures, the trend of G6PDH activity was similar to that of cell growth. Addition of glutamate increased G6PDH activity, while dehydroepiandrosterone (DHEA) decreased G6PDH activity. In elicitor-treated cultures, cell growth was depressed, while G6PDH activity and taxol production were enhanced compared with the control. Glutamate recovered the depression of cell growth, and resulted in further increase in G6PDH activity and taxol production. Contrarily, DHEA exacerbated the depression of cell growth, and decreased G6PDH activity and taxol production induced by fungal elicítor. The results indicated that G6PDH played a critic role of taxol production by affecting cell viability.

Additional key words: cell growth, dehydroepiandrosterone, fungal elicitor, paclitaxel.

Paclitaxel (taxol), a diterpenoid secondary product from the bark of yew species, has been approved as an efficient anti-cancer drug. *Taxus* cell culture is a promising means to obtain taxol and related taxane compounds (Jaziri *et al.* 1996, Lan *et al.* 2002a, Yu *et al.* 2002b). However, taxol content is extremely low in the normal *Taxus* cell cultures. Since the synthesis of secondary metabolites is believed to be associated with the plant's defence reactions against fungal attack, elicitation by biotic elicitors from fungi might enhance taxol content in *Taxus* cells (Jaziri *et al.* 1996, Yu *et al.* 2001, 2002a,b). Before elicitor-induced taxol biosynthesis, an oxidative stress was also observed (Yu *et al.* 2002a). Unfortunately, the oxidative stress caused a decrease in cell viability and in taxol production (Yu *et al.* 2001, 2002a, Lan *et al.* 2003). Some exogenous compounds, such as salicylic acid (Yu *et al.* 2001) and ascorbic acid (Yu *et al.* 2002a), can diminish the oxidative stress and enhance taxol production in *Taxus chinensis* cell cultures.

Activation of the pentose phosphate pathway (PPP) is a part of plant′s defence reactions (Kruger and Schaewen 2003). Glucose-6-phosphate dehydrogenase (G6PDH), the rate-limiting enzyme of PPP, determines the amount of NADPH by controlling the metabolism of glucose *via* the PPP (Kletzien *et al.* 1994, Kruger and Von Schaewen 2003). The work by Pandolfi *et al.* (1995) using G6PDH-deficient cell lines shows that other sources of NADPH do not adequately replace the lack of NADPH-production by G6PDH. That is, the G6PDH-deficient cells were highly sensitive to oxidative stress compared with cells expressing high content of G6PDH.

Previous research found that G6PDH activity was enhanced in *Taxus chinensis* cultures exposed to fungal elicitor (Yu *et al.* 2001), however, the roles of G6PDH in taxol production remain unknown, which is the main subject of the present paper.

Taxus chinensis (Pilg.) Rehd. cell lines, isolated from zygotic embryos, were maintained in modified Murashige and Skoog (MS) medium as previously described (Yu *et al.* 2001, 2002a). About 10 g (f.m.) of 14-d-old cell was incubated in 100 cm^3 fresh medium in a 250 cm^3 Erlenmeyer flask. After 9 d, 40 mg dm^{-3} fungal elicitor,

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Abbreviations: DHEA - dehydroepiandrosterone; G6PDH - glucose-6-phosphate dehydrogenase; PPP - pentose phosphate pathway; f.m. - fresh mass.

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100 μmol dm^{-3} dehydroepiandrosterone (DHEA) plus 40 mg dm^{-3} fungal elicitor and 0.8 g dm^{-3} glutamate plus 40 mg dm^{-3} fungal elicitor were added into the cultures. All the flasks were kept in a Erlenmeyer shaker at 25 ± 1 °C in the dark at a speed of 130 ± 5 rpm. The experiments were performed in quintuplicate.

The fungal elicitor from *Aspergillus niger* isolated from the inner bark of *Taxus chinensis* was prepared according to the method of Yu *et al.* (2002a). The elicitor dose was measured by the total sugar content of the fungal homogenate, which was determined by the phenol sulfuric acid method using glucose as the standard.

For determination of G6PDH activity 2 g (f.m.) of each sample was homogenized with 4 cm^3 of enzymatic extract reagent (5 mM KCl, 5 mM $MgSO_4$, 0.02 M Tris-HCI, pH 7.5) and centrifuged at 10 000 *g* in a refrigerated centrifuge. The supernatant was used as the enzyme extract. The assay of G6PDH activity was done according to the methods of Lan *et al.* (2002b), and by using *UV-1700* spectrophotometer (Japan).

The cell growth was determined by dry mass after the cells were lyophilized to constant mass. Taxol extraction and analysis were done according to Yu *et al.* (2001). Taxol production in the samples was the total of taxol in both cells and medium. Taxol was determined by HPLC (Waters company) using a C18 column. The detector wavelength used was 227 nm.

All the determinations were repeated three times and adopted their averages.

G6PDH activity in *Taxus chinensis* cell cultures was gradually elevated before 24 d to the maximum of 14 U mg^{-1}(protein), and then declined (Fig. 1*B*). Cell growth firstly increased to maximum on day 26 after culture (Fig. 1*A*) and then dropped, similarly as G6PDH. Addition of glutamate enhanced G6PDH activity and cell growth as compared with the control. The maximal G6PDH activity was 16.5 U mg^{-1}(protein) and biomass was 31 g dm^{-3} on day 26 after culture. However, addition of DHEA reduced G6PDH activity and depressed cell growth compared with the control. Maximal biomass was 20.5 g dm^{-3} on day 23 after culture, and maximal G6PDH activity was 10 U mg^{-1}(protein) on day 30 after culture. Addition of glutamate and DHEA had different effects on taxol production. The former enhanced taxol production 20 % of the control, while the latter decreased to taxol production 65 % of the control (Fig. 1*C*).

The fungal elicitor treatment depressed cell growth, but increased G6PDH activity and taxol production compared with the control. The biomass decreased by 19 % of the control on day 14 after treatment. The maximum G6PDH activity was 26 U mg^{-1}(protein) on 16 h and taxol production was 5.6 mg dm^{-3} on day 10 after treatment.

Glutamate addition to elicitor treatment cells almost recovered the depression of cell growth induced by the elicitor, but DHEA exacerbated the depression (Fig. 2*A*). Glutamate also increased G6PDH activity and taxol production. Contrarily, DHEA decreased G6PDH activity and taxol production in the fungal elicitor treated cultures (Fig. 2*B*,*C*).

Previous study showed that fungal elicitor from *Aspergillus niger* could obviously enhanced taxol production in cell suspension cultures of *Taxus chinensis* (Yu *et al.* 2001, 2002a), therefore, the elicitor was

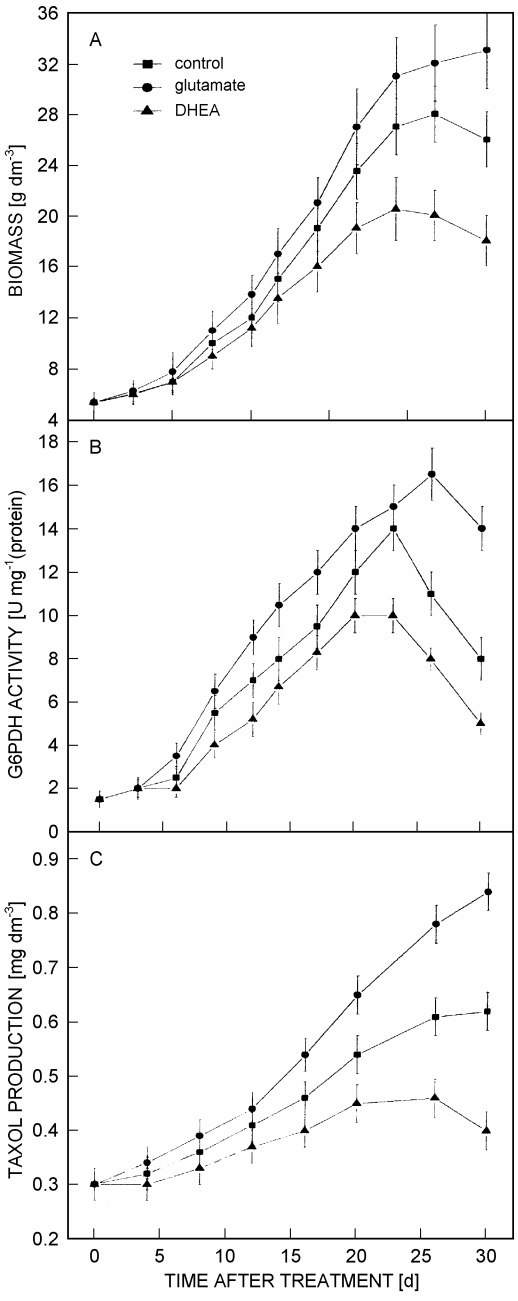

Fig. 1. Time course of cell growth (*A*), G6PDH activity (*B*) and taxol production (*C*) in cell suspension cultures of *Taxus chinensis*. DHEA (100 μM) and glutamate (0.8 g dm^{-3}) were added to the 0-d-old cultures. Means of three independent experiment. *Error bars* represent SE.

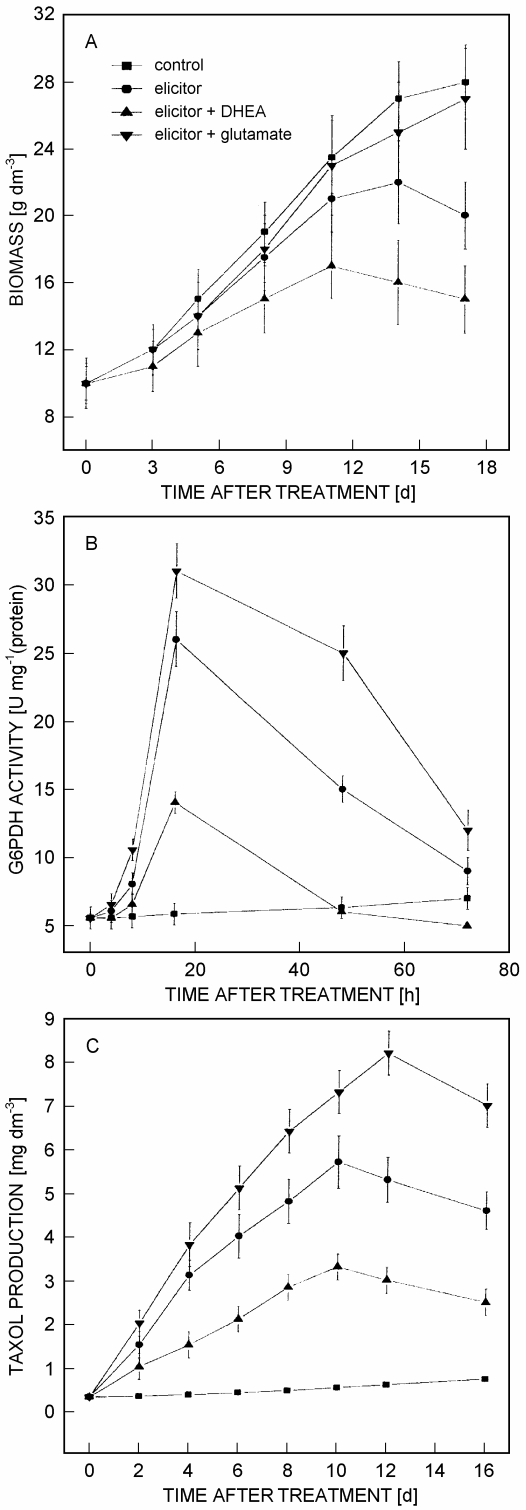

Fig. 2. Effect of fungal elicitor, glutamate and DHEA on cell growth (*A*), G6PDH activity (*B*) and taxol production (*C*) in cell suspension cultures of *Taxus chinensis*. Fungal elicitor (40 mg dm^{-3}) was added to the 9-d-old cultures. DHEA (100 μM) and glutamate (0.8 g dm^{-3}) were added 30 min before fungal elicitor. Means of five independent experiment. *Error bars* represent SE.

selected to analyze the effects of G6PDH on cell growth and taxol production. Moreover, G6PDH activator glutamate and inhibitor DHEA were applied to investigate the roles of G6PDH in cell growth and taxol production. Glutamate synthesis closely links with PPP (Esposito *et al.* 2003), and we found that glutamate enhance G6PDH activity (Lan *et al.* 2002b). Although DHEA has multiple actions, it is known to be an inhibitor of G6PDH (Tian *et al.* 1998, 1999).

The present results demonstrated that in the cell suspension cultures of *Taxus chinensis*, the trend of G6PDH activity was similar to that of cell growth. PPP is a major source of NADPH for biosynthetic processes of compounds necessary for cell growth, such as fatty acid synthesis and the assimilation of inorganic nitrogen, and carbon skeletons for the synthesis of nucleotides (Kruger and von Schaewen 2003). Moreover, Tian *et al.* (1998) found G6PD activity plays a critical role in cell growth by providing NADPH for redox regulation. The present results showed that the cultures treated with glutamate had higher G6PDH activity and cell growth, while the cultures treated with DHEA had lower G6PDH activity and cell growth.

Taxol production was determined by both cellular biomass and taxol content. More cellular biomass by means of enhancing cell growth would bring more taxol production (Jaziri *et al.* 1996, Yu *et al.* 2002a). Exogenous hormones and sugar could accelerate cell growth to enhance taxol and taxane production (Jaziri *et al.* 1996, Lan *et al.* 2002b). The present results showed that appropriate regulation of G6PDH could enhance cell growth and favour taxol production.

Elicitors secreted by or released from microbial or fungal invaders are the primary signals for induction of plant defence responses (Nurnberger and Scheel 2001). Elicitors-induced taxol production and oxidative stress are thought as a part of the defense responses (Yu *et al.* 2002c). Before the activation of biosynthesis of taxol, an oxidative stress is also generally observed (Yu *et al.* 2001, 2002a). This stress decreased cell viability and depressed taxol biosynthesis (Yu *et al.* 2001, 2002a, Lan *et al.* 2003), and was postulated to be a contributor for unstable taxol production (Yu *et al.* 2002b). We found that salicylic acid and ascorbic acid, lessened oxidative stress and enhanced taxol production (Yu *et al.* 2001, 2002a). In the present result we found that G6PDH activity was activated in elicitor-treated *Taxus chinensis* cells, which was similar to the observations in alfalfa (Fahrendorf *et al.* 1995) and tobacco cells upon the environmental stresses (Pugin *et al.* 1997). G6PDH has been found to protect cells against oxidative stress by affecting the redox potential (Tian *et al.* 1999).

The present results showed that fungal elicitor-induced taxol production was in accordance with G6PDH activity. For example, fungal elicitor enhanced G6PDH activity and taxol production, and glutamate further enhanced G6PDH activity and taxol production induced

by the fungal elicitor, and DHEA decreased G6PDH activity and taxol production induced by the fungal elicitor. In the first committed pathway of taxol biosynthesis, the hydroxylation of the taxadiene nucleus is catalysed by a cytochrome P_{450} dependent taxadiene synthase, whose activity also depended on the electron transfer from NADPH (Walker and Croteau 2001). NADPH which is mainly produced by G6PDH (Kruger and Von Schaewen 2003, Pandolfi *et al.* 1995, Tian *et al.* 1999) is needed for taxol biosynthesis. We could not exclude the possibility of G6PDH directly involved in taxol production, besides the roles of G6PDH in keeping cell viability.

References

Esposito, S., Massaro, G., Vona, V., Rigano, V.D.M., Carfagna, S.: Glutamate synthesis in barley roots: the role of the plastidic glucose-6-phosphate dehydrogenase. - Planta **216**: 639-647, 2003.

Fahrendorf, T., Ni, W., Shorroosh, B.S., Dixon, R.A.: Stress responses in alfalfa (*Medicago sativa* L.) XIX. Transcriptional activation of oxidative pentose phosphate pathway genes at the onset of the isoflavanoid phytoalexin response. - Plant mol. Biol. **28**: 885-900, 1995.

Jaziri, M., Zhiri, A., Guo, Y.W., Dupon, J.P., Shimonura, K., Hamada, H., Van Haelen, M., Homes, J.: *Taxus* sp. cell, tissue and organ cultures as alternative sources for taxoids production: a literature survey. - Plant Cell Tissue Organ Cult. **46**: 59-75, 1996.

Kletzien, R.F., Harris, P.K.W., Foellmi, L.A.: Glucose-6-phosphate dehydrogenase: a "housekeeping" enzyme subject to tissue-specific regulation by hormones, nutrients, and oxidant stress. - FASEB J. **8**: 174-181, 1994.

Kruger, N.J., Von Schaewen, A.: The oxidative pentose phosphate pathway: structure and organization. - Curr. Opinion Plant Biol. **6**: 236-246, 2003.

Lan, W.Z., Qin, W.M., Yu, L.J.: Effect of glutamate on arachidonic acid production from *Mortierella alpina.* - Lett. appl. Microbiol. **35**: 357-360, 2002b.

Lan, W.Z., Yu, L.J., Cheng, P., Li, M.Y.: Improvement of cephalomanine production in *Taxus chinensis* cells by the combination of sucrose and methyl jasmonate. - Biotechnol. Lett. **24**: 1253-1255, 2002a.

Lan, W.Z., Yu, L.J., Li, M.Y., Qin, W.M.: Cell death unlikely contributes to taxol production in fungal elicitor-induced cell suspension cultures of *Taxus chinensis.* - Biotechnol. Lett. **25**: 47-49, 2003.

Nurnberger, T., Scheel, D.: Signal transmission in the plant immune response. - Trends Plant Sci. **6**: 372-379, 2001.

Pandolfi, P.P., Sonati, F., Rivi, R., Mason, P., Grosveld, F., Luzzatto, L.: Targeted disruption of the housekeeping gene encoding glucose 6-phosphate dehydrogenase (G6PD): G6PD is dispensable for pentose synthesis but essential for defense against oxidative stress. - EMBO J. **14**: 5209-5215, 1995.

Pugin, A., Frachisse, J.M., Tavernier, E., Bligny, R., Gout, E., Douce, R., Guern, J.: Early events induced by the elicitor cryptogein in tobacco cells: involvement of a plasma membrane NADPH oxidase and activation of glycolysis and the pentose phosphate pathway. - Plant Cell **9**: 2077-2091, 1997.

Tian, W.N, Braunstein, L.D., Apse, K., Pang, J., Rose, M., Tian, X., Stanton, R.C.: Importance of glucose-6-phosphate dehydrogenase activity in cell death. - Amer. J. Physiol. **276**: C1121-C1131, 1999.

Tian, W.N., Braunstein, L.D., Pang, J., Stuhmeier, K.M., Xi, Q., Tian, X., Stanton, R.C.: Importance of glucose-6-phosphate dehydrogenase activity for cell growth. - J. biol. Chem. **273**: 10609-10617, 1998:

Walker, K., Croteau, R.: Taxol biosynthetic genes. - Phytochemistry **58**: 1-7, 2001.

Yu, L.J., Lan, W.Z., Qin, W.M., Jin, W.W., Xu, H.B.: Oxidative stress and taxol production induced by fungal elicitor in cell suspension cultures of *Taxus chinensis.* - Biol. Plant **45**: 459-461, 2002a.

Yu, L.J., Lan, W.Z., Qin, W.M., Xu, H.B.: Effects of salicylic acid on fungal elicitor-induced membrane-lipid peroxidation and taxol production in cell suspension cultures of *Taxus chinensís* - Proc. Biochem. **37**: 477-4.82, 2001.

Yu, L.J., Lan, W.Z., Qin, W.M., Xu, H.B.: High stable production of taxol in elicited synchronous cultures of *Taxus chinensis* cells. - Proc. Biochem. **38**: 207-210, 2002b.

Yu, L.J., Qin, W.M.; Lan, W.Z.: Defense response induced by fungal elicitor and its correlation with taxol biosynthesis in *Taxus* cells. - Chin. appl. environ. Biol. **8**: 255-258, 2002c.