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ENHANCEMENT OF PEROXIDASE ACTIVITY BY STRESS-RELATED CHEMICALS IN SWEET POTATO

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Key Word Index—*Ipomoea batatas*; Convolvulaceae; suspension culture; peroxidase; abscisic acid; calcium chloride; ethephon; paraquat.

Abstract—Stress-related plant hormones, abscisic acid (ABA, 0.5 mg l^{-1}) or ethephon (0.5 ml l^{-1}, an ethylene generating compound), enhanced peroxidase (POD) activity by *ca* 50% in suspension cultures of sweet potato (*Ipomoea batatas*), depending on the time after treatment when added to medium at the late logarithmic growth phase. Calcium chloride (50 mM) also increased POD activity by *ca* 20%, whereas paraquat (1 mg l^{-1}) slightly stimulated it soon after treatment, but inhibited it after 13 days. ABA was the most effective in causing increased POD activity, i.e. 11 247 units g^{-1} dry cell wt (substrate, pyrogallol) 18 days after treatment, which was 1.78 times higher than a normal culture. However, the four chemicals used had no effect on the level of extracellular POD, which was maintained at 5–10% of total POD activity regardless of chemical treatment.

INTRODUCTION

Peroxidase (POD, EC 1.11.1.7) is widely used as an important reagent for clinical diagnosis and micro-analytical immunoassay because of its high sensitivity [1, 2]. New applications for POD have been suggested in the medicinal, chemical and food industries [3]. So far, the major source of commercially available POD is intact roots of horseradish [4].

POD activity in plants increases in response to various biotic and abiotic stresses [5–8]. Presumably plant cultures are considered to be grown under stress conditions, particularly oxidative stress, which suggests that plant cell culture is an ideal system for the production of antioxidant enzymes including POD [9, 10]. In this respect, plant cells and hairy roots have been studied for the production of POD [11–17]. However, there is no cell culture system to substitute for horseradish roots. In the previous paper, we described the selection of a sweet potato (*Ipomoea batatas*) cell line with a high yield of POD, and characterized major POD isoenzymes purified from suspension cultured cells [10, 18]. The POD content in cultured cells of sweet potato showed a significantly higher level compared with other cell lines reported, suggesting that sweet potato POD may be a useful source for commercial use [10].

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Production of secondary metabolites by plant cell cultures has been known to be stimulated by biotic or/and abiotic stress factors [19, 20]. In particular POD activity often increases in response to stress-related hormones such as abscisic acid (ABA) and ethylene [21–24]. Paraquat, a free-radical generating herbicide, also increases oxidative stress, inducing antioxidant enzymes [25, 26]. Several researchers reported that calcium ion activates plant POD, particularly extracellular POD level [27–29]. In this report, we describe the enhancement of POD productivity in suspension-cultured cells of sweet potato by using stress-related hormones such as ABA and ethylene, paraquat, or calcium chloride.

RESULTS AND DISCUSSION

Effect of stress-related hormones on POD activity

To determine the amount and time of elicitation, ABA (0.5, 1, or 2 mg l^{-1}) or ethephon (0.5, 1, 2, or 4 ml l^{-1}) was added to the medium of 0, 8, 12, or 15 days after subculture (DAS). The addition of two chemicals to the cultures before early exponential stage significantly inhibited the cell growth, resulting in decreased total POD activity, even though the POD activity per g cell dry wt was increased (data not shown). The high dose (over 1 mg l^{-1} ABA or 1 ml l^{-1} ethephon) also significantly inhibited the total POD activity regardless of addition stage, suggesting that *ca*

0.5 mg l^{-1} ABA or 0.5 ml l^{-1} ethephon may be a proper concentration for enhancement of total POD activity (data not shown).

According to the above results, ABA (0.5 mg l^{-1}) or ethephon (0.5 ml l^{-1}) added to the medium at 12 DAS, resulted in a marked enhancement of the POD activity in suspension cultures of sweet potato without a significant effect on cell growth. The cell growth of sweet potato reached a maximum on 15 DAS showing a typical sigmoidal curve (the bold line of Fig. 1A). However, there was an initial lag period: i.e. *ca* 5 days intervals between the exponential rises in the cell growth (10 DAS) and POD activity (15 DAS) (Figs 1A and 1B). ABA increased POD activity until 29 DAS showing the maximum activity (11 200 units g^{-1} cell dry weight), which was 1.78 times higher than control, then decreased with further culture. When ethephon was added, POD activity was increased, particularly at the early stage after treatment (20 DAS), by 1.56 times, i.e. 7810 units g^{-1} dry cell weight and maintained at a higher level to the late growth stage. However, ABA (0.5 mg l^{-1}) and ethephon (0.5 ml l^{-1}) added together showed even lower activity than treatment of ABA or ethephon alone.

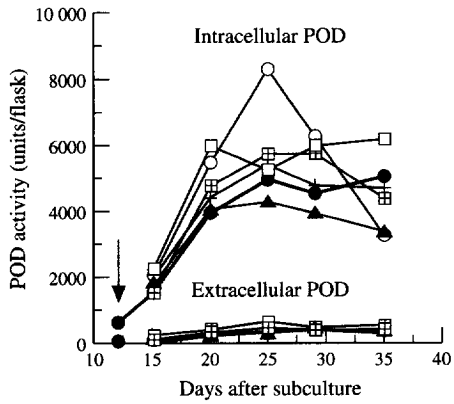

Fig. 2. Effect of stress-related chemicals on intracellular and extracellular POD activity per flask containing 50 ml LS1D medium in cell suspension cultures of sweet potato. Similar results were obtained in two other independent experiments. —●—, Untreated control; —○—, ABA treatment (0.5 mg l^{-1}); —□—, ethephon treatment (0.5 ml l^{-1}); —+—, ABA (0.5 mg l^{-1}) plus ethephon (0.5 ml l^{-1}) treatment; —▲—, paraquat treatment (1 mg l^{-1}); —⊞—, calcium chloride treatment (50 mM).

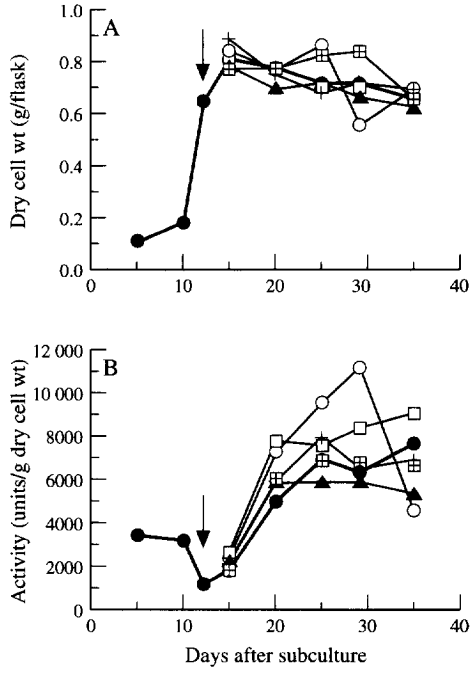

Fig. 1. Effect of stress-related chemicals on the cell growth and intracellular POD activity in cell suspension cultures of sweet potato in LS medium supplemented with 1 mg l^{-1} 2,4-D and 30 g l^{-1} sucrose. Similar results were obtained in two other independent experiments. (A) Time course of cell growth after chemical treatment at 12 days after subculture (↓) on the basis of g dry cell weight per flask containing 50 ml medium. (B) Time course of POD activity on the basis of g dry cell weight after chemical treatment: —●—: untreated control; —○—, ABA treatment (0.5 mg l^{-1}); —□—, ethephon treatment (0.5 ml l^{-1}); —+—, ABA (0.5 mg l^{-1}) plus ethephon (0.5 ml l^{-1}) treatment; —▲—, paraquat treatment (1 mg l^{-1}); —⊞—, calcium chloride treatment (50 mM).

The total POD activity per flask (50 ml cultures in 300 ml conical flask) with or without chemical treatment is shown in Fig. 2. The results of total activity showed a similar effect to that of activity per g dry cell weight, suggesting that the increased total activity was mainly derived from the increased activity per dry cell weight. However, stress-related hormones did not enhance the level of extracellular POD, which was maintained at 5–10% of total POD activity, regardless of chemical used, even though it is slightly increased during cell cultures. Having intracellular POD predicates that the culture has to be sacrificed to obtain POD.

ABA mediates a large number of physiological and developmental processes in plants in response to various environmental stresses [30]. In potato tissue culture, ABA induces an anionic POD associated with suberization [21]. In our study, ABA markedly increased the POD activity, but did not induce a new POD isoenzyme, as demonstrated by gel activity staining (data not shown). Ethylene is known to accelerate maturation, senescence and abscission in plants [31]. These phenomena are often accompanied by an increase of POD activity. Ethylene has been shown to increase POD activity in sweet potato, cucumber, pea, tomato, tobacco [23, 32, 33], and also to affect mRNA expression of POD in suspension cultured cells of peanut [34]. In etiolated pea seedlings, ethylene increases the POD activity at 3 days after treatment (DAT) [32]. The stimulating effect of ethylene also appeared at 3 DAT in suspension cultures of sweet potato, suggesting a similar response to exogenous ethylene in whole plants and cultured cells.

Effect of paraquat and calcium chloride on POD activity

Paraquat (1 mg l^{-1}) stimulated POD activity by 20% during the first 3 days after treatment, whereas it inhibited POD activity at the late growth stage from 25 DAS to the last stage (35 DAS) by 30% with a slight growth inhibition (Figs 1A and 1B). Thus, total POD productivity was significantly inhibited by the paraquat treatment (Fig. 2).

Calcium chloride (50 mM) showed a slight elicitation effect on POD activity with stimulating cell growth during 20–30 DAS (Figs 1A and 1B). The total POD activity was increased by 20% from 20 DAS to 35 DAS on the flask level (Fig. 2). Calcium chloride and paraquat did not increase the extracellular POD activity, which was maintained at a level of 5–10% of total POD activity (Fig. 2). The release of POD into the medium after the addition of calcium ion was reported in cell suspensions of carrot, peanut and radish [28, 29, 35]. The treatment of calcium ion caused a marked increase in extracellular POD in hairy root cultures of horseradish [27]. For commercial production of POD, the extracellular POD is more attractive than intracellular POD. However, our results disagree with other results, which reported that calcium ion increases POD release, suggesting the PODs of sweet potato cells have different chemical characteristics from those of other plant cells.

In a previous paper, higher POD activity at an early growth stage after subculture and at a late growth stage suggested that the POD activity in suspension cultures of sweet potato is closely associated with cell growth and culture stresses derived from subculture (dilution effect) and medium depletion [10]. In conclusion, POD activity was regulated by stress-related chemicals in the cell suspension cultures of sweet potato. ABA significantly increased the POD productivity, although it did not affect the extracellular POD level. These results suggest that ABA may be an effective elicitor for POD production in suspension cultures of sweet potato.

EXPERIMENTAL

Plant cell line. The cell line SP-47 selected by a small cell-aggregated method from cell suspension cultures of sweet potato (*Ipomoea batatas* (L.) Lam. cv White Star) for a high yield of POD was used [18]. Cells subcultured at 10-day intervals (1 g fr. wt) were inoculated into 50 ml of LS (Linsmaier and Skoog) [36] basal medium, supplemented with 1 mg l^{-1} 2,4-D and 30 g l^{-1} sucrose (LS1D medium) in a 300 ml conical flask and cultured at 25° in the dark (100 rpm) as a normal culture.

Elicitation with stress-related compounds. After addition of ABA (0.5 mg l^{-1}), ethephon (0.5 ml l^{-1}), ABA (0.5 mg l^{-1}) plus ethephon (0.5 ml l^{-1}), calcium chloride (50 mM) and paraquat (1 mg l^{-1}) to the normal cultures at 12 DAS, the cell growth and POD activity was measured every 3–5 days from 15 to 35 DAS. Authentic chemicals purchased from Sigma were used except ethephon (Wako Chemicals).

Enzyme assay and protein determination. The POD activity was measured using pyrogallol as a substrate. The standard assay mixture contained, in a total vol. of 3 ml: enzyme soln (0.1 ml), 100 mM K-Pi buffer (pH 6, 0.32 ml), 5% pyrogallol (0.32 ml, w/v), 0.147 M H_2O_2 (0.16 ml) and H_2O (2.1 ml). The reaction was initiated by the addition of H_2O_2 and the increase in $A_{420\,nm}$ recorded in 20 sec. One unit of POD activity is defined as that forming 1 mg of purpurogallin from pyrogallol in 20 sec at pH 6 at 20°. The data of enzyme activity are expressed as an average of three measurements. Protein content was determined according to the method of ref. [37] using Bio-Rad protein assay reagents.

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