THE ROLE OF SALICYLIC ACID IN THE ANTIOXIDANT SIGNAL TRANSDUCTION PATHWAY

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Abstract

Antioxidant enzyme activity was measured in salt tolerant callus tissue derived from the cultivar Coker 312 over an 8 hour period following treatment with either 0.1μ M paraquat, 10 mM H_2O_2 or 100 μM salicylic acid. Paraquat induced an up-regulation of catalase, peroxidase, ascorbate peroxidase, and glutathione reductase activities within 1 hour after treatment. The H_2O_2 and salicylic acid treatments resulted in significant increases in peroxidase and glutathione reductase within 2 hours and in catalase and ascorbate reductase within 8 hours. These data suggest that salicylic acid induces an antioxidant response through a pathway medicated by H_2O_2.

Introduction

Environmentally-induced physiological stress results in a cascade of stress responses, one of which is the up-regulation of the antioxidant defense system. Results from on-going research in our laboratory have shown that NaCl stress results in an up-regulation of ascorbate peroxidase (APX), catalase, glutathione reductase (GR), glutathione S-transferase (GST), peroxidase, and superoxide dismutase (SOD) (Gossett *et al.*, 1994a, 1994b, 1996). Since these enzymes appear to be up-regulated by NaCl-induced oxidative stress, there is a question as to which molecule or molecules may be involved in the signal transduction process. The literature has suggested that abscisic acid (ABA) (Leung and Giraudat, 1998), superoxide (Dole *et al.*, 1994), and H_2O_2 (Foyer *et al.*, 1997) may be involved in the signal transduction pathway. Recent results from our laboratory have shown that reactive oxygen intermediates such as superoxide and H_2O_2 may signal the up-regulation of antioxidant enzyme activity through both ABA-dependent and ABA-independent pathways.

Recent research has shown that salicylic acid induces protection against plant pathogens, primarily through its ability to initiate an oxidative burst (Raskin, 1992). Salicylic acid has also been shown to upregulate antioxidant enzyme activity, and Dat *et al.* (1998) suggested that salicylic acid induced thermotolerance in mustard seedlings through a common signal transduction pathway involving an early increase in H_2O_2 concentration. One of the major questions our laboratory wanted to ask was how the salicylic acid response could fit itor our current signal transduction model for the up-regulation of antioxidant enzymes. Hence, the major objectives of this research was to determine if salicylic acid induces an antioxidant response in cotton callus tissue and if an antioxidant response to salicylic acid was comparable to the antioxidant response to H_2O_2 treatment.

Methods and Materials

Approximately 4 grams of salt-tolerant cotton callus tissue was transferred to culture tubes containing 25 ml of media amended with 150 mM NaCl. Each culture tube was connected to an aerator for constant aeration. Following a 2-hour pre-incubation period, the tubes were left untreated as controls or amended with either 0.1μ M paraquat, 10 mM H_2O_2 or 100 μM salicylic acid. The callus tissue was harvested at 0, 30 min, 1 hr, 2 hr, 4 hr, and 8 hr intervals and stored at -70°C for subsequent antioxidant enzyme analyses.

Samples were prepared for antioxidant analyses by the method previously described by Gossett *et al.* (1996). Catalase activity was determined by monitoring the disappearance of H_2O_2 according to the method of Beers and Sizer (1952). Peroxidase activity was measured by monitoring the H_2O_2-dependent oxidation of 2,3',6-trichloroindophenol after the method of Nickel and Cunningham (1969). GR activity was measured by monitoring the glutathione-dependent oxidation of NADH as described by Schaedle and Bassham (1977). APX activity was assayed by monitoring the ascorbate-dependent reduction of H_2O_2 by the method described by Anderson *et al.* (1992).

Results and Discussion

Enzyme activities are shown in Tables 1-4. Catalase activity (Table 1) did not change signigicantly over the 8-hour time period in the controls. The paraquat treatment induced a significant increase activity in 1 hour, but the salicylic acid and H_2O_2 treatments did not result in significant increases until the 8-hour time point. In the controls, peroxidase activity remained constant over the 8-hour time period (Table 2). The salicylic acid and H_2O_2 treatments induced significant increases in peroxidase activity in 2 hours, but the paraquat produced a significant increase within 1 hour after treatment. APX activity did not change significantly over the 8-hour time period in controls (Table 3). While paraquat induced a significant increase in APX activity within 30 minutes after treatment, the salicylic acid and H_2O_2 treatments did not produce significant increases until the 8-hour time point. In the controls, GR activity remained constant over the 8-hour time period (Table 4). The salicylic acid treatment induced a significant increase in GR activity in 2 hours, and the H_2O_2 treatment resulted in a significant increase in 4

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hours. Treatment with paraquat resulted in a significant increase in GR activity within 1 hour.

Intracellular H_2O_2 concentrations increase under a variety of stress conditions (Foyer *et al.*, 1997), and Singha and Choudhuri (1990) have shown that H_2O_2 and the superoxide radical may play an important role in the mechanism of salt-injury in *Vigna catjang* and *Oryza sativa* leaves. Hence, H_2O_2 may act as both an intracellular and systemic signal for the induction of a subset of the battery of defense genes (Foyer *et al.*, 1997; Dat *et al.*, 1998). In the present study, salicylic acid treatment resulted in significant increases in APX, catalase, GR, and peroxidase in cotton callus tissue. The time course for the antioxidant response to salicylic acid closely resembled the time course observed for the H_2O_2 treatment. Hence, these data support the hypothesis that salicylic acid does induce an antioxidant response through a pathway mediated by H_2O_2.

Summary

Paraquat, salicylic acid, and H_2O_2 treatments all resulted in an up-regulation of APX, catalase, GR, and peroxidase activities over the 8-hour time couse. The close similarity between the salicylic and H_2O_2 treatments suggests that salicylic acid induces this up-regulation through a H_2O_2-mediated signal transduction pathway.

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Table 1. Catalase activity (units/g fresh weight ±SE) in salt-tolerant callus tissue treated for 0, 0.5, 1, 2, 4, and 8 hrs with 150 mM NaCl (controls), 100 mM Salicylic Acid (SA), 0.1 µM Paraquat (PARA), and 10 mM H_2O_2.

	Time					
Treatment	**0**	**0.5 h**	**1 hr**	**2 hr**	**4 hr**	**8 hr**
Control	66±5	60±70	83±4	76±8	87±12	83±7
SA	66±5	81±3	82±8	94±7	107±4	166±12
PARA	66±5	71±7	183±12	86±6	85±12	79±7
H_2O_2	66±5	65±4	72±2	84±6	77±6	155±8

Table 2. Peroxidase activity (units/g fresh weight ±SE) in salt-tolerant callus tissue treated for 0, 0.5, 1, 2, 4, and 8 hrs with 150 mM NaCl (controls),100 mM Salicylic Acid (SA), 0.1µM Paraquat (PARA) , and 10 mM H_2O_2 .

	Time					
Treatment	**0**	**0.5 h**	**1 hr**	**2 hr**	**4 hr**	**8 hr**
Control	111±6	135±4	127±5	132±2	136±3	144±4
SA	111±6	109±5	169±8	385±6	188±8	186±4
PARA	111±6	146±27	286±17	176±23	159±28	151±12
H_2O_2	111±6	119±14	178±37	360±15	176±26	180±27

Table 3. Ascorbate Peroxidase activity (units/g fresh weight ±SE) in salt-tolerant callus tissue treated for 0, 0.5, 1, 2, 4, and 8 hrs with 150 mM NaCl (controls), 100 mM Salicylic Acid (SA), 0.1 µM Paraquat (PARA),and 10mM H_2O_2.

	Time					
Treatment	**0**	**0.5 h**	**1 hr**	**2 hr**	**4 hr**	**8 hr**
Control	210±8	214±12	193±12	216±12	199±3	191±9
SA	210±8	219±15	212±14	202±8	174±10	690±10
PARA	210±8	713±12	682±13	757±27	628±24	776±59
H_2O_2	210±8	236±28	208±28	242±15	166±26	650±27

Table 4. Glutathione reductase activity (units/g fresh weight ±SE) in salt-tolerant callus tissue treated for 0, 0.5, 1, 2, 4, and 8 hrs with 150 mM NaCl (controls), 100 mM Salicylic Acid (SA), 0.1 µM Paraquat (PARA), and 10mM H_2O_2

	Time					
Treatment	**0**	**0.5 h**	**1 hr**	**2 hr**	**4 hr**	**8 hr**
Control	262±13	234±10	346±15	348±8	299±16	303±14
SA	262±13	286±14	381±20	1064±64	856±37	166±12
PARA	262±13	247±7	1133±22	996±16	1085±4	1112±23
H_2O_2	262±13	255±12	357±17	557±26	1010±26	265±8