Free Rad. Res. Comms., Vol. 9, No. 2, pp. 127–133
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ANTIOXIDANT ENZYME LEVEL RESPONSE TO PROOXIDANT ALLELOCHEMICALS IN LARVAE OF THE SOUTHERN ARMYWORM MOTH, *SPODOPTERA ERIDANIA*

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(Received May 17, 1989; in revised form December 21, 1989)

Larvae of the southern armyworm, *Spodoptera eridania*, are highly polyphagous feeders which frequently encounter and feed upon plants containing high levels of prooxidant allelochemicals. While ingestion of moderate quantities of prooxidants can be tolerated by these larvae, ingestion of larger quantities can result in toxicity. Studies were conducted to assess the role of the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) in the protection of *S. eridania* against redox active prooxidant plant allelochemicals. Dietary exposure of mid-fifth-instar larvae to either quercetin (a flavonoid) or xanthotoxin (a photoactive furanocoumarin), which generate superoxide radical, and singlet oxygen, repsectively, resulted in an increase in SOD levels. CAT levels increased in all groups of *S. eridania* including control insects. This may have been due to the sudden exposure to food following an extended fast of 18 h (to insure that larvae would not reject the diet because of the prooxidants' bitter taste) with an eventual lowering of CAT values with time. GR activities did not significantly change except for a slight inhibition at the highest prooxidant concentrations used at 12-h post-ingestion. The data from these studies suggest that SOD responds to prooxidant challenges in these insects and together with CAT and GR contributes to the insect's defense against potentially toxic prooxidant compounds.

KEY WORDS: Catalase, glutathione reductase, southern armyworm, *spodoptera eridania*, superoxide dismutase, quercetin, xanthotoxin.

ABBREVIATIONS: CAT, catalase; GR, glutathione reductase; GT, glutathione transferase; SOD, superoxide dismutase.

INTRODUCTION

Larvae of the southern armyworm, *Spodoptera eridania* (Cramer), are highly polyphagous feeders. While foraging indiscriminately they encounter and thereby eat a variety of plants and often ones containing potentially toxic redox active and prooxidant allelochemicals. This insect appears to be able to tolerate relatively high levels of prooxidant allelochemicals including redox active quercetin[1] although xanthotoxin prevented larvae from maturity past the second instar.[2] In a recent study it was shown

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that mid-fifth-instar *S. eridania* larvae exposed to relatively low dietary levels of the linear furanocoumarin, xanthotoxin (as low as 0.001% w/w) had decreased relative growth rates (RGR's) when compared to the control larvae. Larvae exposed to quercetin (up to 1.0% w/w), however, experienced no decrease in their RGR's.[1] Xanthotoxin is a phototoxic allelochemical which generates both singlet oxygen and superoxide radical upon activation.[3] Quercetin, however, is metabolically activated and autoxidizes with the concomitant production of superoxide, hydrogen peroxide and hydroxyl radical.[4,5] These findings suggest that the southern armyworm larvae which are well-endowed with SOD and CAT are better suited to tolerate superoxide producing plant redox active compounds like quercetin than singlet oxygen producer plant prooxidants like xanthotoxin.

Superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) are three antioxidant enzymes which have been extensively studied in a number of biological species. These enzymes have been shown to be important in the defence against radical toxicity.[6,7] Previously, however, very few studies had been reported regarding these enzymes in insects. Recently, the activities of these enzymes have been described in three insect species, the black swallowtail butterfly, *Papilio polyxenes*,[8] the southern armyworm, *S. eridania*[1,9] and the cabbage looper, *Trichoplusia ni*.[10,11] The findings from these studies show that a relationship exists between the resistance towards prooxidant compounds for these insects and their overall relative antioxidant enzyme activities. *S. eridania*, which can tolerate moderate levels of prooxidants possesses intermediate levels of antioxidant enzyme activities. *P. polyxenes* preferentially feeds on plants with prooxidants and tolerates very high levels of both quercetin and xanthotoxin. *T. ni* is sensitive to plant prooxidants and is a well-known pest for white cabbage which contains low levels of prooxidants. If the antioxidant enzymes, SOD, CAT and GR are important in the defence of *S. eridania* towards dietary prooxidants and redox active compounds then one would expect the ingestion of prooxidants and redox active compounds to induce the antioxidant enzyme activities in these insects in order to protect against oxidative stress. In this report, we tested the effect of dietary exposure of mid-fifth-instar larvae of *S. eridania* to two redox active prooxidant compounds, xanthotoxin and quercetin, on their antioxidant enzyme activities.

MATERIALS AND METHODS

Insects

Eggs of *S. eridania*, were obtained from Dr. Lena B. Brattsten, Dupont de Nemours and Co. Inc., Wilmington, Delaware. Hatchlings were raised on a lima bean foliage containing semi-synthetic diet as previously described.[1]

Enzyme Assays

Mid-fifth-instar larvae were fasted for 18 h prior to exposure to their respective control and pro-oxidant containing diets. The standard diet was supplemented with either control solvent (acetone), xanthotoxin (dissolved in acetone), or quercetin (dissolved in acetone). Xanthotoxin was supplemented into diet to yield either a 0.01 or 0.1% (w/w) diet mixture. Quercetin was added to the diet to give either a 0.1 or 1.0% (w/w) diet mixture.

Xanthotoxin and quercetin were dissolved in acetone such that a maximum of 100 μl of acetone was added per 5 gms of diet. The solvent from each diet was removed under a gentle stream of N_2 – O_2 (70:21 ratio). Larvae receiving the xanthotoxin diet and respective control diet groups were exposed to long-UV light (320–380 nm) during feeding. Feeding duration ranged from 1.4 to 12 h. The exposure to long-UV light was necessary because xanthotoxin being a photodynamic compound is activated by long-UV light to generate activated oxygen species. Previous studies by our group and others[1,2] have shown that xanthotoxin toxicity to *S. eridania* occurs largely in the presence of UV-light. A non UV-light or dark toxicity for xanthotoxin has been observed; however this toxicity is not mediated by activated oxygen and is relatively minor. Larvae receiving the quercetin diet or quercetin control diet were exposed to full solar daylight spectrum generated by General Electric Cool White and Incandescent bulbs. UV-light exposure was not necessary for the quercetin-treated groups as quercetin is activated metabolically rather than photodynamically. Following their time exposure to their respective diets, larvae were homogenized and 850 *g* supernatants were prepared as previously described.[11] The 850 *g* supernatants were used as the enzyme source for assaying SOD, CAT and GR in these studies. SOD was assayed by the method described by McCord and Fridovich[12] and as later modified by Oberly and Spitz.[13] CAT was assayed by following the breakdown of H_2O_2 at 240 nm as described by Aebi.[14] GR activity was monitored by the method described by Racker.[15] Protein concentrations of each sample were determined by the method of Lowry *et al.*[16]

The consumption of dietary prooxidant compounds for each group of insects was determined in the following manner. Following the preparation of each specific diet, aliquots were weighed out and either given to the larvae for feeding or retained as controls. Following each exposure time, the larvae were removed from their food source. The remaining food, together with the pre-weighed control food, was dried overnight in an oven at 80°C. From the control-diet blocks we obtained a wet mass to dry mass ratio for the diet. We used this ratio to determine the exact food consumption for each larval group. Based upon the food consumption for each group, and the concentration of prooxidant (w/w) in the various diets, the dietary intake of prooxidant was computed.

RESULTS AND DISCUSSION

The data presented in Table 1 show that for both prooxidant compounds increasing

TABLE 1
Consumption of Dietary Prooxidant Compounds by Mid-5th Instar *S. Eridania* larvae.

Prooxidant	Prooxidant Conc. (w/w)	Amount of Prooxidant Consumed per Larva* (mg)		
		1 h	4 h	12 h
Quercetin	Control	0	0	0
	0.1 %	0.14	0.17	0.99
	1.0%	1.4	1.7	9.9
Xanthotoxin	Control	0	0	0
	0.01%	0.013	0.016	0.019
	0.1%	0.13	0.16	0.19

*The values represent an average from four separate experiments consisting of five larvae per experiment, with an uncertainty of ± 15%.

TABLE 2
Superoxide Dismutase Activities in 850*g* Supernatants of Mid-5th Instar Larvae of *S. eridania* Following Dietary Exposure to Quercetin and Xanthotoxin.

Pro-oxidant	Pro-oxidant Conc. (% w/w)	Mean Enzyme Units[a] Duration of Exposure (h)		
		1	4	12
Quercetin[b]	Control (none)	1.8	7.7	17.5
	0.1%	4.2	13.6	22.4
	1.0%	4.7	10.6	17.7
Xanthotoxin[c]	Control (none)	2.2	2.9	10.0
	0.01%	10.8	12.4	20.9
	0.1%	1.9	6.0	11.3

[a]Enzyme units are means obtained from two determinations. Units represent specific activities/mg protein-min, as defined by McCord and Fridovich (1959).
[b]Insects on control (solvent) and treated groups were held under normal light regime.
[c]Insects on control (solvent) and treated groups were held under long-UV, 320–380 nm. Solvent in all groups was acetone.

the length of time of dietary exposure and/or proxidant concentration resulted in an increase in the amount of proxidant consumed by the insect. These results were very important as they demonstrated greater dietary intake of the prooxidant compounds, as a consequence of both dietary exposure time and increasing concentration.

SOD activity was measured for larvae exposed to each diet, and the data are summarized in Table 2. In all groups, increasing the dietary exposure time resulted in increased enzyme activity. Increasing the prooxidant dietary concentration also enhanced the enzyme activity relative to the control groups except 12 h exposure of 1.0% quercetin and 0.1% of xanthotoxin.

CAT activities are given in Table 3. It is evident that the activities for the control

TABLE 3
Catalase Activities in 850*g* Supernatants of Mid-5th Instar Larvae of *S. eridania* Following Dietary Exposure to Quercetin and Xanthotoxin.

Pro-oxidant	Pro-oxidant Conc. (% w/w)	Mean + S.D. Enzyme Units[a] Duration of Exposure (h)		
		1	4	12
Quercetin[b]	Control (none)	960 ± 52a	978 ± 77a	296 ± 32a
	0.1%	950 ± 69a	745 ± 61a	208 ± 34b
	1.0%	193 ± 20b	232 ± 10b	195 ± 5b
Xanthotoxin[c]	Control (none)	1165 ± 21a	912 ± 22a	250 ± 62a
	0.01%	990 ± 32b	662 ± 36b	359 ± 17a
	0.1%	219 ± 15c	204 ± 22c	240 ± 67a

[a]Means were derived from four to six pooled replicates of duplicate determinations. Means not accompanied by the same letter for a pro-oxidant in each vertical column are significantly different by Duncan Waller K-ratio T test ($\alpha = 0.05$). One unit of activity = 1 mol H_2O_2 decomposed/mg protein-min, at pH 7.0.
[b]Insects in control (solvent) and treated groups were held under normal light regime.
[c]Insects in control (solvent) and in treated groups were held under long-UV, 320–380 nm. Solvent in all groups was acetone.

TABLE 4
Glutathione Reductase Activities in 850 *g* Supernatants of Mid-5th Instar Larvae of *S. eridania* Following Dietary Exposure Quercetin and Xanthotoxin.

Pro-oxidant	Pro-oxidant Conc. (% w/w)	Mean ± S.D. Enzyme Units[a] Duration of Exposure (h)		
		1	4	12
Quercetin[b]	Control (none)	21.9 ± 11.5a	17.1 ± 4.1a	10.9 ± 2.7a
	0.1%	16.5 ± 2.8a	16.0 ± 1.7a	10.8 ± 3.2a
	1.0%	10.3 ± 2.0a	14.8 ± 2.1a	7.6 ± 1.9ab
Xanthotoxin[c]	Control (none)	11.8 ± 2.8a	11.8 ± 4.5a	9.1 ± 1.7a
	0.01%	10.4 ± 0.6a	16.9 ± 1.8a	9.0 ± 3.8a
	0.1%	11.5 ± 1.8a	12.8 ± 1.4a	3.1 ± 0.5b

[a]Means are derived from a pool of four to six replicates of duplicate determinations. Means not accompanied by the same letter for a pro-oxidant in each vertical column are significantly different by Duncan Waller K-ratio T test ($\alpha = 0.05$). One unit of activity = 1 nmol NADPH oxidized/mg protein-min.
[b]Insects in control (solvent) and treated groups were held under normal light regime.
[c]Insects in control and treated groups were held under long-UV, 320–380 nm. Solvent in all groups was acetone.

and lower dietary prooxidant groups at 1-h post-ingestion were exceedingly high, ca. 10-fold greater, than 80 units of the constitutive levels of this enzyme reported for unfasted insects.[1] The untreated control activities declined slightly at 4-h post-ingestion, and more drastically by ca. 3-fold, at 12-h epxosure time. This unusual response of CAT of *S. eridania* is difficult to rationalize at this time.

In a recent study, dietary restriction (60% *ad libitum* diet compared to 100% in controls) of male rats initiated at six weeks of age, resulted in significantly higher SOD and CAT.[17] It was concluded that dietary restriction appears to enhance the expression of free radical detoxification enzymes and, therefore, reduces radical damage. The consequence, however, of feeding full dietary rations to rats after a period of restricted feeding on antioxidant enzyme levels was not explored in this study. It is conceivable, therefore, that the extended fasting of insects with a larval life span of less than one month caused an unusual oxidative stress associated with the change in their metabolic activity.

The data on GR activities presented in Table 4 show that for up to 4-h dietary exposure to prooxidants, the activities are unaffected with respect to insects or control diets. Ony at the highest concentration tested and 12-h exposure time was any modification (more so for xanthotoxin that quercetin) observed. The GR activities in these regimes were slightly suppressed at exposure to 1.0% quercetin for 12 h. A significant depression of GR was observed when *S. eridania* was exposured to 0.1% xanthotoxin for 12 h. Partial inhibition of GR activity has also been reported for *P. polyxenes*[8] and *T. ni*[18] in response to prooxidant challenge by quercetin, but not by xanthotoxin. The GR of this insect may be somewhat different than in the other two insect species. Nevertheless, sufficient GR activity remained capable of catalyzing the reduction of GSSG to GSH.

It appears that SOD induction is the primary response to the prooxidant challenges of mid-fifth-instar larvae of *S. eridania*. High SOD activity accompanied by the retention of sufficiently high CAT activity would likely provide protection from

superoxide radical, and its dismutation product, H_2O_2. The peroxidase activity of GT together with GR activity would provide a primary line of defense against highly reactive, hence deleterious organic peroxides arising from oxidative stress. A lack of significant alteration in GR activity suggests at this time that SOD-CAT enzyme couple is quite likely very efficient in terminating the oxygen radical cascade by scavenging both superoxide and H_2O_2, thus minimizing the threat of formation of organic peroxides. More studies are needed to clarify the extent of lipid/organic peroxidation in this insect in support of the contention.

In summary, based on responses of the antioxidant enzymes to prooxidant challenges, we believe that these enzymes play a crucial role in the highly polyphagous feeding habit of *S. eridania*, which increases the risk in this insect of encountering and consuming redox active prooxidant rich plants.

Acknowledgements

This work was supported in part by the USDA Science and Education grant 88-37153-3475 and by the Natural Products Laboratory, University of Nevada-Reno, Department of Biochemistry and is a contribution of the Nevada Agricultural Experiment Station.

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Accepted by Prof. B. Halliwell