Research Article

STRESSORS INDUCED GLUTATHIONE PEROXIDASE ACTIVITY IN LEPIDOPTERAN INSECT SILKWORM (*BOMBYX MORI*)

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ABSTRACT

The present study, discuss the role of antioxidant enzyme glutathione peroxidase to overcome free radicals produced under the influence of various stressors. A 24 hours exposure of silkworm, *Bombyx mori* to cold, hypoxia and nuclear polyhedral virus resulted in a significant increase in Glutathione peroxidase in the various tissue of both IV and V instars. However, upon recovery from stress, antioxidant enzyme activity returned to base value and larvae did not show any age dependent difference in glutathione peroxidase activity. All the results are discussed in the light of immediate response of poikilotherms to various stressors.

INTRODUCTION

All organisms possess mechanisms to maintain homeostasis which are essential for survival. Insects are often prone to burden of oxidative stress which disturbs its homeostasis on exposure to various environmental stressors. Stressors such as temperature have been reported to act, at least in part, via oxidative stress related mechanism. Cold hardiness induced free radical formation is evident in European corn borer *Ostrinianubulalis* (Jovanovic- Galovic, 2007). In a state of hypoxia when oxygen demand exceeds supply, a physiological response is mounted to meet the oxygen debt. Oxygen play a critical role in the existence of life, it also produces a highly reactive molecule that damage living organism by producing reactive oxygen species (ROS) (Davies, 1995). Viral infection in insects results in increased level of oxidative stress (Wang *et al*., 2001; Lee *et al*., 2005) resulting in the formation of free radicals and oxidative stress markers (Li *et al*., 2011).

To protect against the toxicity of free radicals, organisms have evolved protective enzyme system. Antioxidant metabolites and enzymes form a complex network that work together to prevent oxidative damage to cellular components (Vertuani *et al*., 2004). Insects process an antioxidant enzyme (AOEs) suite which includes Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (Gpx) (Ahmad *et al*., 1990; Felton and Summer, 1995). These AOEs acts as a mutually supportive defense complex against ROS. SOD catalyses the dismutation of superoxide radicals to H_2O_2 and oxygen (Ahmad and Pardini, 1990) and CAT reduces H_2O_2 to water and oxygen (Ahmad *et al*., 1991). However, Gpx metabolises H_2O_2 and deleterious lipid peroxides by using reduced glutathione as a substrate (Ahmad *et al.*, 1989).

The main aim of the present work is to determine the role of Glutathione peroxidase that are involved in scavenging the stress induced free radicals in silkworm *Bombyx mori*.

MATERIALS AND METHODS

Insects and experimental design

The present study was approved by the Institutional Animal Ethics Committee (IACE), Bangalore University, Bangalore, India. Earlier instar larva were procured from Kunigal seed area, Karnataka, India and were maintained in laboratory until V instar and were fed ad libitum on M5 variety mulberry leaves (Vyjayanthi and Subramanyam, 2002a, 2002b). The uniformly grown healthy larvae were made into six groups and each group consisted of hundred and were maintained at 24–25ºC, relative humidity of 70-75 %. Experimental animals of group I was not subjected to any stress and was considered as control. Group II larva was subjected to cold at 5ºC for 24h, whereas group III was also

subjected to cold treatment however, the larva were retrieved at 24h and were maintained at room temperature for additional period of 12h and considered as cold recovery. Group IV was subjected to hypoxia for 24h and group V larva were subjected to hypoxia for the same period and were allowed to recover for an additional period of 12h. The hypoxia was induced by closure of 4 pairs of posterior spiracles with dental wax and during recovery period all the spiracles were in open state. Group VI larvae were inoculated with 10 µl / g body weight of 1 x 10^6 *Bombyx mori* nuclear polyhedra virus (Bm NPV).

Isolation of midgut

Whole midgut was excised from larva and 10% homogenate was prepared (after separating the malphigian tubules, fat bodies, trachea and other tissue fragments adhering to the gut) in ice cold buffer (pH 7.4) solution using a Potter-Elvehjem homogenizer. The homogenates were centrifuged at 3000g at 4ºC for 10 min and the supernatant was used as source for various studies with appropriate dilutions.

Isolation of haemolymph and haemocytes

Haemolymph was collected by cut opening the caudal horn into a pre- cooled centrifuge tubes that were coated with 2% EDTA. Haemolymph was diluted with equal volume of ice cold buffer (pH- 7.4) solution and centrifuged at 3000g at 4ºC for 10 min. Haemocytes pellets were later resuspended and 10% homogenate was prepared using the same buffer and has used for further studies.

Glutathione peroxidase (Gpx, E. C. 1.11.1.9)

GPx was analysed by the method of Flohe and Gunzler (1984). 50 µl of 0.1 M phosphate buffer (pH 7.0), 100 µl enzyme sample, 100µl glutathione reductase (0.24 units) and 100 µl of 10 mM GSH were mixed. The mixture was pre incubated for 10 min at 37ºC followed by the addition of 100µl 1.5 mM NADPH in 0.1% $NaHCO_3$. 50 µl of 12 mM t-butylhydroperoxide was added to monitor the hydrogen peroxide independent concentration of NADPH for 3 min. The overall reaction was started by adding 100 µl of pre warmed H_2O_2, and the decrease in absorption at 340 nm was monitored for 5 min. The enzyme activity was expressed as µm NADPH oxidized / min / mg protein.

Statistical analysis

Data are shown as mean ± SD of six observations. Changes between the groups were analysed by ANOVA and further tested by Bonferroni Post HOC test using Statistical package for Social Science (SPSS) software and p value of less than 0.05 was considered significant. Statistically significant data were presented in the text.

RESULTS AND DISCUSSION

Exposure to low temperature induced a significant upregulation of glutathione peroxidase by 58.13% and 33.87% in haemolymph of IV and V instar silkworm respectively, whereas 21.29% and 42.9% increase in Gpx was observed in IV and V instar silkworm larvae respectively on exposure to hypoxia. On recovery after treatment the enzyme activity was reverted back to that of control group. Viral infection induced a significant increase in Gpx activity in haemolymph by 59.71% in IV instar and by 46.44% in V instar silkworm (Fig 1 and 2). However, age dependent decrease observed in SOD and CAT activity was absent in Gpx.

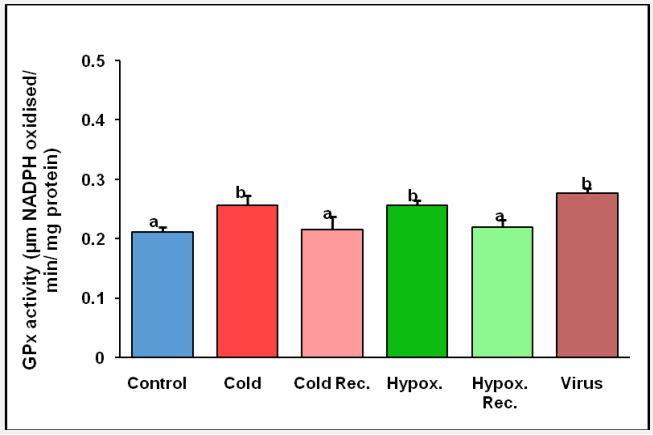

Fig. 1. GPx activity in haemolymph of IV instar silkworm *B. mori* subjected to cold, cold recovery, hypoxia, hypoxia recovery, and viral infection. Data are means ± SE (*n* = 6). *P* <0.05 was considered significant. Values between the stressors are represented in lower cases (a, b). Those not sharing the same letters are significant.

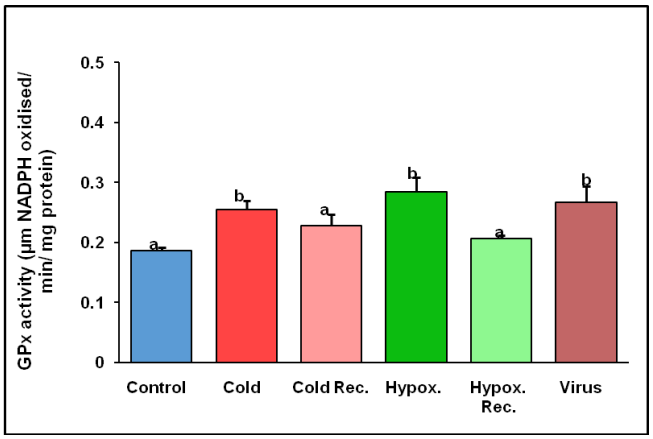

Fig. 2. GPx activity in haemolymph of V instar silkworm *B. mori* subjectedto cold, cold recovery, hypoxia, hypoxia recovery, and viral infection. Data are means ± SE (*n* = 6). *P* <0.05 was considered significant. Values between the stressors are represented in lower cases (a, b). Those not sharing the same letters are significant.

Similarly, exposure to low temperature and hypoxia induced a significant increase of GPx activity in midgut tissue and reverted to control value during recovery period. GPx activity was significantly increased on viral infection in midgut tissue of IV instar silkworm (Fig 3.). Though, all

the stressors induced a significant increase of GPx activity in V instar larval midgut tissue, hypoxia and viral infection showed a relatively higher GPx activity compared to that of cold stress (Fig 4). However, age dependent decrease observed in SOD and CAT activity was absent in GPx activity of midgut tissue.

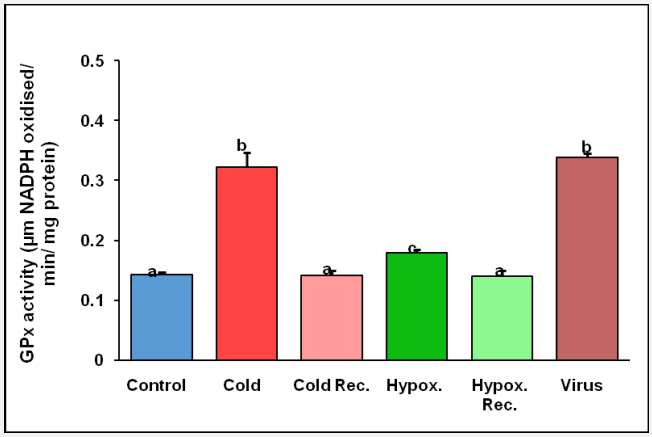

Fig. 3. GPx activity in midgut tissue of IV instar silkworm *B. mori* subjected to cold, cold recovery, hypoxia, hypoxia recovery, and viral infection. Data are means ± SE (*n* = 6). *P* <0.05 was considered significant. Values between the stressors are represented in lower cases (a, b, c). Those not sharing the same letters are significant.

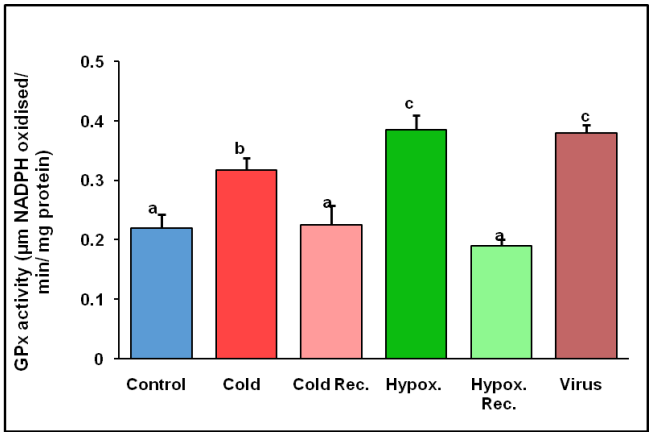

Fig. 4. GPx activity in midgut tissue of V instar silkworm *B. mori* subjected to cold, cold recovery, hypoxia, hypoxia recovery, and viral infection. Data are means ± SE (*n* = 6). *P* <0.05 was considered significant. Values between the stressors are represented in lower cases (a, b, c). Those not sharing the same letters are significant.

Glutathione peroxidase activity was also significantly increased during exposure to stresses such as cold, hypoxia and virus in haemocytes of IV instar larvae. Among the stressors, low temperature and viral infection showed relatively higher GPx activity (Fig 5). A similar increase in GPx activity was observed in haemocytes of V instar larvae on exposure to all stressors and viral infection showed significantly higher GPx activity (Fig 6). Age dependent decrease in GPx activity was observed only in the haemocytes of control group.

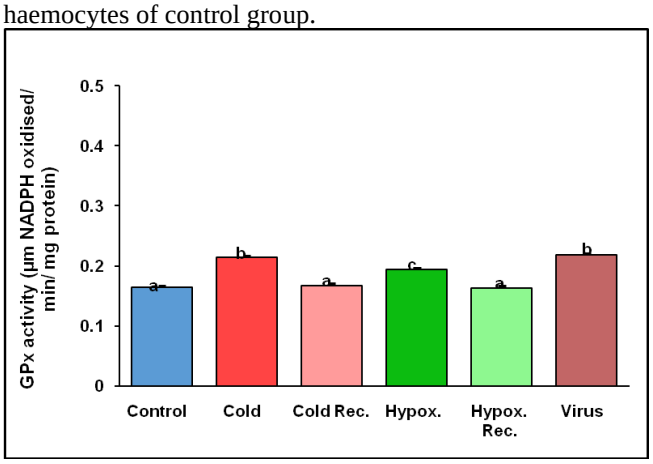

Fig. 5. GPx activity in haemocytes of IV instar silkworm *B. mori* subjectedto cold, cold recovery, hypoxia, hypoxia recovery, and viral infection. Data are means ± SE (*n* = 6). *P* <0.05 was considered significant. Values between the stressors are represented in lower cases (a, b, c). Those not sharing the same letters are significant.

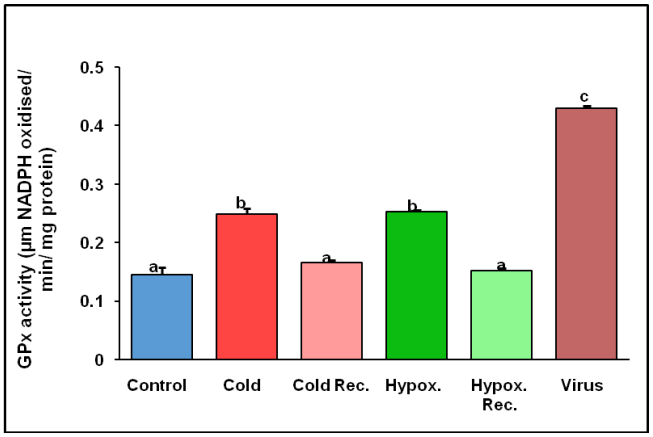

Fig. 6. GPx activity in haemocytes of V instar silkworm *B. mori* subjected to cold, cold recovery, hypoxia, hypoxia recovery, and viral infection. Data are means ± SE (*n* = 6). *P* <0.05 was considered significant. Values between the stressors are represented in lower cases (a, b, c). Those not sharing the same letters are significant.

In the present study, the antioxidant enzymes Gpx in haemolymph, midgut and haemocytes has shown a significant increase in activity when *B. mori* subjected to low temperature, hypoxia and virus. The study depicts that Gpx activity has no relation with reference to age of the silkworm. However, in our earlier study we have observed a

significant increase in ROS and other antioxidant enzyme activity in tissues of silkworm subjected to various stressors and also in aged worms. Build up of reactive oxygen species (ROS) and reactive nitrogen species (RNS) under various stressors in cells and tissues were reported earlier (Droge, 2002) and these oxidants also increase with the age (Sohal *et al*., 2002). Organisms have evolved a defense mechanism against the oxidants in the form of antioxidant enzymes (Imlay, 2008; Joanisse and Storey, 1996; Li *et al*., 2011; Sim and Dehlinger, 2011). The increase in the AOEs activities in the present study are immediate response to oxidative stress and diminish soon after stress period. Whereas, lepidopteran larvae infected with NPV (Li *et al*., 2011) or with antibiotics (Buyukguzel and Kalender, 2007) have shown reduction of AOEs, which are contrary to our findings. Increased Gpx activity could be attempt of the organism to resist against the adverse effect of stress. The present study clearly indicates enhanced Gpx activity may function as an immediate defense mechanism to overcome the oxidative insult induced by single exposure to cold, hypoxia, and virus.

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