Research Paper

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Stressors Induced Antioxidant System in Silkworm *Bombyx Mori*

Zoology

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Ann Sandhya Micheal — Department of Life Science, Bangalore University, Bangalore 560 056, INDIA

Muthangi Subramanyam — Department of Life Science, Bangalore University, Bangalore 560 056, INDIA

ABSTRACT

The present study, discuss the role of antioxidant enzymes (AOEs) 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 AOEs, Superoxide dismutase, catalase and glutathione peroxidase in the haemolymph of both IV and V instars. However, upon recovery from stress, antioxidant enzymes activity returned to base value and larvae showed an age dependent decrease in antioxidant system. All the results are discussed in the light of immediate response of lepidopterans to various stressors.

Introduction

All organisms possess mechanisms to maintain homeostasis which are essential for survival. Insects are often prone to 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 Ostrinia nubulalis (Jovanovic- Galovic, 2007). In 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.

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 pocess an antioxidant enzyme (AOEs) suite which includes Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (Gpx) (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 and CAT reduces H_2O_2 to water and oxygen. However, Gpx metabolizes H_2O_2 and deleterious lipid peroxides by using reduced glutathione as a substrate.

The main aim of the present work is to determine the role of antioxidant enzymes 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, 2002). The larvae were made into six groups and were maintained at 24 – 25°C, relative humidity of 70-75 %. Experimental animals of group I were not subjected to any stress and was considered as control. Group II larva were subjected to cold at 5°C for 24h, where as group III was also subjected to cold treatment however, the larva were retrived 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 whereas 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).

Antioxidant enzymes

Superoxide dismutase

SOD activity was measured according to Misra and Fridovich (1972) with slight modification. Briefly 100 µl of 5% tissue extract was added to 880 µl of carbonate buffer (0.5 M, pH 10.2). 20 µl of epinephrine was added to the mixture and measured spectrophotometrically (Genway Genova, UK) at 480nm for 4 min. SOD activity was measured as the amount of enzyme that inhibits oxidation of epinephrine by 50%, which is equal to 1 unit.

Catalase

CAT was determined by method of Abei (1984). Briefly, 100 µl enzyme sample with 10 µl of absolute alcohol was incubated for 30 min at 0°C followed by addition of 10 µl Triton X-100. An aliquot of 50 µl was taken up in 1.25 ml of 0.066 M H_2O_2 in phosphate buffer and decrease in absorbance was measured at 240 nm for 60 s in a spectrophotometer. An extinction coefficient of 43.6 M cm^{-1} was used to determine enzyme activity and was expressed as one µmoles of H_2O_2 degraded/min/mg protein.

Glutathione peroxidase

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 37°C 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 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

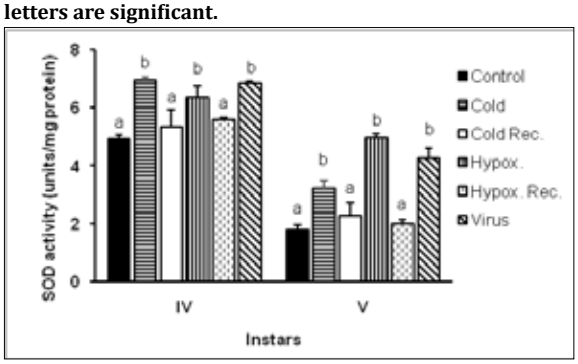
Data shown are mean ± SD of six observations. Changes among groups were analysed by MANOVA and tested by Bonferroni Post HOC test using Statistical package for Social Science (SPSS) and p value of less than 0.05 was considered significant.

Results

SOD activity in the haemolymph of silkworm B. mori significantly increased by 27.5% in IV instar and by 64.12% in V instar on exposure to cold stress. The exposure to hypoxia induced a significant increase in SOD by 20.69% and 63.18% in IV and V instar silkworm respectively. The increased activity returned to control value in the recovery period of 12h after exposure

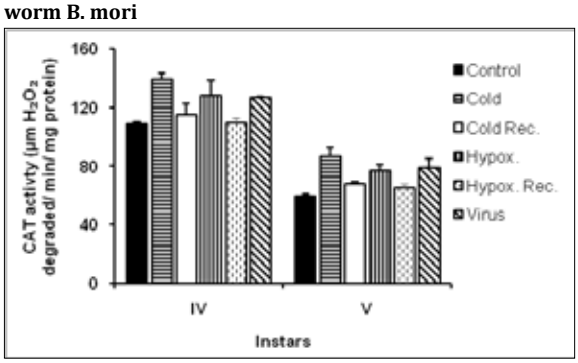
to 24 h of cold and hypoxia. On viral infection, the SOD activity in haemolymph of silkworm was significantly increased by 29.08% in IV instar by 58.6% in V instar over their repective control groups (Fig 1).However, in control worms the SOD activity was reduced by 43% in V instar larvae when compared to IV instar.

Figure 1. SOD activity in haemolymph of IV and V instar silkworm B. mori to cold, cold recovery, hypoxia, hypoxia recovery and viral infection. Values among the stressors are represented in lower cases. Those not sharing the same letters are significant.



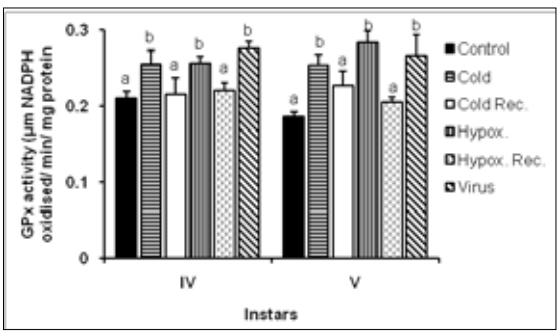
Significant increase in haemolymph CAT activity was observed in Bombyx mori larvae subjected to all stressors and its activity was found to be higher under cold exposure when compared to hypoxia and viral infection. Cold exposure induced 22.05% increase in IV instar and a 25.31% increase in V instar silkworm. Hypoxia resulted in a significant increase of CAT activity by 13.45% and by 19.62% in IV and V instar silkworm larvae respectively. The increased CAT activity reverted to control value during 12h recovery period after treatment. Increase in activity induced by virus was 14.81% and 22.43% in IV and V instar silkworm (Fig 2). Irrespective of the treatment the CAT activity per assay in IV instar was higher over V instar.

Figure 2. CAT activity haemolymph of IV and V instar silkworm B. mori



Exposure to low temperature induced a significant upregulation of Gpx 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, the enzyme activity was reverted back to control values. Virus induced a significant increase in Gpx activity in haemolymph by 59.71% and 46.44% in IV and V instars (Fig 3). However, age dependent decrease observed in SOD and CAT activity was absent in Gpx.

Figure 3. Gpx activity haemolymph of IV and V instar silkworm B. mori



Discussion

In the present study, the antioxidant enzymes SOD, CAT, Gpx in haemolymph has shown a significant increase in activity when B. mori subjected to various stressors. Enzymic activity of SOD and CAT were significantly higher in IV instar when compared to V instar. However, Gpx activity has no relation with age of the silkworm. Buildup of ROS under various stressors in cells was 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; 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 or with antibiotics (Buyukguzel and Kalender, 2007) have shown reduction of AOEs, which are contrary to our findings. Increased AOEs could be attempt of the organism to resist against the adverse effect of stress. The decreased activity of AOEs in V instar may be due to high levels of ROS since older organisms suffer from more of ROS and also may be due to the age related down regulation of AOEs.

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