Journal of Invertebrate Pathology 132 (2015) 157–164

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Differential oxidative stress responses in castor semilooper, *Achaea janata*

Ayinampudi Pavani, R.K. Chaitanya, Vinod K. Chauhan, Anwesha Dasgupta, Aparna Dutta-Gupta *

Department of Animal Biology, School of Life Sciences, University of Hyderabad, Hyderabad 500046, India

ARTICLE INFO

Article history:
Received 29 July 2015
Revised 25 September 2015
Accepted 8 October 2015
Available online 8 October 2015

Keywords:
Oxidative stress
Antioxidants
Insect immunity
Integrated pest management

ABSTRACT

Balance between reactive oxygen species (ROS) and the antioxidant (AO) defense mechanisms is vital for organism survival. Insects serve as an ideal model to elucidate oxidative stress responses as they are prone to different kinds of stress during their life cycle. The present study demonstrates the modulation of AO enzyme gene expression in the insect pest, *Achaea janata* (castor semilooper), when subjected to different oxidative stress stimuli. Antioxidant enzymes' (*catalase* (*Cat*), *superoxide dismutase* (*Sod*), *glutathione-S-transferase* (*GST*) and *glutathione peroxidase* (*Gpx*)) partial coding sequences were cloned and characterized from larval whole body. Tissue expression studies reveal a unique pattern of AO genes in the larval tissues with maximum expression in the gut and fat body. Ontogeny profile depicts differential expression pattern through the larval developmental stages for each AO gene studied. Using quantitative RT-PCR, the expression pattern of these genes was monitored during sugar-induced (D-galactose feeding), infection-induced (Gram positive, Gram negative and non-pathogenic bacteria) and pesticide-induced oxidative stress (*Bt* Cry toxin). D-Galactose feeding differentially modulates the expression of AO genes in the larval gut and fat body. Immune challenge with *Escherichia coli* induces robust upregulation of AO genes when compared to *Bacillus coagulans* and *Bacillus cereus* in the larval fat body and gut. Cry toxin feeding predominantly induced *GST* upregulation in the gut. The current study suggests that though there are multiple ways of generation of oxidative stress in the insect, the organism tailors its response by insult- and tissue-specific recruitment of the antioxidant players and their differential regulation for each inducer.

1. Introduction

Oxygen is an essential element and plays important role in oxidative metabolism which facilitates the generation of ATP in various living organisms (Tourmente et al., 2015). The biological processes while using oxygen, also generate reactive molecules called reactive oxygen species (ROS). Under normal physiological conditions, the organisms essentially maintain a homeostatic balance of ROS generation and degradation. Occasionally, this balance can be perturbed causing oxidative stress (Finkel and Holbrook, 2000).

Oxidative stress is a universal phenomenon observed in all aerobic living forms and has been implicated and associated with many diseases/disorders in humans and other species (Ye et al., 2015). Harmful effects of oxidative stress are mainly due to the volatile nature of ROS and their interaction with the macromolecules like proteins, lipids and DNA causing damage to the cells and tissues (Stadtman, 1992). Although ROS are potentially harmful to the organisms, they also play important roles in normal physiology serving as immune effector molecules and sometimes as secondary messengers in the intracellular signaling pathways (D'Autréaux and Toledano, 2007). Hence, the organisms have evolved a complex system of antioxidant (AO) defenses to achieve homeostasis between ROS generation and their elimination thereby making this system to maintain the balance critical for the survival of the organism (Valko et al., 2007).

Insects have been valuable research models in the discovery of many scientific principles owing to the numerous advantages they provide. Throughout the life cycle, many of them thrive in pathogen-rich environments, manage harsh weathers and are exposed to a number of allochemicals. Their remarkable ability to cope up with the enormous oxidative stress generated in all these circumstances, make them attractive models in this field of research. Beneficial effects of ROS characterized in insects include immune pathways like melanization and respiratory burst where in ROS-mediated microbe evasion, ROS or ROS-derived intermediates

* Corresponding author.
E-mail addresses: apdgsl@uohyd.ernet.in, aparnaduttagupta@gmail.com (A. Dutta-Gupta).

http://dx.doi.org/10.1016/j.jip.2015.10.002
0022-2011/

sequester and kill the invading microbes (Christensen et al., 2005; Nappi et al., 2009; Nappi and Vass, 1998). Not only to the invading pathogens but ROS are equally damaging to the insect tissues as well. Hence, insects have evolved a suite of AO enzymes that function in concert to maintain a fine redox balance and protect the insects from the harmful effects of ROS. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferases (GSTs) are some of the few prominent AO enzymes in insects (Ahmad et al., 1991). A thorough understanding of the AO defense responses in insects might help in gaining insights into their survival strategies through the course of their successful evolution and also exploit this information for designing integrated pest management modules.

Majority of the existing studies on antioxidant systems in insects report the changes in the AO enzyme activities in response to any given oxidative insult. Though the activity changes seem to be enough to reach upon a conclusion, it is important to understand if the change is by *de novo* transcript synthesis or by enhanced transcript stability. Also, as the changes at the transcription levels are indicative of the long term responses, these are therefore more useful in understanding the key phenomena like adaptations for survival and resistance development. The current study is such an attempt to monitor the AO gene changes at the transcriptional level for a given stress stimulus. Using various stress inducers like D-galactose, bacteria and Cry toxin to analyze AO gene expression provides a better outlook on the importance of this AO defense system and helps to identify AO gene markers, if any.

2. Materials and methods

2.1. Insect rearing

Achaea janata belongs to the insect order Lepidoptera, which is the most destructive group of insect pests and is widespread throughout the tropical and subtropical regions of the world. This pest causes considerable yield loss of its primary host castor, which is an important non-edible oil seed crop in many countries including India. Occasional hosts include economically important plants like mustard, sugar cane, cabbage, rose, tomato, banana and tea (Lakshminarayana and Raoof, 2005).

A. janata larvae were obtained from Indian Institute of Oil Seeds Research (Hyderabad, India) and fed on castor leaves at 25 ± 1 °C and 70% relative humidity with a photoperiod of 12:12 h light: dark cycle (Budatha et al., 2011). The larvae were anesthetized on ice for 15 min and dissected in cold insect Ringer solution to isolate the tissues. The collected tissues were snap frozen using liquid nitrogen and stored at −80 °C till further use.

2.2. Cloning of AO genes from larval whole body

Total RNA was isolated from the whole body using TRI reagent™ (Sigma–Aldrich, USA). The quality of the isolated RNA was analyzed by formaldehyde–agarose gel electrophoresis and the concentration was estimated using NanoDrop-1000 spectrophotometer (Thermo Scientific Nanodrop 2000). First strand cDNA synthesis was performed using Verso cDNA synthesis kit (Thermo Scientific, Rockford, USA) according to the manufacturer's protocol. Based on the reported nucleotide sequences from Lepidoptera, degenerate primers were designed in the conserved regions of catalase (*Cat*), superoxide dismutase (*Sod*), glutathione S-transferase (*GST*) and glutathione peroxidase (*Gpx*). Amplicons obtained using degenerate primers were further confirmed using gene specific primers. Real time primers were designed for the respective AO genes to carry out further studies. All the primers used are listed in Table 1.

2.3. qRT-PCR of AO genes, in various tissues, during development and different stress conditions

For tissue expression studies, gut, fat body, salivary glands, Malpighian tubules and hemocytes were collected from 5th instar (last instar) larvae. Whole body samples of 1st to 5th instar larvae, prepupa and pupa were used for ontogeny analysis. Using the same method, larval gut and fat body samples were collected from the D-galactose fed and bacteria-challenged insect groups. As fat body was degenerated in Cry toxin exposed insects, only gut tissue was obtained. Total RNA was isolated from the respective samples and first strand cDNA was synthesized using 1 μg of total RNA as mentioned above. Gene expression was monitored using gene specific primers and SYBR green qRT-PCR kit (Applied Biosystems, CA, USA) in ABI-7500 fast real-time PCR system (Applied Biosystems, CA, USA). A 40-cycle two-step PCR was carried out in triplicates with 10 μL reaction volume containing the following components: 2.0 μL cDNA template (1:40 dilution), 1.0 μL of forward and reverse primers (10 pmol) each, and 5.0 μL of 2X SYBR green PCR mix. Melting curve analysis was performed for each gene to check for specific amplification. During each cycle of the PCR, fluorescence accumulation resulting from DNA amplification was analyzed and converted into cycle threshold (C_t) by the sequence detection system software (Applied Biosystems, CA, USA). Relative quantification results were normalized with ribosomal protein S7 as endogenous control. All the results were represented as fold change in the transcript levels relative to the reference values obtained for their respective controls using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) except for tissue distribution and ontogeny profiles where relative transcript levels with respect to the endogenous control were represented.

2.4. D-Galactose treatment

D-Galactose has been widely employed to generate ageing, diabetes, hyperglycemia models in rats and to induce oxidative stress (Gong and Xu, 1991 and Li et al., 1995). However, few studies have reported its application in insect models (Cui et al., 2004 and Gaikwad et al., 2010). For our current study, we fed the insects

Table 1
List of primers used for cloning and real-time analysis of *A. janata antioxidant genes:* catalase (*Cat*), superoxide dismutase (*Sod*), glutathione S-transferase (*GST*) and glutathione peroxidase (*Gpx*).

Gene	Primer	Cloning primers (5′-3′)	Real time primers (5′-3′)
Cat	Forward	GATGGATACAGGCATATGAACGG	GGTCCATCACGCTGATAGTTGG
	Reverse	CCCAACTCCTTCTCTGGTCC	CGAGTTACGGTCCGTGTTTC
Sod	Forward	GACAACACAAATGGGTGCACGTCTG	CGTCTTGCTAAGTTCGTGGC
	Reverse	GCAAGACGACTCGGCAACG	CTCTAGAGGCTGCACTGCCTAC
GST	Forward	CCACAACACACAGTTCC	GCGAGCCATCAGCCGATATCTAG
	Reverse	GGAGATAAACTGACCTTCGC	CCGGTTTGACTTCTTCGACCTCC
Gpx	Forward	GTAGACGTGCAGGTGGCCG	GGAACTTCCATAGTGGACTGG
	Reverse	GTGTCATCCCTTTTGGTGC	CTCTTACGCATTAGGGAAAAGAAGTC