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Short Communication

Levels of Glutathione S - Transferase In Different Larval Tissues of Papilio Demoleus

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Abstract

Papilio species are of economic importance, as their larvae are important defoliator of citrus known from almost all the citrus growing areas of the world. As a result, the overall vigour and vitality of the citrus plant is reduced considerably. The aim of the present article is to study the glutathione S-transferase in different larval tissues of Papilio demoleus. CDNB - conjugation activity was compared in various fifth instar larval tissues of laboratory reared and field collected Papilio demoleus using CDNB (1-chloro-2, 4-dinitrobenzene) as substrate. Different tissues estimated for GST include the midgut, fat bodies, cuticle, haemolymph and the whole body of two days old fifth instar larvae. The GST activity was found highest (648.93 ± 0.08 µM mg protein^{-1}min^{-1}) in fat bodies of field-collected larvae while the corresponding value in the laboratory-reared larvae was 80.19 ± 0.05 µM mg protein^{-1}min^{-1}, where it showed 8.09 fold increases in GST activity. The fat bodies showed highest GST activity suggesting that the fat body is the major site of metabolism of insecticide, especially the organophosphate class, whiles the gut, cuticle and haemolymph also plays an important role in the metabolism of insecticide.

Keywords: Glutathione S - transferase, Papilio demoleus, insecticide resistance, life cycle, GST

Introduction

Glutathione S - transferase (GST; E.C. 2.5.1.18) is a family of multifunctional enzymes, which catalyze the conjugation reaction of the xenobiotics (insecticides) with an endogenous factor viz. reduced glutathione (GSH). The conjugates were further metabolized to mercapturic acids and excreted[1]. GSTs act by catalyzing the conjugation of large variety of compounds bearing an electrophonic site, with reduced glutathione. The conjugates were then eliminated from the cell via the glutathione S - conjugate export pump[2,3]. It detoxifies many insecticides including organophosphate pesticides and acaricides[4]. It has been already studied by various workers that indiscriminate use of insecticides, multiple generations of insect per annum and year round availability of host crops have contributed to the development of resistance in different insect pest to almost all kinds of insecticides[5]. The faster degradation of insecticides by metabolic enzyme GST is one of the mechanisms commonly associated with insecticide resistance in different insects[6].

Extensive indiscriminate use of organophosphate insecticides (one of the most important insecticide in wide-scale use) has led to the development of resistance in P. Demoleus to this insecticide. In view of the importance of GST in the insecticide resistance of many insects and their direct involvement in their management, present studies were undertaken to characterize the GST mediated biochemical mechanism of organophosphate resistance in P. demoleus. For that the levels of GST enzyme were studied in different tissues of laboratory reared and field collected P. demoleus.

Material and Methods

P. demoleus were reared in cages for many generations in laboratory during three consecutive years. They were also collected from the citrus orchards of Babulgaon block, Dr. PDKV, Akola. The orchards were sprayed from time to time with 0.3% dimethoate insecticide for the control of this pest. The stages that were found on the plants on the tenth day after spraying insecticide were collected and were used for enzymological studies.

Estimation of protein using BSA as a standard[7]: One mg / ml solution of BSA was taken in test - tubes in different amount ranging from 1 µl to 10 µl, to make the concentration of BSA from 1 µg to 10 µg. Each concentration was prepared in triplicate. Volume of the protein solution was adjusted to 20 µl by using 0.15 N NaCl. Ready to use Bradford reagent (200 µl) was added to each test-tube and then each test-tube was diluted five times. Test tubes were incubated for 15 min at room temperature. The absorbance was measured at 595 nm. Calibration curve was plotted between mean value of concentration on X-axis and mean value of absorbance on Y-axis.

Isolation of GST enzyme[8]: Two days old first to fifth instar larvae reared in the laboratory and collected from field were chilled in refrigerator, separately. The larvae were pinned dorsally at head and anal region in wax plate and their midguts were dissected out. Dissections were carried out with the help of sterilized dissecting needles in ice-cold sodium phosphate buffer (SPB) (0.1M pH 6.5). Fat bodies and food particles were removed from the midguts with the help of soft brush. Removed midguts were placed in glass homogenizing tubes containing 1ml ice- cold SPB (0.1M pH 6.5) containing 0.1 M of EDTA, PTU, PMSF each and 10% Glycerol. The different developmental stages of *P. demoleus* were homogenized in ice-cold condition in the Teflon tissue homogenizer at 1500 rpm, separately. The homogenate thus obtained were centrifuged at 15,000 rpm for 15 min at 4°C in high-speed refrigerated centrifuge. Solid debris and cellular material was discarded. The resultant post - mitochondria supernatant obtained was stored at –20°C and used as the enzyme source.

Estimation of GST[9]: 50 µl of 50 mM 1 chloro-2, 4 dinitrobenzene (CDNB) and 150 µl of 50 mM reduced glutathione (GSH) were added in 2.77 ml (2770 µl) of phosphate buffer (100 mM pH 6.5, 0.1 mM PTU). 30 µl of enzyme stock was added in the above mixture. Reaction was carried out in triplicate set. The content were gently shaken and incubated for 2 minutes at 24^{o}C and then transferred to cuvette placed in sample cuvette slot of the UV Spectrophotometer. 3 ml of the reaction mixture without enzyme was placed in the cuvette present in reference slot. Absorbance was read for 5 min at 340 nm by employing time scan menu of the spectrophotometer. The GST activity was calculated as follows:

$$\text{CDNB - GSH conjugate } (\mu M\ min^{-1} mg\ protein^{-1}) = \frac{\text{Abs (increase in 5 min) x 3 x 1000}}{9.6^{*} \text{ x 5 x mg of protein}}$$

9.6 mM / cm – extinction coefficient for CDNB – GSH conjugate.

Materials and Disscussion

CDNB - conjugation activity was compared in various fifth instar larval tissues of laboratory reared and field collected P. demoleus using CDNB as substrate. Different tissues estimated for GST include the midgut, fat bodies, cuticle, haemolymph and the whole body of two days old fifth instar larvae. The GST titers were found to be 101.13, 80.19, 17.87, 10.77 and 87.36 µM mg protein^{-1}min^{-1} in the midgut, fat bodies, cuticle, haemolymph and whole body of the laboratory reared fifth instar larvae, respectively. Different tissues of fifth instar larvae collected from field showed 616.81, 648.93, 53.60, 42.15 and 579.16 µM mg protein^{-1}min^{-1} in the midgut, fat bodies, cuticle, haemolymph and whole body, respectively. table – 1

The field collected P.demoleus showed high levels of GST. It showed 6.09, 8.09, 2.99, 3.91 and 6.62 fold increase of GST activity in midgut, fat bodies, cuticle, haemolymph and whole body, respectively. Among these tissues, the highest activity was found in the fat bodies (figure - 1). In the present studies, GST activity was determined in the fat bodies, whole body, midgut, haemolymph and cuticle of P. demoleus. Among different tissues, the highest activity was found in the fat bodies of P. demoleus. The field collected P. demoleus showed high levels of GST than the laboratory reared P. demoleus. It showed 8.09, 6.62, 6.09, 3.91 and 2.99 fold increase of GST activity in fat bodies, whole body, midgut, haemolymph and cuticle, respectively. In Lucilia cuprina also elevated GST level were found in the larval fat body[10]. The blood component probably included some enzyme released from the fat body, gut and cuticle as they were cut during dissection.

Table – 1
Levels of GST in different tissues of fifth instar larvae

Name of the tissue	GSH-CDNB CONJUGATION (µM mg protein^{-1}min^{-1})		
	Laboratory reared	Field collected	Field collected/Lab. reared*
Midgut	101.13 ± 0.02	616.81 ± 0.05	6.09
Fat bodies	80.19 ± 0.05	648.93 ± 0.08	8.09
Cuticle	17.87 ± 0.02	53.60 ± 0.06	2.99
Haemolymph	10.77 ± 0.06	42.15 ± 0.04	3.91
Whole body	87.36 ± 0.12	579.16 ± 0.33	6.62

Field collected / Lab. reared: fold increase in enzyme activity

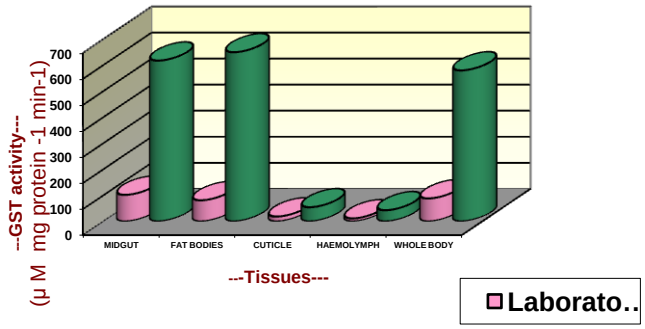

Figure – 1
GST activity in different tissues of
Fifth instar larvae of P. demoleus

Similarly, it was found that GST activity was highest in fat bodies of H. armigera indicating the major site of metabolism of organophosphate insecticide[11]. It is a common feature that the fat body is the major site of metabolism of many insecticides, especially the organophosphate class, while the gut, cuticle and haemolymph also plays an important role in the metabolism of xenobiotics. Low enzyme activity in whole body extract is probably due to the effect of endogenous inhibitors such as polyphenols and quinines released during homogenization[12].

Conclusion

Among different tissues, the highest activity was found in the fat bodies of P. demoleus collected from the field, suggesting that fat body is the major site of metabolism of insecticide while the gut, cuticle and haemolymph also plays an important role in the metabolism of insecticide.

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