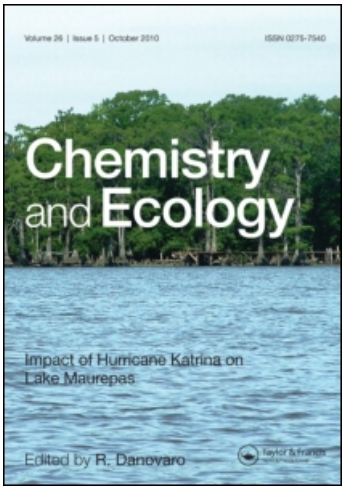

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Oxidative Metabolism and Glucose-6-Phosphate Dehydrogenase in the Detoxification of Phosphamidon and Methylparathion in Prawn, *Metapenaeus Monoceros*

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To cite this Article Sreenivasulu, Y. and Reddy, M. Srinivasulu(1997) 'Oxidative Metabolism and Glucose-6-Phosphate Dehydrogenase in the Detoxification of Phosphamidon and Methylparathion in Prawn, *Metapenaeus Monoceros*', Chemistry and Ecology, 14: 1, 69 — 77
To link to this Article: DOI: 10.1080/02757549708035540
URL: http://dx.doi.org/10.1080/02757549708035540

Chemistry and Ecology, 1997, Vol. 14, pp. 69–77
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OXIDATIVE METABOLISM AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN THE DETOXIFICATION OF PHOSPHAMIDON AND METHYLPARATHION IN PRAWN, *METAPENAEUS MONOCEROS*

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(Received 21 January 1997; Revised 30 July 1997)

The changes in the soluble and insoluble protein contents, glucose and activity levels of glucose-6-phosphate dehydrogenase were studied in hepatopancreas, muscle and gill tissues of penaeid prawn, *Metapenaeus monoceros*, following its exposure to sublethal concentrations of phosphamidon and methylparathion, organophosphorus insecticides. Both soluble and insoluble protein fractions increased, whereas tissue glucose content decreased. Oxidative metabolism and glucose-6-phosphate dehydrogenase activity levels was significantly elevated. The results obtained in the present study throws light on the enhanced protein synthetic potentiality and detoxification of insecticide molecules are the adoptive measures to counteract the insecticide toxicity.

Keywords: Glucose-6-phosphate; *Metapenaeus monoceros*; insecticides

INTRODUCTION

Organophosphorus insecticides (OPI) are now increasingly used on account of lower persistance and highly degradable in biological system and nature. The mishandling of these chemicals may result in ecological imbalances and are also known to induce physiological and

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biochemical changes in several non-target organisms including crustaceans, some of which are important members of the food chain (Nimmo, 1979, Couch, 1979). Our previous reports indicate that even sublethal concentrations of OPI like phosphamidon and methylparathion appear to be neurotoxic, by inhibiting acetylcholinesterase (AChE) (Reddy and Rao, 1988a), subsequently leading to alterations in the certain metabolic profiles, as a secondary manifestation (Reddy and Rao, 1988b, 1991). Several workers restricted their studies to mechanism of action of various OPI compounds and little attention has been paid to trace out the study of oxidative metabolism and the detoxification processes in organisms. With this perspective background, the present study aims to probe into the possible role of oxidative metabolism and glucose-6-phosphate dehydrogenase, a key enzyme of hexose monophosphate shunt pathway in the detoxification of both phosphamidon and methylparathion in the selected tissues of penaeid prawn, *Metapenaeus monoceros*. *M. monoceros* in the present investigation is a sensitive indicator of marine and estuarine pollution (Butler, 1966) and also forms an important fishery in India.

MATERIALS AND METHODS

The details of collection and maintenance of prawns was described earlier (Reddy and Rao, 1988b, 1991). Technical grade methylparathion (O,O-dimethyl, O-4-nitrophenyl thiophosphate) and phosphamidon (O,O-dimethyl-O-(1-methyl-2-chloro-2-diethyl-carbomoyl-vinyl) phosphate) were used as test chemicals. A stock solution of 1000 ppm and appropriate working concentrations were prepared by dilution with sea water. LC_{50} values were found to be 1.20 mgl^{-1} for phosphamidon and 0.12 mgl^{-1} for methylparathion for 96 h exposure period in a static bioassay system (Doudoroff *et al.*, 1951). Laboratory acclimatized prawns were exposed to sublethal concentration of 0.4 mgl^{-1} of phosphamidon and 0.04 mgl^{-1} for methylparathion for 4 days. After 4 days of exposure, the metabolically active tissues like hepatopancreas, muscle and gill tissues were isolated quickly and kept in crustacean Ringer (Van Harreveld, 1936) and were subsequently subjected to biochemical analysis. For proteins, the tissues were

homogenized in 0.25 M cold sucrose solution and centrifuged at 1000 × g for 10 minutes. The supernatant was used for the estimation of soluble proteins and the residue for insoluble proteins. The protein content was estimated with folin-phenol reagent (Lowry *et al.*, 1951) using bovine serum albumin as standard. Tissue glucose was estimated by the method of Kemp and Mayers (1954). For G6PDH assay, the tissue homogenates were prepared in 0.25 M cold sucrose solution and the supernatants were used. The G6PDH activity (glucose-6-phosphate, NAD oxidoreductase, EC.1.1.1.49) was determined by the method of Georg and Waller (1965). Acetylcholinesterase (AChE; acetylcholine acetylhydrolase, EC.3.1.1.8, Ellman *et al.*, 1961), succinate dehydrogenase (SDH, succinate oxidoreductase, EC.1.3.99.1, Nachlas *et al.*, 1960), pyruvate dehydrogenase (PDH, pyruvate oxidoreductase, EC.1.2.3.3, Srikanthan and Krishnamoorthy, 1955), isocitrate dehydrogenase (ICDH, isocitrate oxidase, Kornberg and Pricer, 1951), lactate dehydrogenase (LDH, L-lactate; NDH oxidoreductase, EC.1.1.1.27, Srikanthan and Krishnamoorthy, 1955), cytochrome-c-oxidase, (EC.1.9.3.1, Oda *et al.*, 1958). Each experiment was replicated six times and data were subjected to statistical analysis following Bailey (1965).

RESULTS AND DISCUSSION

The changes in activity levels of AChE, SDH, ICDH, PDH, LDH and cytochrome-c-oxidase of hepatopancreas and muscle tissues of prawn, *M. monoceros*, after exposure to sublethal concentrations of phosphamidon and methylparathion (Tables I and II). The sucrose soluble and insoluble protein fractions showed significant increase, whereas glucose content was considerably decreased in both phosphamidon (PE) and methylparathion (MPE) exposed prawn, *M. monoceros* (Tables III and IV). G6PDH activity levels were also significantly elevated in all the tissues of prawn following its exposure to sublethal concentrations of phosphamidon and methylparathion.

The AChE activity was significantly inhibited in both the tissues after acute exposure to sublethal concentrations of phosphamidon and methylparathion. The rate of inhibition is more under methylparathion

TABLE I Levels of oxidative enzymes in hepatopancreas of prawn ***M.monoceros*** after exposure to phosphamidon (PE) and methylparathion (MPE)

Enzyme	*Control*	*PE*	*MPE*
AChE[1]	2.64 z±0.12	1.29 ±0.10 (−51.0)	1.18 ±0.07 (−55.0)
SDH[2]	0.922 ±0.021	0.512 ±0.017 (−45.0)	0.472 ±0.013 (−49.0)
ICDH[2]	0.425 ±0.038	0.182 ±0.019 (−57.0)	0.171 ±0.018 (−60.0)
PDH[2]	0.428 ±0.039	0.153 ±0.018 (−64.0)	0.129 ±0.015 (−70.0)
LDH[2]	0.312 ±0.015	0.131 ±0.012 (−58.0)	0.105 ±0.010 (−66.0)
Cytochrome-c-oxidase[3]	95.78 ±6.79	56.75 ±3.12 (−41.0)	47.95 ±3.15 (−50.0)

Each value is mean ± SD of six individual observations.
Values in parenthesis are percent change over respective controls.
Values expressed in (1) μ moles of acetylcholine hydrolysed/mg protein/hr.
Values expressed in (2) μ moles of formazan formed/mg protein/hr.
Values expressed in (3) μ moles of diformazan formed/mg protein/hr.
All values are significant at $P < 0.001$.

exposure compared to phosphamidon exposure. The general decrement in the AChE activity during selected OPI exposure suggest that acetylcholine hydrolysis was depressed considerably. The majority of compounds in use as insecticides contain the (=S) thionomoiety and are either phosphorothionates or phosphorodithioates. Further studies with a variety of compounds now have established that conversion of phosphorothionate and phosphorodithionate insecticides to their corresponding oxygen analogues is a necessary prerequisite for their action as cholinesterase inhibitors. The group of NADPH-dependent mixed function oxidases, the enzyme system present in the microsomes are responsible for the catalysis of the reaction for the conversion of =S to =O. These oxygen analogues are more potent inhibitors of AChE than the parent insecticide compounds (Casida *et al.*, 1983). The inhibition in the AChE activity in the present investigation may be attributed to the binding

TABLE II Levels of oxidative enzymes in muscle of prawn, ***M.monoceros***, after exposure to phosphamidon (PE) and methylparathion (MPE)

Enzyme	*Control*	*PE*	*MPE*
AChE[1]	2.03 ±0.13	1.13 ±0.05 (−44.0)	0.92 ±0.04 (−55.0)
SDH[2]	0.078 ±0.004	0.043 ±0.003 (−45.0)	0.039 ±0.003 (−50.0)
ICDH[2]	0.105 ±0.012	0.058 ±0.011 (−45.0)	0.051 ±0.008 (−51.0)
PDH[2]	0.135 ±0.032	0.075 ±0.012 (−45.0)	0.068 ±0.011 (−50.0)
LDH[2]	0.068 ±0.011	0.047 ±0.005 (−31.0)	0.036 ±0.005 (−47.0)
Cytochrome-c-oxidase[3]	31.14 ±4.12	18.19 ±2.17 (−42.0)	15.45 ±1.42 (−58.0)

Each value is mean ± SD of six individual observations.
Values in parenthesis are percent change over respective controls.
Values expressed in (1) μ moles of acetylcholine hydrolysed/mg protein/hr.
Values expressed in (2) μ moles of formazan formed/mg protein/hr.
Values expressed in (3) μ moles of diformazan formed/mg protein/hr.
All values are significant at $P < 0.001$.

TABLE III Levels of soluble, insoluble proteins, glucose (mg g^{-1} wet weight of tissue) and glucose-6-phosphate dehydrogenase (G6PDH) activity (μ moles of formazan formed/mg protein/h) in control (C) and phosphamidon exposed (PE) prawn, ***M.monoceros***

Parameter	*Hepatopancreas*		*Muscle*		*Gill*	
	C	*PE*	*C*	*PE*	*C*	*PE*
Soluble Proteins	112.13 ±8.31	176.42 ±9.78 (+57.0)	61.18 ±6.52	82.85 ±7.49 (+35.0)	77.42 ±6.49	93.32 ±7.03 (+21.0)
Insoluble Proteins	68.19 ±5.84	91.45 ±5.98 (+34.0)	139.13 ±10.19	185.49 ±11.15 (+33.0)	50.49 ±4.18	67.85 ±5.02 (+34.0)
Glucose	6.692 ±0.145	3.682 ±0.049 (+45.0)	0.714 ±0.048	0.559 ±0.027 (+22.0)	0.583 ±0.045	0.412 ±0.029 (+29.0)
G6PDH	2.420 ±0.154	4.381 ±0.349 (+81.0)	0.656 ±0.024	0.982 ±0.089 (+50.0)	0.902 ±0.089	1.439 ±0.175 (+60.0)

Each value is mean ± SD of six individual observations.
Values in the parentheses are percent change over respective controls.
All values are statistically significant at $P < 0.001$.

TABLE IV Levels of soluble, insoluble proteins, glucose (mg g^{-1} wet weight of tissue) and glucose-6-phosphate dehydrogenase (G6PDH) activity (μ moles of formazan formed/mg protein/h) in control (C) and methylparathion exposed (MPE) prawn, ***M.monoceros***

Parameter	*Hepatopancreas*		*Muscle*		*Gill*	
	C	*MPE*	*C*	*MPE*	*C*	*MPE*
Soluble proteins	112.13 ±8.31	189.84 ±8.95 (+49.0)	61.18 ±6.52	92.14 ±7.45 (+51.0)	77.42 ±6.49	98.42 ±6.89 (+27.0)
Insoluble proteins	68.19 ±5.84	94.48 ±4.45 (+34.0)	139.13 ±10.19	194.95 ±12.04 (+40.0)	50.49 ±4.18	71.12 ±4.84 (+41.0)
Glucose	6.692 ±0.145	3.402 ±0.049 (−49.0)	0.714 ±0.048	0.501 ±0.024 (−30.0)	0.583 ±0.045	0.401 ±0.028 (−31.0)
G6PDH	2.420 ±0.154	4.589 ±0.351 (+90.0)	0.656 ±0.024	1.014 ±0.094 (+55.0)	0.902 ±0.089	1.514 ±0.182 (+68.0)

Each value is mean ± SD of six individual observations.
Values in parenthesis are percent change over respective controls.
All values are statistically significant at $P < 0.001$.

of electrophilic insecticide group to the active site of the enzyme, AChE, thus blocking the ACh hydrolysis by the enzyme. Coppage and Mathews (1975) observed a similar kind of inhibitor pattern of AChE in *Penaeus monodon*, after exposure to different insecticides.

The present investigation concludes that sublethal phosphamidon and methylparathion exposure causing significant inhibition of oxidative metabolism in the hepatopancreas and muscle tissues of *M. monodon*. There is a clear shift in the metabolism from aerobiosis to anaerobiosis during sublethal OPI induced stress condition. When the oxidative metabolism is impaired, the rate of production of NADPH/NADH/FADH will also diminish, and will hamper the detoxification process.

The decrease in tissue glucose content during insecticide exposure signifies their possible utility to meet the higher energy demands to counteract the toxic effects of phosphamidon and methylparathion. Reports also indicate that besides tissue glucose, the glycogen precursors are being trapped and utilized to meet the energy demands warranted by toxic environment (Reddy and Rao, 1991). Besides the

tissue glycogen synthesis and utilization was impaired during insecticide exposure in prawns (Reddy and Rao, 1988b, 1991). Reports are also available about the tissue damage (unpublished data) and elevation in blood or haemolymph glucose levels during organophosphate insecticide exposure (Reddy *et al.*, 1986). The decrement in the tissue glucose levels may be attributed to either utilization in energy yielding pathways or its leakage into haemolymph causing hyperglycemia under phosphamidon and methylparathion exposure in prawn, *M. monoceros*. The another possible reason for a decrease in the tissue glucose might be due to feeding of glucose into hexose monophosphate (HMP) shunt pathways for further oxidation and production of NADPH and pentose sugars. The increased G6PDH activity levels in the tissues of prawns during insecticide exposure suggests the increased operation of HMP shunt pathway, possibly to generate not only NADPH but also pentose sugars. The NADPH generated through the HMP pathway will be utilized for fatty acid synthesis (Harper *et al.*, 1970) and also plays an important role in the detoxification of organophosphorus insecticides (O'Brien, 1967). The possibility of utilization of NADPH is in the fatty acid production in prawns (Reddy and Rao, 1989). Hence, it is evident that NADPH produced in the present study may be diverted for the detoxification of insecticide molecules. Besides the production of NADPH, G6PDH also contributes to the synthesis of pentose sugars, which forms the skeleton for the synthesis of nucleic acids. Reports also indicate the increased amounts of DNA and RNA in the prawn species under insecticide exposure (Vijayalakshmi, 1987). The increased nucleic acid synthesis further confirms the increased protein synthetic potentiality of the prawn tissues, which is visualized through increased levels of both soluble and insoluble fractions under insecticide exposure. The increase in the soluble proteins may be justifiable during insecticide exposure. Since the soluble protein fraction represents enzymes, hormones and free peptides, the increase in tissue soluble proteins should represent an increase in the synthesis of enzymes, which are responsible for the detoxification of organophosphorus insecticides including phosphamidon and methylparathion.

The G6PDH is playing a dual role in prawn, *M. monoceros*, by maintaining higher G6PDH levels, the production of more amounts of NADPH for detoxification and pentose sugars for the synthesis of

substances needed for detoxification purposes. These prawns are showing certain physiological and biochemical adaptive responses like increased operational efficiency of HMP pathway and enhanced protein synthetic potentiality to counteract the toxicity of phosphamidon and methylparathion. All these biochemical adaptive responses will pave the way for the successful survival of prawns during insecticide polluted environments.

Acknowledgements

This work was supported by a research grant awarded to Dr. Y. S. by CSIR, New Delhi.

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