Bull. Environ. Contam. Toxicol. (1988) 41:344–351

Bulletin of Environmental Contamination and Toxicology

Changes in Nitrogen Metabolism of Penaeid Prawn, *Penaeus indicus*, during Sublethal Phosphamidon and Methylparathion–Induced Stress

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Hazards of environmental contamination through indiscriminate use of a variety of pesticides have attracted global attention. Increased use of organophosphorous (OP) pesticides in recent years due to suspended registration of chlorinated hydrocarbon pesticides, generates a need for information on their effects on the aquatic environment. Very few reports are available on the effects of OP insecticides on nitrogen metabolism in non-target organisms (Reddy et al. 1982; Reddy & Rao 1986b). Changes in carbohydrate metabolism in the selected tissues of prawns during phosphamidon induced toxicity have suggested the possible alterations in the energy metabolism and nitrogen metabolism (Reddy & Rao 1986a; b). Hence in the present investigation an attempt has been made to probe into certain aspects of ammonia production and its utilization in the selected tissues of penaeid prawn, Penaeus indicus exposed to sublethal concentrations of phosphamidon and methylparathion, organophosphorous insecticides. Phosphamidon and methylparathion are toxic to several aquatic biota, including crustaceans (Couch 1979). P. indicus is considered to be a sensitive indicator of marine and estuarine pollution (Butler 1966) and also forms one of the commercially important fishery of India.

MATERIALS AND METHODS

Prawns, P. indicus (H.Milne Edwards) were collected from the Buckingham canal, near Kavali seacoast, Andhra Pradesh, India. Only intermolt prawns (75 ± 5 mm in length and weight 2.5 ± 0.5 g) were selected and acclimatized to laboratory conditions for a period of one week under constant salinity of 15 ± 1 ppt, pH 7.1 ± 0.2 and temperature of 23 ± 2°C. They were fed ad lib diet of oil cake powder. The media in which they were placed was changed for every 24 h.

Technical grade phosphamidon (92% w/v; 0,0-dimethyl-0-(1-methyl-2-chloro-2-diethyl-carbomoyl-vinyl) phosphate) and methylparathion (80% w/w; 0-0-dimethyl, 0-4 nitrophenyl thiophosphate) were used as test chemicals. A stock solution of 1000 ppm (1 mg/ 1 ml) and appropriate working concentrations were prepared by dilution with seawater. Toxicity evaluation studies were conducted in static bioassay system (Doudoroff et al. 1951) and the results were tabulated for computization of LC_{50} values as per Finney (1964). LC_{50} values were found to be 0.9 ppm for phosphamidon and 0.095 ppm for methylparathion to the intermolt prawn for 48 h exposure. Laboratory acclimatized prawns were exposed to sublethal concentrations of phosphamidon (0.3 ppm) and methylparathion (0.03 ppm) for 72 h in the present study and aerated twice a day to prevent hypoxia. Midgut gland, muscle and gill tissues were isolated from control, phosphamidon exposed and methylparathion exposed prawns and were used for biochemical analysis.

NAD-dependent glutamate dehydrogenase (GDH) assay was estimated by the method of Lee and Lardy (1965). AMP-deaminase assay was estimated by the method of Weil-Malherbe and Green (1955) with slight modification of Waegelin et al (1978). Adenosine deaminase assay was estimated by the method of Galanti and Glusti (1974). Arginase and glutamine synthetase were assayed by the methods of Campbell (1964) and Wu (1963) respectively. Glutamine, urea and ammonia were estimated by the methods of Colowick and Kaplan (1971), Natelson (1971) and Bergmeyer (1965) respectively.

The protein content in the enzyme source was estimated with the Folin phenol reagent (Lowry et al. 1951) using bovine serum albumin as standard. The data were subjected to statistical analysis as per Bailey (1965).

RESULTS AND DISCUSSION

Changes in certain biochemical parameters of nitrogen metabolism in selected tissues of P. indicus exposed to sublethal concentrations of phosphamidon and methylparathion were presented in Tables 1, 2 and 3.

The increment in the AMP deaminase and adenosine deaminase activity levels indicates the augmented purine catabolism resulting in an elevation of ammonia content. The activity levels of glutamate dehydrogenase (GDH), a mitochondrial enzyme was also increased in muscle and gill tissues and diminished in midgut gland. The increased GDH in gill and muscle

Table 1. Levels of ammonia, urea and glutamine in the selected tissues of control (C), phosphamidon exposed (PE) and methylparathion exposed (ME) prawns.

Midgut gland			Muscle			Gill		
C	PE	ME	C	PE	ME	C	PE	ME
				Ammonia				
11.18 ±1.32	17.13 ±1.48 (+53)	19.49 ±1.64 (+74)	5.48 ±0.89	8.72 ±0.93 (+59)	9.03 ±0.88 (+65)	6.83 ±0.85	10.93 ±1.08 (+60)	11.42 ±1.14 (+67)
				Urea				
1.24 ±0.25	1.78 ±0.33 (+44)	2.02 ±0.38 (+63)	1.08 ±0.17	1.43 ±0.25 (+32)	1.62 ±0.28 (+50)	0.93 ±0.05	1.38 ±0.25 (+48)	1.43 ±0.34 (+54)
				Glutamine				
20.48 ±2.34	31.35 ±3.92 (+53)	38.30 ±3.85 (+87)	6.83 ±0.75	9.92 ±0.82 (+45)	10.14 ±0.85 (+49)	11.32 ±0.99	15.48 ±1.15 (+37)	16.59 ±1.18 (+47)

Values are mean ± S.D of 6 individuals. Values are expressed as μ moles/g wet wt. tissue. Values in parentheses are % change over their respective controls. All values are significantly different at $P < 0.001$ over their respective controls.

Table 2. Activity levels of AMP and adenosine deaminase and glutamate dehydrogenase in tissues of control (C), phosphamidon exposed (PE) and methylparathion exposed (ME) prawns.

Midgut gland			Muscle			Gill		
C	PE	ME	C	PE	ME	C	PE	ME
AMP-deaminase (μ moles of ammonia formed/mg protein/ h)								
0.18 ±0.02	0.32 ±0.04 (+78)	0.34 ±0.05 (+89)	0.45 ±0.03	0.75 ±0.08 (+67)	0.78 ±0.08 (+73)	0.32 ±0.03	0.53 ±0.05 (+66)	0.55 ±0.05 (+72)
Adenosine deaminase (μ moles of ammonia formed/mg protein/ h)								
1.08 ±0.17	1.65 ±0.19 (+53)	1.75 ±0.20 (+63)	0.80 ±0.12	1.28 ±0.17 (+60)	1.34 ±0.19 (+68)	0.48 ±0.09	0.74 ±0.11 (+54)	0.77 ±0.12 (+60)
Glutamate dehydrogenase (μ moles of formazan formed/mg protein/ h)								
1.18 ±0.10	0.60 ±0.04 (-49)	0.50 ±0.04 (-58)	0.38 ±0.05	0.46 ±0.05 (+21)	0.48 ±0.04 (+26)	0.72 ±0.09	0.99 ±0.09 (+38)	0.99 ±0.09 (+38)

Values are mean ± S.D. of 6 individuals. Values in parentheses are % change over their respective controls. Values are significantly different at $P < 0.001$ over their respective control values.

Table 3. Activity levels of glutamine synthetase and arginase in tissues of control (C), phosphamidon exposed (PE) and methylparathion exposed (ME) prawns.

Midgut gland			Muscle			Gill		
C	PE	ME	C	PE	ME	C	PE	ME
Arginase (μ moles of urea formed / mg protein/ h)								
0.99 ±0.04	1.67 ±0.10 (+69)	1.74 ±0.09 (+76)	0.28 ±0.02	0.49 ±0.04 (+75)	0.50 ±0.05 (+79)	0.33 ±0.02	0.47 ±0.03 (+42)	0.48 ±0.03 (+45)
Glutamine synthetase (μ moles of Y glutamyl hydroxamate/mg protein/h)								
0.74 ±0.04	1.28 ±0.13 (+73)	1.30 ±0.13 (+76)	0.23 ±0.05	0.32 ±0.05 (+39)	0.39 ±0.05 (+70)	0.35 ±0.05	0.46 ±0.06 (+31)	0.48 ±0.05 (+37)

Values are mean ± S.D. of 6 individuals. Values in parentheses are % change over their respective control. Values are significantly different at $P < 0.001$ over their respective controls.

indicates increased glutamate oxidation resulting in ammonia production (Vijayalakshmi 1987) and decreased GDH activity in midgut gland suggests decreased oxidation of glutamate (Natarajan 1983). In evidence to this, the glutamate levels were found to be increased in the midgut gland and decreased in muscle and gill tissues under phosphamidon induced stress. Ammonia, a byproduct of purine and amino acid catabolism, is a toxic compound (Lowenstein 1972). The cells can not tolerate high concentrations of ammonia. In the present investigation, ammonia levels are found to be elevated significantly in the phosphamidon exposed (PE) and methylparathion exposed (ME) prawn tissues. The enzymes such as agrinase and glutamine synthetase involved in the synthesis of the less toxic nitrogenous substance namely urea and glutamine were studied. Midgut gland arginase responded similar to the other two tissues and increased significantly in all the tissues. Glutamine synthatase, a mitochondrial enzyme was also found to be elevated under both phosphamidon and methylparathion induced stress and the elevation was found to be more in the midgut gland as compared to muscle and gill tissues. In consonance to this observation, glutamine and urea levels were found to be increased significantly in all the PE and ME prawn tissues studied. The presence of urea and glutamine in non-hepatic tissues could be due to its transport from the midgut gland. This indicates that phosphamidon and methylparathion induced the triggering of ammoniagenesis. To avert the toxicity of ammonia, the tissues have mobilized this toxic metabolite into the synthesis of comparatively safer substances like urea and glutamine as evidenced through the increased specific activities of arginase and glutamine synthetase respectively. All these metabolic changes were more pronounced in ME prawn tissues when compared to PE prawn tissues.

It may be concluded from the present investigation that ammoniagenesis was triggered by increased deamination of purines and oxidative deamination of glutamate under phosphamidon and methylparathion induced stress. This results in the production of ammonia. As a consequence of this the PE and ME prawn tissues have adopted the mechanisms to detoxify the ammonia by enhancing the synthesis of urea and glutamine. These changes in the tissues will pave way for the successful survival of prawns in the phosphamidon and methylparathion polluted environments.

Acknowledgments Dr. MSR is grateful to the Council of Scientific and Industrial Research, New Delhi, for providing financial support. We also thank Prof. K. Sasira Babu for his encouragement.

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Received November 9, 1987; accepted March 22, 1988.