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Changes in Acid Phosphatase Activity in Tissues of Crab, *Oziotelphusa senex senex*, Following Exposure to Methyl Parathion

P. Sreenivasula Reddy, A. Bhagyalakshmi, and R. Ramamurthi

Department of Zoology, Sri Venkateswara University, Tirupati 517 502, India

The use of organophosphatase pesticides is being increased in the recent years due to the ban or restrictions on chlorinated hydrocarbons. These organophosphate pesticides degrade rapidly in biological systems and their metabolic products persist in irrigation and drainage canals and affects aquatic fauna. Decreased limb bud growth, moulting rate and ovarian maturation were reported in crabs exposed to organophosphates (Sreenivasula Reddy et al 1982a, 1983a, 1983b). Acute and chronic exposure to Sumithion (an organophosphate pesticide) would lead to a chain of metabolic changes at cellular level in the crab (Bhagyalakshmi et al. 1982, 1983a, 1983b, Sreenivasula Reddy et al. 1982b, 1982c). The present report deals with the effect of exposure to Methyl parathion [0,0-dimethyl-0-(4-nitrophenyl) monothiophosphate] on acid phosphatase activity in the tissues of crab (Oziotelphusa senex senex).

Acid phosphatase, a typical lysosomal enzyme, located in lysosomes in a latent state, can be activated not only by the disruption of particle membrane in vitro, but also by a number of stress conditions in vivo (De Duve et al. 1955). Increase in acid phosphatase after pesticide exposure seems to be characteristic of tissues damage and became a useful diagnostic and experimental tool (Tietz 1970).

MATERIALS AND METHODS

Healthy and active specimens of Oziotelphusa senex senex (standard body weight, 30-32g; carapace length, 30-35mm) were collected from local rice fields and irrigation canals and fed ad libitum with frog muscle. Prior to use they were acclimatized to laboratory conditions (temperature 30±2°C; relative humidity 75% and a light period of 12 h) for one week. The media in which they were placed were changed every 24 h. Technical grade (95% W/V) Methyl parathion obtained gratis

from Bayer (India) Ltd was used. Procedures for preparation of stock solution and other test conditions were described earlier (Sreenivasula Reddy et al. 1982b). The crabs were exposed to 0.1, 0.2, 0.5 and 1.0 mg/L of Methyl parathion. One hundred crabs were placed in each concentration for 30 days. During experimentation and acclimation the crabs were provided with frog muscle to avoid any effects of starvation on enzyme activity. Controls were maintained. Ten crabs from each container were killed at 1-, 3-, 5-, 10-, 15- and 30 days.

The hepatopancreas and muscle tissues were isolated in cold (4°C), rinced thoroughly in cold crustacean ringer, minced into small pieces in chilled petri dish and homogenised in cold 0.25 M sucrose solution and centrifuged at 3000 g for 10 min to removal cell debris. The clear supernatant was used for enzyme assay. Acid phosphatase (orthrophosphoric monoester phosphohydrolase, EC 3.1.3.2) was estimated by the method of Bodansky (1933) and the liberated inorganic phosphatase was estimated by the method of Fiske and Subba Rao (1925). The protein content in the enzyme source was estimated following Folin phenol reagent (Lowry et al. 1951) using Bovine serum albumin as standard.

RESULTS AND DISCUSSION

The changes in the activity of the enzyme acid phosphatase after exposure to Methyl parathion are presented in Tables 1 and 2. Enzyme stimulation and inhibition is expressed as percentage of control activities. Acid phosphatase activity was significantly ($P < 0.001$) higher than the control levels in both the tissues in all four experimental groups on day 1. The higher the Methyl parathion concentration, the greater the enzyme activity. The extent of elevation was found to increase gradually in both the tissues on further exposure leading to maximum on day 3 in the 0.1 ppm group and on day 5 in 0.2-, 0.5- and 1.0 ppm groups. Then there was a gradual decrease in elevation on further exposure leading to a non-significant difference from control from day 15 onwards in the 0.1 ppm group and from day 30 onwards in the 0.2-, 0.5- and 1.0 ppm groups.

Lysosomal hydrolases are thought to contribute to the degradation of damaged cells and hence to facilitate their replacement by normal tissue (De Duve et al. 1955) The intracellular digestion of damaged material by lysosomes could be prompted by Methyl parathion damage to subcellular structures (Nagarathnamma 1982). The elevation in acid phosphatase activity in the present study could be attributed to similar pesticide induced changes in lysosomal activity. The higher the concentration,

Table 1. Alteration in acid phosphatase activity in hepatopancreas after exposing the crab to 0.1, 0.2, 0.5 and 1.0 ppm of methyl parathion

Concentration exposed	Enzyme activity (µg of Pi liberated/mg protein/h) Time after exposure (days)					
	1	3	5	10	15	30
Control	56.4 ± 3.6*	58.8 ± 6.1	57.6 ± 4.3	56.6 ± 4.1	58.1 ± 4.1	55.1 ± 5.1
0.1 ppm	73.1 ± 6.0[1] (+29.61)	78.1 ± 5.0[1] (+32.72)	74.8 ± 4.7[1] (+29.89)	61.1 ± 5.4 (+7.86)	60.0 ± 4.8 (+3.32)	58.4 ± 4.1 (+6.08)
0.2 ppm	74.6 ± 2.3[1] (+32.27)	80.1 ± 6.4[1] (+36.11)	94.0 ± 8.1[1] (+63.18)	75.9 ± 6.0[1] (+33.97)	63.4 ± 2.1 (+9.12)	54.8 ± 6.0 (-0.51)
0.5 ppm	77.8 ± 3.1[1] (+37.94)	89.7 ± 5.6[1] (+52.38)	101.9 ± 8.1[1] (+76.91)	84.1 ± 5.4[1] (+48.46)	70.1 ± 4.1[1] (+20.66)	56.6 ± 4.1 (+2.76)
1.0 ppm	79.5 ± 4.1[1] (+40.96)	94.1 ± 8.1[1] (+59.89)	112.7 ± 6.1[1] (+95.56)	110.1 ± 5.4[1] (+94.40)	84.1 ± 6.7[1] (+44.76)	59.6 ± 8.1 (+8.26)

*Values are mean ± S.D. of 10 individuals. Values in parentheses are % change over their respective controls.

[1]Values are significantly different at $P < 0.001$. The remaining values are not significant.

Table 2. Alterations in acid phosphatase activity in muscle after exposing the crab to 0.1, 0.2, 0.5 and 1.0 ppm of methyl parathion

Concentration exposed	Enzyme activity (µg of Pi liberated/mg protein/h) Time after exposure (days)					
	1	3	5	10	15	30
Control	37.1 ± 3.1*	39.6 ± 4.2	36.6 ± 3.7	37.1 ± 6.6	35.5 ± 4.3	36.2 ± 3.1
0.1 ppm	43.1 ± 4.2[1] (+16.12)	66.4 ± 5.1[1] (+67.52)	59.5 ± 3.9[1] (+62.71)	42.0 ± 3.0 (+13.27)	37.4 ± 5.1 (+5.47)	37.7 ± 5.5 (+4.15)
0.2 ppm	49.7 ± 5.0[1] (+33.89)	73.4 ± 5.7[1] (+85.24)	79.9 ± 3.1[1] (+118.55)	60.1 ± 3.1[1] (+62.91	45.5 ± 4.8 (+28.25)	38.1 ± 6.7 (+5.31)
0.5 ppm	61.7 ± 6.1[1] (+66.27)	75.4 ± 6.2[1] (+90.36)	88.1 ± 7.3[1] (+141.01)	60.3 ± 4.9[1] (+62.66)	50.1 ± 5.1[1] (+41.22)	34.1 ± 4.1 (-5.80)
1.0 ppm	70.1 ± 4.1[1] (+88.97)	89.7 ± 4.4[1] (+126.24)	102.6 ± 6.8[1] (+180.79)	100.1 ± 8.1[1] (+169.86)	89.0 ± 4.3[1] (+150.94)	54.1 ± 6.1[1] (+49.46)

*Values are mean ± S.D. of 10 individuals. Values in parentheses are % change over their respective controls.

[1]Values are significantly different at $P < 0.001$. The remaining values are not significant.

the greater will be the tissue damage and more of the damaged cells will have to be removed; this may be reflected in the increase in acid phosphatase activity.

With in a period of 15 days in the 0.1- ppm group and of 30 days in the 0.2-, 0.5- and 1.0 ppm groups, the crabs successfully acclimatized to the stress imposed, since the acid phosphatase, a marker of lysosomal activity was stabilized almost reaching the level of the control crabs. This suggests that the crabs exposed to sublethal concentrations ($\leq$1.0 ppm) of Methyl parathion for a period of 30 days might counteract the deleterious effects of the Methyl parathion at the tissue level.

Nagarathnamma (1982) observed histological damage in all the tissues of fish (Cyprinus) after exposure to sublethal concentrations of Methyl parathion. In all the groups, the tissues showed the first signs of histological recovery on day 10. With the beginning of recovery the activity of enzyme started declining and it gradually approached control values, thereby indicating that the major role of the enzyme during the damage rather than in the recovery process.

Several mechanisms have been suggested for the release of hydrolases from lysosomes. In the present case an increase in acid phosphatase after Methyl parathion exposure could be due to (i) alteration in osteoblasts resulting in more production and liberation of acid phosphatases (Cantarow and Schepartz 1967). (ii) proliferation of smooth endoplasmic reticulum in the parenchymatous cells, that leads to more production and release of microsomal enzymes resulting in an increased enzyme activity (Hart and Fouts 1965). (iii) peroxidation of lysosomal membrane leading to membrane breakdown or increase in permeability of lysosomal membrane or both results in liberation of acid phosphatases there by causing increased level (Novikoff 1961). (iv) degeneration and necrosis induced in tissues (Nagarathnamma 1982) results in release of acid phosphatases. Tissue necrosis and elevated acid phosphatase activity has also been observed by Onikieno (1963).

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