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Effect of carbaryl on esterases in the air-breathing fish *Channa punctatus* (Bloch)

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Abstract. Electrophoretic analyses of liver and muscle of *Channa punctatus* revealed that they contain atleast six and eight fractions of esterase, respectively. Characterization of esterase was made on the basis of their responses towards certain inhibitors. Liver esterase consists of acetylesterase and carboxyl esterases, whereas the muscle esterase has three types namely acetylesterase, carboxylesterase and cholinesterase. The liver and muscle of *C. punctatus* subjected to maximum sublethal concentration of carbaryl were electrophoretically analysed and it was found that both liver and muscle showed only three fractions of esterase.

Keywords. *Channa punctatus*; esterases; characterization; liver; muscle.

1. Introduction

Although a number of workers have studied the effects of industrial pollutants and agricultural pesticides on the activity of hepatopancreatic enzymes in many vertebrates (Bhattacharya and Mukherjee 1976; Thomas and Murthy 1976), they have not reported the findings of the electrophoretic analysis on these enzymes. The present study deals with the electrophoretic characterization of liver and muscle esterases of a freshwater air breathing fish *Channa punctatus* and the effect of carbaryl upon this enzyme.

2. Material and methods

Channa punctatus were collected from the local pond and acclimated to laboratory condition and feeding schedule keeping them in a large glass aquarium.

For separation and characterization of the enzyme, two groups of fish were reared: one in pesticide-free water and another in a medium containing 5 ppm concentration of carbaryl (N-methyl carbamate) (Union Carbide India Ltd) (sublethal level: Arunachalam *et al* 1984) for 15 days. The liver and muscle were separated after killing the fish in each group and homogenized in 40 % sucrose solution. The homogenate was centrifuged at 5000 g for 10 min and the supernatant was used as the enzyme source.

Disc gel electrophoresis was carried out as previously described by Balasubramanian *et al* (1982). Esterases were visualized by staining the solution containing 1 % 1-napthyl acetate and 1 % fast blue RR in phosphate buffer (M/15, at pH 7) at 37°C for 15 min. For characterizing the enzymes, gels were incubated in different inhibitors of varying strength solutions for 30 min and then stained for esterase. By comparing with the control gel, different types of esterases have been identified.

3. Results and discussion

Electrophoretic analyses of liver and muscle of *C. punctatus* revealed that they contain atleast six and eight esterase fractions, respectively. Based on the mobility of the enzyme fractions they have been designated as LEst-1 to LEst-6 and MEst-1 to MEst-8, respectively (figure 1a, c), indicating that the esterase of both liver and muscle of *C. punctatus* exists in multiple form. This is in accordance with the findings of Varma and Frankel (1980).

Effects of certain inhibitors on esterase fractions of liver and muscle of *C. punctatus* are presented in tables 1 and 2. Characterization of esterases was made on the basis of its responses towards certain inhibitors. Among the liver esterase fractions of *C. punctatus*, LEst-1 and LEst-2 were partially inhibited by silver nitrate and other chemicals like p-CMB, EDTA, eserine sulphate and organophosphate had no effect on these two fractions which are acetylesterases (Bergmann and Rimon 1958; Dickinson and Johnson 1978; Balasubramanian *et al* 1982). LEst-3, 4, 5 and 6 were inhibited by

Table 1. Effect of inhibitors on various fractions of esterases in the liver of *C. punctatus*.

Inhibitors	LEst 1	LEst 2	LEst 3	LEst 4	LEst 5	LEst 6
Control	+ +	+ +	+ + +	+ + +	+ +	+ +
p-CMB 10^{-2}M	+ +	+ +	+ + +	+ + +	+ +	+ +
EDTA 10^{-2}M	+ +	+ +	+ + +	+ + +	+ +	+ +
Organophosphate 10^{-4}M	+ +	+ +	–	–	–	–
Eserine sulphate 10^{-4}M	+ +	+ +	+ + +	+ + +	+ +	+ +
$AgNO_3$ 10^{-2}M	+/–	+/–	+	+	+/–	+/–
	acetyl	acetyl	carboxyl	carboxyl	carboxyl	carboxyl

– represents inhibition of enzyme activity; + 25% activity; + + 50% activity; + + + maximum activity or 100% activity.

Table 2. Effect of inhibitors on various fractions of esterases in the muscle of *C. punctatus*.

Inhibitors	MEst 1	MEst 2	MEst 3	MEst 4	MEst 5	MEst 6	MEst 7	MEst 8
Control	+ + +	+ +	+ +	+ +	+ +	+ + +	+ + +	+ +
p-CMB 10^{-2}M	+ + +	+ +	+ +	+ +	+ +	+ + +	+ + +	+ +
EDTA 10^{-2}M	+ + +	+ +	+ +	+ +	+ +	+ + +	+ + +	+ +
Organophosphate 10^{-4}M	+ + +	–	–	–	–	–	–	–
Eserine sulphate 10^{-4}M	+ + +	+ +	–	–	–	+ + +	+ + +	+ +
$AgNO_3$	+ +	+	+ +	+ +	+ +	+ +	+ +	+
	acetyl	carboxyl	choline	choline	choline	carboxyl	carboxyl	carboxyl

– represents inhibition of enzyme activity; + 25% activity; + + 50% activity; + + + maximum activity or 100% activity.

organophosphate, but not by p-CMB, EDTA and eserine sulphate and these fractions are the carboxylesterase (Ahmad 1976; Payne 1978; Varma and Frankel 1980).

Regarding the fish muscle esterases, MEst-1 is partially inhibited by silver nitrate and not by other chemicals. This fraction is acetylesterase (Bergmann and Rimon 1958; Dickinson and Johnson 1978; Balasubramanian *et al* 1982). MEst-3, 4 and 5 which were inhibited by organophosphate and eserinesulphate are probably cholinesterases (Augustinsson 1961; Holmes and Masters 1968) and MEst-2, 6, 7 and 8 which were inhibited by organophosphate but not by p-CMB and EDTA are carboxylesterase (Ahmad 1976; Payne 1978; Varma and Frankel 1980).

When fishes are exposed to pollutants whether industrial or agricultural, organs like liver and kidney are affected much (Brown 1970), since most of the toxic substances passing through these organs may cause histopathological and enzymatic changes.

The esterases of liver and muscle of fish reared in sublethal concentration of carbaryl are shown in figure 1. In liver, only three fractions *i.e.* LEst-3, 4 and 6 were identified, while others (LEst-1, 2 and 5) were not exhibited (figure 1b). In the fish muscle also there are only three fractions *i.e.* MEst-2, 6 and 8 (figure 1d), and the other fractions were not exhibited. Relative mobility of enzyme fractions, LEst-3, 4 and 6 and MEst 2, 6 and 8 in comparison with that of fish reared in pesticide-free water showed that these are carboxylesterases.

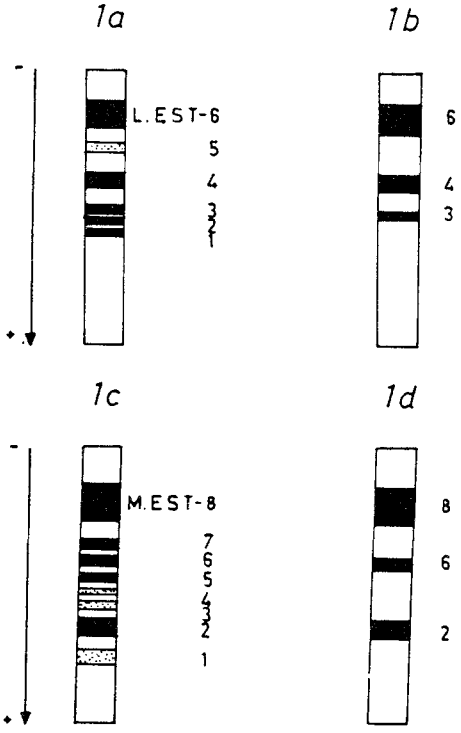

Figure 1. Zymogram pattern of esterases in *Channa punctatus*. (1a—Liver; 1b—Liver of treated animal; 1c—Muscle; 1d—Muscle of treated animal).

Therefore it appears that acetylesterases of liver and acetylesterases and cholinesterases of muscle in *C. punctatus* were inhibited. Such inhibition on esterases in different vertebrates due to certain pesticides has been reported (Mendoza and Hatina 1970). Industrial effluents like sodium sulphide, phenol, ammonia and copper sulphate have similar effects on liver esterase in *C. punctatus* and *Clarias batrachus* (Bhattacharya and Mukherjee 1976). The intensity of the activity of the esterases of liver and muscle of the treated fish was low when compared with that of fish reared in pesticide free-water.

Previous studies reported that carbaryl present in the medium decreased the growth rate of fishes (Arunachalam and Palanichamy 1982; Arunachalam *et al* 1984). Inhibition of acetylesterase and the consequent low activity observed in the present study may be the reasons for the decreased growth. Cholinesterase is an important enzyme in the excitable tissues of brain and muscle of teleost fishes (Nachamanson *et al* 1941; Weiss 1958; Lundin 1959). Inhibition of cholinesterase may lead to changes in the normal behaviour. This may be the reason for the erratic movements and imbalance in the fish exposed to pesticides (Arunachalam *et al* 1984).

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