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Regulation of Phosphorylases and Aldolases in Tissues of the Teleost (*Tilapia mossambica*) Under Methyl Parathion Impact

K. Siva Prasada Rao[1] and K. V. Ramana Rao

Department of Zoology, S.V. University P.G. Center, Kavali—524 202, A.P. India

Today, pesticides have occupied all segments of the environment (JENSEN et al. 1969) due to their use in field operations as potent and economically useful poisons (DATTA & DIKSHITH 1973). Consequently, untold hazards to several nontarget species like fish have been reported (HOLDEN 1972). The organophosphates are neurotoxic and they inhibit acetylcholinesterase activity with subsequent disruption of nervous functions (FEST & SCHMIDT 1973; RAINSFORD 1978), there by interfering with some of the vital physiological functions (RAO et al. 1980). Among them, energy metabolism has a key role as the animal is forced to expend more energy to mitigate toxic stress. Based on this, an attempt was made to study the sublethal effect of methyl parathion (a widely used organophosphorus pesticide) on the glycolytic path way, taking the key enzymes, phosphorylases and aldolases in selected tissues of the fish, Tilapia mossambica. This species is selected because of its wide availability and edibility in India.

MATERIAL AND METHODS

The fish were collected from unpolluted tanks of Tirupati, India. They were acclimatised to laboratory conditions for one week. During acclimation, water was changed daily and they were fed ground nut cake. Technical grade methyl parathion (O-O-dimethyl O-4-nitrophenyl thiophosphate) was obtained gratis from Bharat Pulverising Mills Pvt. Ltd., Bombay.

The standard stock solution of methyl parathion was prepared as described earlier (RAO & RAO 1979). Lethal concentration (LC) values were determined by probit analysis (FINNEY 1971) and the LC_{50} of methyl parathion to Tilapia mossambica was found to be 0.266 ppm for 48 hours. Hence the fish were exposed at 0.09 ppm concentration of methyl parathion for 48 hours, since this represents sublethal level which is normally one third to two thirds the LC_{50} value (KONAR 1969). Equal number of fish were kept in tap water without the addition of pesticide for the same period, served as controls.

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[1]Present address and all correspondence should be made to: Department of Neurology, The University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, U.S.A.

After the exposure, four tissues viz. muscle, gill, liver and brain were isolated and the homogenates were prepared quickly in 10% trichloroacetic acid for the estimation of total carbohydrates and glycogen (CARROL et al. 1956). For phosphorylase assay, the tissue homogenates were prepared in a medium containing sodium flouride (0.1M) and ethylenediamine tetraacetic acid (0.037M) at pH 6.5 as suggested by GUILLORY & MOMMAERTS (1962) to prevent enzymatic interconversions of the two phosphorylases. The supernatents were used for the assay. The glycogen phosphorylase (α-1,4- glycogen: orthophosphate glucosyl transferase.E.C 2.4.1.1) activity was estimated by the method of CORI et al.,(1955) in the direction of glycogen synthesis by determining the amount of inorganic phosphate (Pi) formed from glucose-1-phosphate. The Pi liberated was estimated by the method of FISKE & SUBBA ROW (1925).

For aldolase assay, the tissues were homogenised in cold distilled water and the supernatent was used for the enzyme assay. The aldolase (Fructose 1,6-diphosphate:D-glyceraldehyde-3-phosphate lyase.E.C 4.1.2b) activity was estimated by the method of BRUNS & BERGMEYER (1965) wherein the triosephosphates formed were estimated using 2,4-dinitrophenyl hydrazine and the activity was calculated according to BRUNS (1954). The protein content was determined by the method of LOWRY et al. (1951). The statistical correlations were made using student 't' test (BAILEY 1965).

RESULTS AND DISCUSSION

The carbohydrates were decreased significantly in the muscle (-52%; $P<0.001$), gill (-42%; $P<0.001$),liver (-37%; $P<0.001$) and brain (-28%: $P<0.001$) tissues of methyl parathion exposed (MPE) fish (Table 1). The glycogen content also decreased by 68% in muscle ($P<0.001$), 44% in gill ($P<0.001$), 57% in liver ($P<0.001$) and 38% in brain ($P<0.001$) tissues (Table 1), while the activity levels of aldolase showed an increasing trend (Table 2). The increase was 13%, 17%, 16% and 9% for muscle, gill, liver and brain tissues. The active phosphorylase 'a' increased significantly in the gill (22%; $P<0.025$), liver (28%; $P<0.001$) and brain (16%; $P<0.005$) tissues, but showed a significant decrease in the muscle tissue (26%; $P<0.005$) of the MPE fish. The inactive phosphorylase 'b' showed a significant decrease in the gill (38%; $P<0.005$), liver (43%; $P<0.001$) and brain (43%; $P<0.001$) tissues, while an insignificant decrease was observed in muscle phosphorylase 'b' (10%; NS) (Table 2).

The decrease in tissue carbohydrates signifies their utility possibly to meet the higher energy demands under methyl parathion stress condition. Of the carbohydrates, glycogen decreased rapidly indicative of its immediate utilisation by these tissues. It also suggests that such of those carbohydrates that are convertible or related to glycogen are being trapped extensively during methyl parathion impact. At the tissue level, the glycogen content decreased considerably in the muscle and liver tissues (68% and 57%). Since these tissues store glycogen, the greater breakdown of the animal starch in these tissues show active utilisation of the product for metabolic purposes. A similar alteration in

carbohydrate metabolism was reported in liver cytoplasm (OSTROUKHOVA 1965; YAKUSKO 1969).

Table 1. Levels of total carbohydrates and glycogen (mg/g wet wt.) in the tissues of control and methyl parathion exposed (MPE) fish.

Muscle		Gill		Liver		Brain	
Cont.	MPE	Cont.	MPE	Cont.	MPE	Cont.	MPE
			TOTAL CARBOHYDRATES				
3.886 ±0.242	1.863 ±0.249 (-52)[a]	1.058 ±0.152	0.615 ±0.060 (-42)[a]	65.03 ± 4.38	40.64 ± 2.68 (-37)[a]	0.864 ±0.058	0.621 ±0.022 (-28)[a]
			GLYCOGEN				
1.868 ±0.164	0.602 ±0.144 (-68)[a]	0.582 ±0.062	0.326 ±0.053 (-44)[a]	46.88 ± 2.04	20.36 ± 2.80 (-57)[a]	0.436 ±0.051	0.269 ±0.026 (-38)[a]

Values in parentheses are percent change over control (N = 6; Mean ± S.D) a = $P \leq 0.001$.

The increased phosphorylase 'a' activity in gill, liver and brain tissues of MPE fish confirms the active breakdown of tissue glycogen for metabolic purposes to meet the augmented stress condition. The increase in phosphorylase 'a' form suggests rapid conversion of the inactive phosphorylase 'b' into active phosphorylase 'a' form (HARPER et al. 1978), thereby quantitatively elevating phosphorylase 'a' to manifest its activity under toxic stress. Correspondingly, there is a decrease in the phosphorylase 'b' activity in the tissues of MPE fish. In contrast to other tissues, muscle phosphorylase 'a' decreased considerably (Table 2), which is not in consonance with the decrease in the muscle glycogen content (68%; Table 1). It can be inferred from this that phosphorylase 'a' should have been active in degrading glycogen in the initial stages of exposure, but when sufficient quantity of glycogen was exhausted, the phosphorylase 'a' activity should have been decreased indicating the operation of a regulatory mechanism to avoid further breakdown of glycogen, since the glycogen content cannot be utilised completely under any stress condition. The aldolases, that cleaves the hexoses into trioses in the glycolysis increased significantly in all the tissues of MPE fish further suggesting that the animal attempts to gear up the mobilisation of the reserves as an attempt to maintain high energy potentials.

From this, it can be inferred that there is a high energy demand during methyl parathion impact and the animal tries to withstand the toxic stress by operating some regulatory pathways.

Table 2. Levels of phosphorylase 'a','b' (μ moles of Pi formed/ mg protein/h) and aldolase (μ moles of FDP cleaved/mg protein/h) in the tissues of control and methyl parathion exposed (MPE) fish.

Muscle		Gill		Liver		Brain	
Cont.	MPE	Cont.	MPE	Cont.	MPE	Cont.	MPE
PHOSPHORYLASE 'a' (active)							
5.628 ±0.556	4.188 ±0.665	2.480 ±0.334	3.033 ±0.216	7.420 ±0.898	9.471 ±0.972	3.507 ±0.193	4.056 ±0.272
	(-26)[b]		(+22)[c]		(+28)[a]		(+16)[b]
PHOSPHORYLASE 'b' (inactive)							
2.406 ±0.548	2.157 ±0.636	1.194 ±0.152	0.738 ±0.178	4.039 ±0.375	2.307 ±0.716	2.920 ±0.340	1.651 ±0.206
	(-10)[d]		(-38)[b]		(-43)[a]		(-43)[a]
ALDOLASE							
47.76 ± 1.10	53.85 ± 2.01	5.847 ±0.425	6.874 ±0.342	5.007 ±0.242	5.821 ±0.189	14.20 ± 0.85	15.49 ± 0.35
	(+13)[a]		(+17)[b]		(+16)[a]		(+9)[c]

Values in parentheses are percent change over control (N = 6; Mean ± S.D). a = $P<0.001$; b = $P<0.005$; c = $P<0.025$; d = not significant.

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