

***In Vivo* Response of ATPases in Few Tissues of the Fish *Mystus vittatus* (Ham.) to the Synthetic Detergent Swacofix® E45 (ABS)**

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A great deal of literature is available, related to the toxicity of synthetic detergents (syndets) to fish and other aquatic organisms but there is a paucity of information about the mechanism of their toxic action, especially on ATP bound active transport, involving ATPase across the cellular membranes. Recently, VERMA et al. (1978a and b) studied the alterations in the membrane bound ATPase system in Channa punctatus, in vivo and in vitro exposed to various concentrations of different syndets.

In the present paper, results of the response of ATPase system to syndet Swascofix[®] E45 in a fish Mystus vittatus have been communicated.

MATERIALS AND METHODS

Fish Mystus vittatus with size range, 154-165 mm and weight from 95-108 gm collected from the neighbouring fresh water bodies were brought to laboratory and acclimatized for a period of one week. Fish were continuously exposed to sub-lethal concentrations of an anionic syndet Swascofix[®] E45 with alkyl benzene sulphonate (ABS) as active matter, supplied by Innosearch Pvt. Ltd., New Delhi for a period of 30 and 60 days respectively. LC(50) value for 96 hr of fish to the syndet was determined by adopting dilution techniques (STANDARD METHODS, 13th Ed. 1971). Then the sublethal concentrations, 1/2 1/3 and 1/6th of the LC(50) values were calculated. During experimentation fish were fed with an artificial diet to avoid any possible effect of starvation on enzyme activity. Controls were set side by side.

Brain, gill, liver and kidney tissues were dissected from untreated and treated fish after 30 and 60 days exposure and homogenized in cold 0.32 M sucrose solution containing 1 mM EDTA and 10 mM Imidazole buffer (pH 7.5). The homogenates were fractionated according the procedure described by KOCH (1969) and adopted by VERMA et al. (1978a and b). The 'B' fraction obtained at 13,000 g

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centrifugation, containing nerve ending particles, endoplasmic reticulum and mitochondria was diluted and divided into small aliquots for study.

ATPase activity was measured as per method described by FULLMAN et al. (1960), and FRITZ and HAMRICK (1966). Protein concentration was determined by the method of LOWRY et al. (1951), using bovine serum albumin as standard. Mg^{2+} ATPase activity was estimated when 1 mM ouabain was present in reaction mixture, containing Na^+ , K^+ and Mg^{2+} . $Na^+ - K^+$ ATPase is the total of ATPase activity minus Mg^{2+} ATPase activity.

The data obtained were subjected to student's t test (FISHER, 1950) for comparison with control.

RESULTS AND DISCUSSION

The response of ATPase system in brain, gill, liver and kidney from Mystus vittatus, chronically exposed to different sublethal concentrations of Swascofix^R E 45 was determined in vivo and the results so obtained have been compiled in Tables 1 and 2. The inhibition pattern of all ATPases assayed at all the concentrations was quite similar in all the four tissues. It was concentration dependant. The highest inhibition was noted in brain, followed by gill, kidney and liver, respectively. The similar pattern of ATPases inhibition was also noted by VERMA et al. (1978b) in fish Channa punctatus exposed in vitro to different sublethal levels of sodium lauryl sulphate (SLS).

It is observed that the lowest concentration in some cases enhance the enzyme activity to some extent. ROUFOGALIS (1973) also reported enhanced ($Mg^{2+} + Ca^{2+}$)-ATPase activity in microsomal fraction of bovine brain cortex, treated with sodium deoxycholate and lubrol-WX. KOCH et al. (1972) observed the stimulation in all the types of ATPases at lower concentrations of polychlorinated biphenyls in different tissues of Pimphales promelas. Further, VERMA et al. (1978a) reported an insignificant enhancement in the activities of $Na^+ - K^+$ and oligomycin-sensitive Mg^{2+} ATPases in fish Channa punctatus, in vivo exposed to syndets Idet 5L and Swanic 6L.

$Na^+ - K^+$ activated ATPase activity is associated with the active transport system which is responsible for the extrusion of Na^+ from animal cells and the accumulation of K^+ within these cells (SKOU 1961 and 1964), this enzyme is therefore, fundamental to such functions as the generation of membrane potentials,

TABLE 1

In vivo sensitivity of ATPases in the brain and gill of Mystus vittatus, exposed to Swascofix^R E45.

Exposure days	Conc. mg/L	Specific activity (μ moles Pi mg^{-1} protein hr^{-1})	
		Na ⁺ -K ⁺ ATPase	Mg ²⁺ ATPase
BRAIN			
15	Control	18.68 \pm 1.52	12.43 \pm 1.28
	0.880	8.15 \pm 0.95 (56.35)***	6.39 \pm 0.65 (48.54)**
	0.586	15.45 \pm 1.88 (17.27)	10.24 \pm 0.77 (17.65)
	0.352	19.88 \pm 1.91 (+6.41)	11.13 \pm 0.74 (10.46)
30	Control	19.17 \pm 1.55	11.54 \pm 0.83
	0.880	7.81 \pm 0.62 (59.25)**	5.73 \pm 0.49 (50.31)***
	0.586	15.03 \pm 1.12 (21.57)*	8.95 \pm 0.71 (22.45)*
	0.352	21.43 \pm 1.71 (+11.78)	11.95 \pm 0.87 (+3.18)
GILL			
15	Control	9.88 \pm 0.73	12.94 \pm 0.97
	0.880	5.51 \pm 0.61 (44.27)**	7.60 \pm 0.62 (41.24)**
	0.586	7.72 \pm 0.65 (21.88)	10.02 \pm 0.91 (22.55)*
	0.352	10.19 \pm 0.92 (+3.17)	13.74 \pm 1.13 (+6.21)
30	Control	8.96 \pm 0.69	14.12 \pm 1.02
	0.880	4.25 \pm 0.45 (52.52)**	6.94 \pm 0.59 (50.83)***
	0.586	7.06 \pm 0.67 (21.14)*	10.55 \pm 0.97 (25.26)*
	0.352	9.87 \pm 0.91 (+10.20)	15.15 \pm 1.37 (+7.32)

Values are the mean \pm S.E. (3 observations).

Values in parenthesis indicate percent inhibition and (+) indicates percent activation from control.

Values are significant at * $P < 0.5$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 2

In vivo sensitivity of ATPases in the liver and kidney of Mystus vittatus, exposed to Swascofix^R E45.

Exposure days	Conc. mg/L	Specific activity (μ moles Pi mg^{-1} protein hr^{-1})	
		$\text{Na}^{+} - \text{K}^{+}$ ATPase	Mg^{2+} ATPase
LIVER			
15	Control	6.11 \pm 0.37	11.85 \pm 1.76
	0.880	4.49 \pm 0.41(26.51)*	7.78 \pm 0.64(34.36)*
	0.586	4.94 \pm 0.48(19.22)*	9.31 \pm 0.87(21.43)
	0.352	6.55 \pm 0.45(+7.24)	13.18 \pm 1.05(+11.25)
30	Control	5.92 \pm 0.41	11.94 \pm 1.82
	0.880	4.17 \pm 0.38(29.52)*	7.68 \pm 0.52(35.65)*
	0.586	4.58 \pm 0.51(22.67)*	9.14 \pm 0.75(23.42)
	0.352	6.48 \pm 0.51(+9.46)	13.55 \pm 1.04(+13.54)
KIDNEY			
15	Control	18.97 \pm 1.88	17.10 \pm 1.93
	0.880	10.88 \pm 0.85(42.68)**	10.01 \pm 0.92(41.43)**
	0.586	16.75 \pm 1.27(13.77)*	13.95 \pm 1.11(18.43)
	0.352	20.35 \pm 1.76(+7.24)	19.44 \pm 1.87(+13.71)
30	Control	19.22 \pm 1.85	16.97 \pm 1.91
	0.880	11.15 \pm 1.54(41.98)**	10.20 \pm 0.95(39.87)**
	0.586	16.04 \pm 1.31(16.53)	13.72 \pm 1.00(19.15)
	0.352	21.38 \pm 1.97(+11.24)	18.66 \pm 1.76(+9.94)

Values are the mean \pm S.E. (3 observations).

Values in parenthesis indicate percent inhibition and (+) indicates percent activation from control.

Values are significant at * $p < 0.5$, ** $p < 0.01$.

and the regulation of cell volume and electrolyte composition. As such, inhibition of enzymatic activity by 'Swascofix[®] E45 could cause alteration of nerve transmission. Further, the reported action of Swascofix[®] E45 in these tissues might be related to the ability of this compound to alter the cellular membrane configuration by binding with lipid portion of the membrane. Since, ATPase is an integral component of the membrane, the active site of the enzyme would be altered. Thus, the energy needed to pump out the toxicant would like-wise be reduced.

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REFERENCES

- APHA, AWWA, and WPCF: Standard methods for the examination of water and waste water 13th edition, Washington, U.S.A. (1971).
- FISHER, R.A.: Statistical Methods for Research Workers, ed. Oliver and Boyd 11th ed. London (1950).
- FRITZ, P.J. and M.E. HAMRICK: Enzymol. Acta Biocat. 30, 57 (1966).
- KOCH, R.B.: J. Neurochem. 16, 145 (1969).
- KOCH, R.B., D. DESAIAH, H.H. YAP, and L.K. CUTKOMP: Bull. Environ. Contam. Toxicol. 7, 87 (1972).
- LOWRY, O.H., N.J. ROSEBROUGH, A.L. FARR, and R.J. RANDALL: J. biol. Chem. 193, 265 (1951).
- PULLMAN, M.E., H.S. PENEFSKY, A. DATTA, and E. RACKER: J. biol. Chem. 235, 3322 (1960).
- ROUFOGALIS, B.D.: Biochem. et Biophys. Acta 318, 360 (1973).
- SKOU, J.C.: In: Membrane Transport and Metabolism (A. Kleinzeller and A. Kotyk eds.). Academic Press, New York, 228 (1961).
- SKOU, J.C.: Progr. Biophys. 14, 131 (1964).
- VERMA, S.R., A.K. TYAGI, N. PAL, and R.C. DALELA: Arch. Environ. Contam. Toxicol. (In Press) 1978a.
- VERMA, S.R., A.K. TYAGI, N. PAL, and R.C. DALELA: Toxicol. Letter (Communicated) 1978b.