ESTERASES IN *AMBLYPHARYNGODON MOLA*

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ABSTRACT

Esterase patterns in the three tissues viz skeletal muscle, brain and liver tissue of *Amblypharyngodon mola* are discussed. Physostigmine, pCMB, paraoxon and DFP are used to classify the esterases into different categories.

INTRODUCTION

ESTERASES are a complex group of enzymes, catalysing the hydrolysis of organic esters. They have been used as important gene markers in a wide variety of organisms. The polymorphic nature of esterases of teleostean fishes has been studied only in a few genera and none from the Indian species have been reported. Studies on esterase polymorphisms in cyprinid fishes have been initiated to assess their role in environmental stress and the taxonomic relationships of this group of fishes. The present study describes the patterns and classification of esterases in three tissues of *Amblypharyngodon mola*.

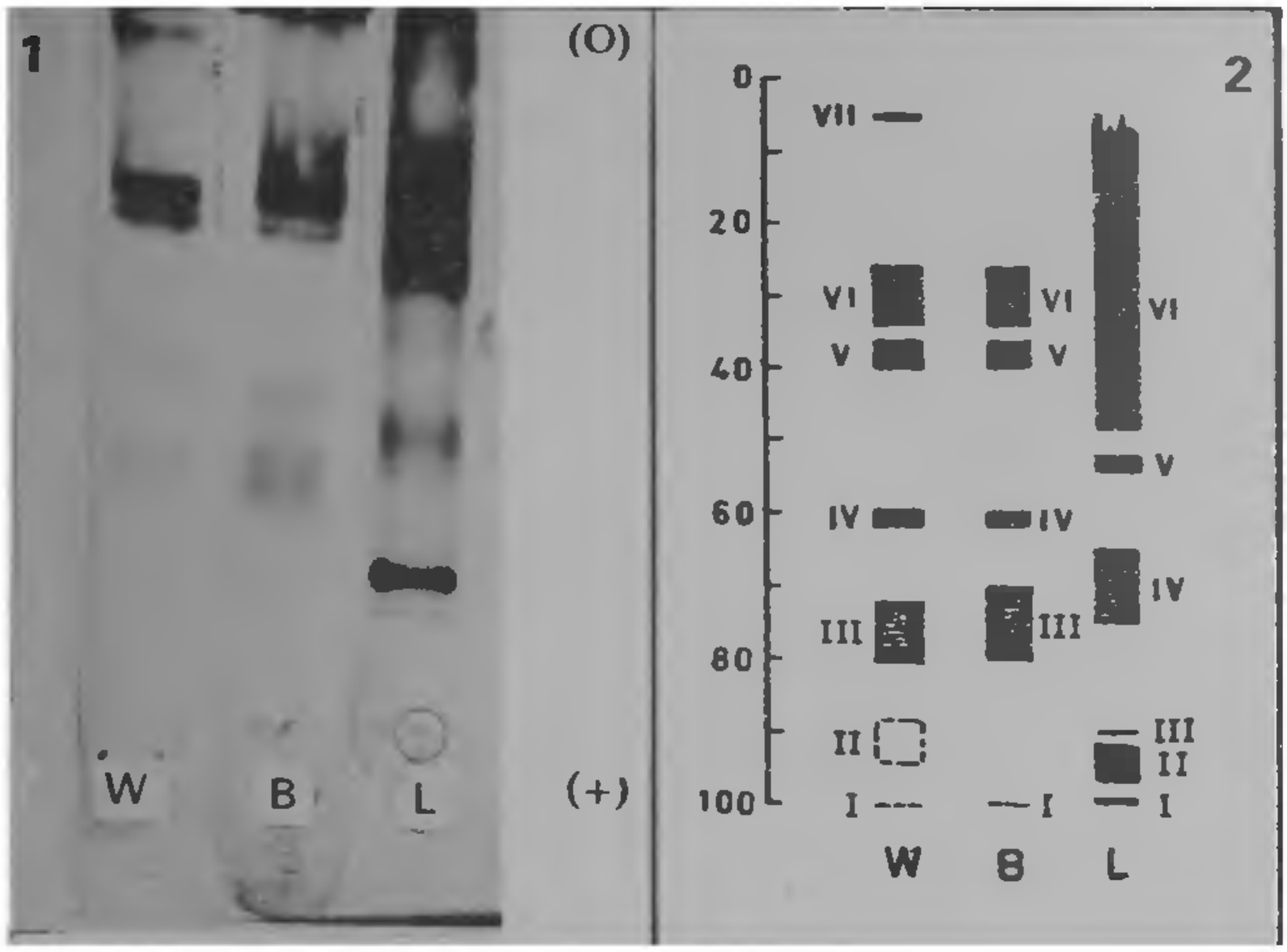
MATERIALS AND METHODS

Adult fishes were collected, immobilized and transported with ice to the laboratory from Nagaram tank about 15 km away. White skeletal muscle (epoxial muscle, dorsal to the lateral line avoiding the red muscle), brain and liver tissues were quickly removed from the animals. The muscle and brain had been frozen in the deep freezer until electrophoresed. Since stored liver tissue demonstrated the loss of some bands and alterations in their mobility, this tissue was processed immediately after dissection. Homogenates of the muscle (20%) and the brain (10%) were prepared in 0.1 M tris HCl buffer (pH 7.4) containing 0.9% NaCl. Liver gave better patterns with 1% homogenates in 0.9% NaCl solution. The homogenates were centrifuged at 2000 rpm at room temperature for 10 min. One ml of the supernatant was added to 1 ml of the 20% sucrose solution containing bromophenol blue as a marker dye. An aliquot of this mixture (0.1 ml) was added directly to the separating gel. Simplified disc gel electrophoresis[1] was carried out at 10°C on 7.5% polyacrylamide gels. Tris (0.05 M)–Glycine (0.38 M), buffer (pH 8.3) were used as the gel buffer. The same buffer (1:9 dilution) was used as the electrode buffer. An initial current of 2 mA per tube was used for the first 5 min followed by 4 mA per tube and the run was terminated when the tracking dye migrated to a distance of 5 cm from the origin. After rinsing out, the gels were stained for 20 min at 30°C following the procedures of Holmes and Masters[2]. One-naphthyl esters of acetate, propionate, butyrate and laurate were used for substrate specificity studies. EDTA (10^{-3} M), physostigmine (10^{-4} M), pCMB (parachloromercuribenzoate; (10^{-3} M), paraoxon (O, O-diethyl O-(4-nitrophenyl) phosphate; 2×10^{-5} M) and DFP (diisopropyl phosphorofluoridate; 10^{-4} M) were used for inhibition studies.

RESULTS AND DISCUSSION

The pattern of esterases found in the three tissues, white skeletal muscle(W), brain(B) and liver(L), are presented in figure 1. The zymograms of the same are presented in figure 2. In all, there are seven zones of activity in white muscle, five in brain and six in liver tissues. Results obtained on the substrate specificity of these zones and the inhibition of enzyme activity are presented in table 1.

Esterases are classified into four categories depending upon their substrate specificity and sensitivity to various inhibitors[3-5]. Carboxyl esterases (EC 3.1.1.1) hydrolyse the aliphatic esters and are sensitive to inhibition by the organophosphate compounds. Aryl esterases (EC 3.1.1.2) preferentially hydrolyse the aromatic esters and are inhibited by sulphydryl reagents such as pCMB. Choline esterases (including acetyl choline and pseudo choline esterase; EC 3.1.1.7 and 3.1.1.8) hydrolyse the choline esters and are sensitive to physostigmine as well as OP compounds. Acetyl esterases (EC 3.1.1.6), on the other hand, hydrolyse the acetate esters and are not affected by any of these inhibitors. Three new classes of esterases have been added to this list by Hart and Cook[6]: *ER* esterases which are resistant to inhibition by physostigmine, DFP and pHMB/pCMB; *ESe* enzymes susceptible only to



Figures 1 and 2. **1.** Electrophoretic patterns of esterases in three tissues. W = White muscle, B = brain, L = liver; (O) = origin, (+) = anode; **2.** Zymograms of the enzyme patterns in three tissues. The scale 0 to 100, indicate the R_f values (from origin (0) to the distance migrated by the tracking dye (100)). I – VII represent the zones of enzyme activity. The broken lines indicate weak activity or activity recorded with other substrates.

eserine sulphate and *Esdp* enzymes which are inhibited by pHMB/pCMB with sensitivity to organophosphate inhibitors. Haritos and Salamastrakis[7] suggest the inclusion of yet another group of enzymes, *CHsp* i.e. *Choline esterase-like* enzymes with sulphydryl groups essential for their activity. EDTA was routinely included in the present investigation as it was shown atleast to partially inhibit the activity of esterases in Tuna[8].

The pattern observed in the present study (table 1) indicates that muscle has two zones of activity, zones V and VII, which are inhibited both by physostigmine and OP compounds. They can be classified as choline esterases. All other zones in muscle can be classified as carboxyl esterases as they are inhibited by OP compounds but are not affected by other inhibitors. In brain, zone III is not inhibited by the OP compounds but it is affected by pCMB. This zone is classified as aryl esterases. Zone V is a choline esterase, while zones IV and VI are carboxyl esterases; the fate of zone I could not be determined as the band showed very feeble activity in stored tissues. In liver, zone I is not affected by any of the inhibitors; although with DFP the activity is feeble paraoxon, the other OP compound, has negligible influence on its activity. This band is also active when propionate and butyrate were used as substrates. Hence it can be classified as *ER* esterase following the model of Hart and Cook[6]. Zone V is a choline esterase, but all other bands inhibited by OP compounds alone can be classified as carboxyl esterases.

Susceptibility to eserine sulphate inhibition among the bands inhibited by DFP has been used as a criterion for identifying choline esterases by several authors[6,7,9–13]. There are, however, conflicting reports on the existence of choline esterases in teleostean fishes. Several workers[4,5,8,12,14], did not record choline esterases in their investigations on fishes. Others[6,7,13] however, reported weak choline esterase activity in their investigations. A prominent band at zone V in muscle, brain and liver and zone VII of muscle are recorded as choline esterase in the present investigation.

Neuraminidase was reported to reduce the migration of esterase bands[7,15]. It remains to be investigated whether hydrolysis of sialic acid moiety or any other group takes place in the enzyme of stored liver tissues, which recorded reduced migration rates of zones V and VI in the present investigation.

McCabe and Dean[8] used the substrate specificity to group esterases into acetyl, propionyl and butyryl esterases. Choudhury[16] suggested that an esterase capable of hydrolysing the ester with a given length

Table 1 *Results of substrate specificity and inhibition studies of different zones in the three tissues of Amblypharyngodon mola*

	White muscle							Brain						Liver					
Zones	I	II	III	IV	V	VI	VII	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Relative mobility	100	90	78	60	40	30	5	100	–	78	60	40	30	100	95	90	70	54*	48*
Substrate specificity:																			
∝-Naphthyl acetate	+	±	2+	3+	4+	5+	2+	±	–	3+	2+	4+	5+	+	4+	+	3+	2+	5+
∝-Naphthyl propionate	+	3+	3+	+	4+	5+	+	2+	–	2+	2+	5+	5+	2+	2+	3+	3+	+	5+
∝-Naphthyl butyrate	±	2+	+	+	3+	4+	±	±	–	+	+	2+	4+	+	±	2+	+	–	3+
∝-Naphthyl laurate	–	–	–	–	±	±	–	±	–	–	–	+	+	+	–	–	–	–	±
Inhibition studies:																			
DFP (10^{-4}M)	–	–	–	–	–	–	–	–	–	2+	–	–	–	±	–	–	–	–	–
Paraoxon (2×10^{-5}M)	–	–	–	–	–	–	–	±	–	2+	–	–	–	+	–	–	–	–	–
pCMB (10^{-3} M)	+	+	+	3+	4+	4+	+	±	–	–	2+	4+	4+	+	+	3+	+	+	5+
Physostigmine (10^{-4} M)	+	+	+	3+	–	4+	–	±	–	+	2+	–	4+	+	+	3+	+	–	5+
EDTA (10^{-3} M)	2+	2+	2+	3+	4+	5+	+	±	–	3+	2+	4+	5+	+	+	3+	2+	2+	5+
Classification	CE	CE	CE	CE	CHE	CE	CHE	CE	–	ArE	CE	CHE	CE	ER	CE	CE	CE	CHE	CE

Note: Relative mobility is shown as per cent migration of the zone from the origin to that of tracking dye; * relative mobility of zones V and VI was observed to decrease to 40 and 30 when stored livers were used for study. 5+ indicates high activity; + low activity; ± doubtful activity; – no activity, residual activity is shown in case of inhibition studies. Classification: CE – carboxyl esterases; CHE – choline esterase; ArE – aryl esterase; ER – enzymes resistant to OP compounds, physostigmine and pCMB.

of carbon chain in its acyl group can also hydrolyse the corresponding esters of shorter chain lengths but not *vice versa*. Certain esterase bands have been reported to hydrolyse preferentially the esters of propionate to those of acetate and suggested that utilization of substrates is an inconclusive means of classification[6,13]. However, it was suggested[7] that this disagreement can be avoided if one assumes that each esterase band is expected to show a maximal activity for a certain length of acyl moiety with low or no activity with shorter or longer acyl moiety esters. In the present investigation propionate appears to be a preferred substrate at zone II of muscle, zone VI of muscle and brain and zone III of liver. The activation of zone II in muscles with acetate in the presence of EDTA, physostigmine and pCMB needs further investigation.

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1. Clarke, *Ann. N. Y. Acad. Sci.*, 1964, **121**, 428.
2. Holmes, R. S. and Masters, C. J., *Biochem. Biophys. Acta*, 1967, **132**, 379.
3. Augustinsson, K. B., *The enzymes*, Vol. 4, Academic Press, New York, 1960, p. 521.
4. Augustinsson, K. B., *Ann. N. Y. Acad. Sci.*, 1961, **94**, 844.
5. Holmes, R. S., Masters, C. J. and Webb, E. C., *Comp. Biochem. Physiol.*, 1968, **26**, 837.
6. Hart, N. H. and Cook, M., *Comp. Biochem. Physiol.*, 1976, **B54**, 357.

7. Haritos, A. A. and Salamastrakis, S. S., *Comp. Biochem. Physiol.*, 1982, **B72,** 477.
8. McCabe, M. M. and Dean, D. M., *Comp. Biochem. Physiol.*, 1970, **34,** 671.
9. Lundin, J. J., *Experientia*, 1958, **14,** 131.
10. Ecobichon, D. J. and Kalow, K., *Can. J. Biochem.*, 1965, **43,** 73.
11. Knowles, C. O., Arurkar, S. and Hogan, J., *J. Fish Biol. Bd. Can.*, 1968, **25,** 1517.
12. Holmes, R. S. and Whitt, G. S., *Biochem. Genet.*, 1970, **4,** 477.
13. Varma, A. K. and Frankel, J. S., *Comp. Biochem. Physiol.*, 1980, **B65,** 267.
14. Metcalf, R. A., Whitt, G. S., Childers, W. F. and Metcalf, R. L., *Comp. Biochem. Physiol.*, 1972, **B41,** 27.
15. Simonarson, B. and Watts, D. C., *Comp. Biochem. Physiol.*, 1969, **31,** 309.
16. Choudhury, S. R., *J. Histochem. Cytochem.*, 1972, **20,** 507.

ANNOUNCEMENT

NATIONAL SEMINAR ON "ATOMIC INNER-SHELL IONIZATION AND ITS ANALYTICAL APPLICATIONS"

The above seminar is being organised to commemorate the Silver Jubilee of Punjabi University, Patiala, and the centenary of the discovery of photo-ionization during February 19–20, 1988. The main purpose of this seminar is to understand various processes and experimental techniques involved in the phenomenon of photo-ionization and ionization by charged particles of inner atomic shells. The following topics are being discussed: 1. Inner-shell photo-ionization; 2. X-ray fluorescence with particular reference yield to K-shell; 3. EDXRF studies at BARC/NRL, Srinagar; 4. Photon-atom scattering; 5. Heavy ion radiation damages in solid and single activation energy model; 6. Rutherford back-scattering and ion channelling technique; 7. Production and attenuation of bremsstrahlung; 8. PIXE: Analysis of biological samples, and 9. Compton scattering from inner-shell electrons.

In addition, two poster-sessions will be organized. Contributions to these poster-sessions are invited. Authors are requested to send an extended abstract of their papers.

Abstracts in duplicate may be sent to Dr B. S. Ghumman, Department of Physics, Punjabi University, Patiala 147 001, to reach him not later than 15 January 1988.