Asian Fisheries Science **9** (1997): 325-331.
Asian Fisheries Society, Manila, Philippines

https://doi.org/10.33997/j.afs.1997.9.4.010

Retinal Specific Esterases in Thirteen Freshwater Fish Species

V. RAJAIAH and V. LAKSHMIPATHI

Department of Zoology
Kakatiya University
Warangal - 506 009
India

Abstract

Distribution of retinal specific esterases were studied in 13 freshwater fish species belong-ing to the orders Siluriformes, Channiformes, Atheriniformes and Perciformes by thin layer poly-acrylamide gel electrophoresis. The inhibitor sensitivity of the enzymes towards paraoxon, pCMB and eserine was used to classify the individual zones of esterases.

Introduction

Retina of teleostean fishes has attracted the attention of comparative biochemists and geneticists because of the presence of a unique lactatedhydrogenase isozyme, the C4-isozyme (Markert et al. 1975; Whitt 1984; Rao et al. 1989). On a comparative basis, studies on distribution of other enzymes, especially of esterases, in this tissue are scanty. Esterases are a complex group of enzymes catalyzing the hydrolysis of carboxylic acid esters. These enzymes are ubiquitous in nature and are thought to be products of several polymorphic loci (Masters and Holmes 1975). Epigenetic modifications introduce further complexity to the multiple forms of these enzymes (Bellen et al. 1984). Within the fish groups, the enzymes have been used to characterize the tissue-specific and species-specific variations existing in enzyme forms (Holmes et al. 1968; Metcalf et al. 1972; Hart and Cook 1976; Verma and Frankel 1980; Haritos and Salamastrakis 1982; Reddy and Lakshimpathi 1988; Lakshimpathi and Reddy 1989). Most of these studies were made on tissues such as liver, intestine, skeletal muscle and brain. Few reports are available on the esterase patterns of whole homogenates of the eyes of fish. Ahuja et al. (1977) compared the esterase patterns of two xiphophorine fish species. Shaklee et al. (1974), Champion and Whitt (1976), and Hart and Cook (1977) compared the esterase patterns of developing embryos with those of adult tissues. Eye was one of the tissues examined by these authors. None of these studies were made on isolated retinas. Results obtained on the electrophoretic patterns of esterases of isolated retinas of 13 freshwater fish species belonging to four different orders are presented in this paper.

Materials and Methods

Fish were collected from ponds located within a radius of 40 km of Kakatiya University, Warangal, India. The fish were immobilized on ice, their eyeballs were cut open on ice-jacketed petri dishes, and the retinal layer along with the pigment epithelium was carefully removed. Retinas isolated from at least 10 eyeballs were blotted free of the attached fluids, weighed quickly to the nearest milligram, and homogenized (10%) in 0.01 M Tris-HCl buffer (pH 7.5), vortexed for one minute and centrifuged at 2,000 rpm in a clinical centrifuge at room temperature (30 $\pm$ 2°C). The supernatants were mixed with equal volumes of 20% sucrose containing 0.01% bromophenol blue as the tracking dye. Esterase patterns were separated on thin layer (1.5 mm thick) polyacrylamide gels (7.5%, 14 x 14 cm). The gel mixture was prepared according to the procedures of Clarke (1964). Gelling was allowed for 45 minutes and the samples were loaded directly onto the separating gel, overlaid with electrode buffer, and the gel plates were connected to the electrophoretic tank. Tris (0.05 M) glycine (0.38 M) buffer (pH 8.3) was used as the gel buffer. The same buffer diluted (1:9) with distilled water was used as the electrode buffer. A constant current of 20 mA for the first 15 minutes, followed by 40 mA for the rest of the run was supplied during electrophoresis. The electrophoretic run was terminated when the tracking dye reached a distance of 12 cm from the origin. Esterases were visualized on the gels by adopting the staining procedures of Holmes and Masters (1967) and as described by Lakshmipathi and Reddy (1989). Physostigmine (10^{-4} M) pCMB (para chloromercuribenzoate 10^{-3} M) and paraoxon (0,0 diethyl-[4-nitrophenyl] phosphate, 2 x 10^{-3} M) were used for inhibitor sensitivity studies. The gels were preincubated in the buffer containing the above concentrations of inhibitors for half an hour, then they were stained for esterase activity with 1-naphthyl acetate as the substrate. The inhibitors in concentrations that were used for preincubation were added to the stain buffer to prevent reversal of inhibitory action of the compounds.

Results

Results obtained on the electrophoretic patterns of esterases are presented in Fig. 1. The relative mobilities of individual esterase zones and their classification is given in Table 1. Naphthyl-1-acetate was used as a substrate to score the activity of non-specific esterases on gels. Based on the sensitivity of individual zones towards the inhibitors, paraoxon, pCMB and physostigmine, these non-specific esterases were further classified. Zones which were inhibited by paraoxon were classified as caboxylesterases (CE), zones inhibited by paraoxon and physostigmine were classified as choline esterases (ChE), zones inhibited by all the three inhibitors were (CHsp) choline esterase-like enzymes, and esterases ER were the zones which were not affected by any of the three inhibitors. A detailed method of classification of esterases in fishes was given in earlier studies from this laboratory (Reddy and Lakshmipathi 1988; Lakshmipathi and Reddy 1989, 1990).

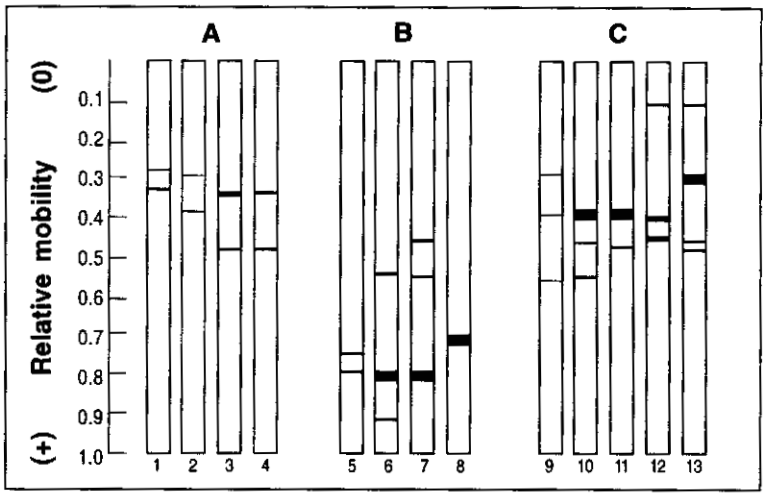

Fig. 1. Esterase patterns of retinal tissue of 13 freshwater fishes.
Siluriformes: 1. *Mystus vittatus*, 2. *Clarias batrachus*, 3. *Heteropneustes fossilis*.
Atheriniformes: 4. *Strongylura strongylura*
Channiformes: 5. *Channa marulius*, 6. *Channa orientalis*, 7. *Channa punctatus*, 8. *Channa striatus*
Perciformes: 9. *Chanda nama*, 10. *Etroplus maculatus*, 11. *Oreochromis mossambicus*, 12. *Glossogobius giuris* and 13. *Colisa fasciatus*.

0=Origin, + = Anode, ↓= Direction of current flow

A summary of the results presented in Fig. 1 and Table 1 indicate that, except for *Channa striatus* which had only one zone and *Colisa fasciata* which had four zones, fishes belonging to Siluriformes, Atheriniformes and Channiformes had two zones, and those of Perciformes had three zones. Oreochromis, the tilapia, had only two zones among the Perciforms. Electrophoretic mobilities of the zones indicate that fishes belonging to Channiformes had fast-moving zones (Rm > 0.54) while those of Siluriformes had slow-moving zones (Rm 0.27-0.47). Fishes belonging to Perciformes and Atheriniformes had zones of intermediate mobilities (Rm 0.32-0.54). Among the types of esterases found in different fishes, carboxylesterases are the predominant enzymes in almost all the fishes examined. These enzymes were not noticed in one fish, *Heteropneustes fossilis*. Cholinesterase (ChE) and cholinesterase-like enzymes were noticed in Channiformes, Siluriformes and in two fishes, *Glassogobius* and *Colisa* of Perciformes. Carboxylesterases are the only enzymes found in *Strongylura* and in three of the five fishes of Perciformes. ER esterases were noticed in two of the three species, *Mystus* and *Heteropneustes* of Siluriformes. Comparative study of the distribution of individual zones indicates that there is not a single zone found common in all the fishes. Within individual groups, Perciformes exhibited one zone (Rm 0.38) which had similar electrophoretic mobility in four of the five species. Since it was inhibited by the organophosphate compound (Paraoxon), it is a carboxylesterase in all the four fishes. Similarly, one zone (Rm 0.73) exhibited the same electrophoretic mobility in three species of Channiformes, but in *Channa marulius*, the zone was weak and disappeared in the presence of all the inhibitors. In *C. punctatus* and

Table 1. Esterase patterns of retina of 13 freshwater fishes.

Fish species	Rm	0.90	0.78	0.73	0.69	0.54	0.47	0.45	0.38	0.32	0.29	0.27	0.10
I. Siluriformes:													
1. *Mystus vittatus*										++ CE		+ ER	
2. *Clarias batrachus*									± CHsp		+ CE		
3. *Heteropneustes fossilis*								+ CHsp		++ ER			
II. *Atheriniformes*:													
4. *Strongylura strongylura*							+ CE			+ CE			
III. Channiformes:													
5. *Channa marulius*			+ CE	± CHsp									
6. *Channa orientalis*		± CHsp		+++ CE									
7. *Channa punctatus*				++ CE		+ ChE		+ CE					
8. *Channa striatus*					+++ CE								
IV. Perciformes:													
9. *Channa nama*						++ CE			+ CE			++ CE	
10. *Etroplus maculatus*						+ CE		+ CE	+++ CE				
11. *Oreochromis mossambicus*							+ CE		+++ CE				
12. *Glossogobius giurus*								++ CE	++ CE				+ ChE
13. *Colisa fasciatus*								+ ChE	± CHsp		+++ CE		+ ChE

Relative mobility (Rm) is given as a fraction of distance migrated by the zone compared to that of tracking dye.
Activity (visual observation of stain deposited on the gel) is shown as: +++ high activity; ++ moderate activity; + low activity and ± very low activity.
Abbreviations: CE - carboxyl esterases; ChE - choline esterases; CHsp - choline esterase-like enzymes, ER - esterases resistant to inhibitors.
Arrangement of fishes is in accordance with the handbook of Jayaram (1981).

C. orientalis, the zone is a carboxylesterase. None of the zones were found to be common in the fishes belonging to Siluriformes. The patterns presented indicate that the fast-moving zones in Channiformes distinguish the group from others. Similarly, the presence of three zones of intermediate mobility and the presence of carboxylesterases distinguish Perciformes from other groups. Among the Perciformes fishes, only *Colisa* exhibits variation from others in having four zones on the zymogram, three of which are Cholinesterase-like enzymes. Glossogobius is the only other fish which exhibited the presence of slow-moving cholinesterase.

Discussion

Earlier reports on eye-specific esterases in adults (Ahuja et al. 1977) as well as in embryos of fishes (Shaklee et al. 1974; Champion and Whitt 1976; Hart and Cook 1977) indicate the presence of two to three zones of esterases on the electrophoretic zymogram. Only carboxylesterases were reported in the eyes of xiphophorine fishes (Ahuja et al. 1977). Hart and Cook (1977), however, reported the presence of a cholinesterase during the differentiation of retinal epithelium in *Brachydanio* species. Eye-specific esterases found in adult fish of *Erimyzon sucetta* (Shaklee et al. 1974) and *Lepomis cyanellus* (Champion and Whitt 1976) were reported to appear in the homogenates of embryos during the differentiation of retinal epithelium. Our studies on the isolated retinas of fishes have indicated the presence of one to four zones of esterases on electrophoretic zymogram. The arrangement of the fishes in different groups presented in Table 1 is in accordance with the scheme adopted by Jayaram (1981). There seems to be some difference of opinion in the systematic positions of Channiformes, Atheriniformes and Perciformes. Jayaram followed the classification of Greenwood et al. (1966) in arranging Channiformes as a separate order between Gasterosteiformes and Synbranchiformes considering them as Preperciformes. Nelson (1984) placed Channiformes as one of the suborders of Preperciformes, Channoidei. The five fishes studied under Perciformes in the present investigation were grouped into three separate suborders. *Chanda nama, Etroplus maculatus* and *Oreochromis mossambicus* were placed under suborder percoidei. *Glossogobius* was placed in suborder Goboidei, and *Colisa* was placed in suborder Anabontoidei. Anabontoidei along with Channiformes were considered to be the early offshoots of Perciformes. *Strongylura* was placed under order Atheriniformes and super order Atherinomorpha, which was given the rank equal to that of Acanthopterygii by Greenwood et al. (1966). Nelson (1984) followed the classification of Rosen and Patterson (1969) in arranging Atherinomorpha as one of the series under superorder Acanthopterygii. The pattern of esterases of the five Perciformes fishes indicates that the three placed under suborder Percoidei exhibit similarities in having zones, which are all carboxylesterases and have intermediate mobilities. Similar patterns were noticed in *Strongylura*. Presence of a slow-moving cholinesterase in *Glossogobius* distinguishes this fish from other Perciformes. *Colisa* exhibits a distinct pattern in having two cholinesterases and one CHsp esterase and one carboxylesterase. With the exception of

Channa striatus, which has only one zone of carboxylesterase in its retina, all other fishes in Channiformes had one zone of Cholinesterase. The fishes are distinct in having fast-moving zones. The esterase patterns of the fishes examined alone are insufficient to comment upon their taxonomic divisions. A detailed survey on the genetic polymorphisms of these enzymes *vis-a-vis* studies on other enzyme loci are required to present a credible discussion on the taxonomic positions of these fishes. Nevertheless, the enzyme patterns indicated broad similarities in fishes arranged in specific groups and differed from the patterns of fishes whose taxonomic position is under debate.

The exact physiological roles of esterases and the natural substrates utilized by these enzymes in different tissues are not clearly understood. Within retinal tissue, retinols deesterified from their esters are oxidized and are bound to species-specific opsins (Wald 1967; Tsin and Santos 1985). Upon exposure to light, retinols are released from opsins and are reesterified and stored in pigment epithelium (Tsin 1986). Retinyl esters, thus, play a vital role in the biochemistry of vision. It can be suggested that esterases may have an important role in the hydrolysis and storage of retinylesters. The cyclic GMP phosphodiesterase was shown to be extractable in low ionic strength media and plays an important role in visual excitation of retina (Chabre 1985). Rod outer segment contains several proteins, interacting and participating in the visual cycle (Chabre 1985). These proteins include the transducins, kinases, rim-proteins and enzymes. Several non-enzymatic proteins like egasyn (Medda and Swank 1985), metmyoglobins (Breslow and Gurd 1962) and lipoproteins (Kaminisky and Dubois 1972; Whitmore and Gilbert 1972) are reported to be involved in esterolytic activity. It is possible that some of the membrane proteins present in rod cells may be involved in esterolytic activity. Esterification of retinols liberated from rhodopsins on photoexcitation was demonstrated to take place in the pigment epithelium (Tsin 1986).

Acknowledgements

V.R. is thankful to the Social Welfare Department of A.P. for fellowship. The facilities provided by the Head, Department of Zoology, Kakatiya University, are gratefully acknowledged.

References

Ahuja, M.R., M. Schwab and F. Anders. 1977. Tissue specific esterases in the Xiphophorine fish, *Platypoecilus maculatus Xiphophorus helleri* and their hybrid. Biochemical Genetics 15(5/8): 601-610.

Bellen, H., A. Van de Weghe, Y. Bouguet and L.F.M. Van Zutphen. 1984. Heterogeniety of Es-1 esterases in the rabbit (*Oryctolagus cuniculus*). Biochemical Genetics 22: 853-870.

Breslow, E. and F.R.N. Gurd. 1962. Reactivity of sperm whale metmyoglobin towards hydrogen ion and p-nitrophenylacetate. Journal of Biological Chemistry 237: 371-381.

Chabre, M. 1985. Trigger and amplification mechanism in visual phototransduction. Annual Review of Biochemistry 14: 331-360.

Champion, M.J. and G.S. Whitt. 1976. Differential gene expression in multilocus isozyme systems of the developing green sun fish. Journal of Experimental Zoology 196: 263-282.

Clarke, J.T. 1964. Simplified disc (polyacrylamide gel) electrophoretic technique. Annuals of New York Academy of Sciences 124: 428-436.

Greenwood, P.H., D.F. Roosen, S.H. Weitzman and G.S. Myers. 1966. Phyletic studies of teleostan fishes with a provisional classification of living forms. Bulletin of the American Museum of Natural History 131: 339-456.

Haritos, A.A. and S.S. Salamastrakis. 1982. A comparison of muscle esterases in the fish genus *Trachurus* by vertical gel electrophoresis. Comparative Biochemistry and Physiology 72B: 477-480.

Hart, N.H. and M. Cook. 1976. Comparative analysis of tissue esterases of the zebra danio (*Brachydanio rerio*) and the pearl danio (*B. albolineatus*) by disc gel electrophoresis. Comparative Biochemistry and Physiology 54B: 357-364.

Hart, N.H. and M. Cook. 1977. Esterase isozyme patterns in developing embryos of *Brachydanio rerio* (Zebra danio), *B. albolineatus* (Pearl danio) and their hybrids. Journal of Experimental Zoology 199: 109-128.

Holmes, R.S. and C.J. Masters. 1967. The developmental multiplicity and isozyme status of cavian esterases. Biochimica et Biophysica Acta 132: 379-399.

Holmes, R.S., C.J. Master and E.C. Webb. 1968. A comparative study of vertebrate esterase multiplicity. Comparative Biochemistry and Physiology 26: 837-852.

Jayaram, K.C. 1981. The fresh water fishes of India, Pakistan, Bangladesh, Burma and Sri Lanka - a handbook. In: Zoological survey of India (ed. Z.S.I. Director). Calcutta, India.

Kaminisky, M. and P. Dubois. 1972. Are serum lipoproteins esterases? European Journal of Biochemistry 29: 175-183.

Lakshmipathi, V. and T.M. Reddy. 1989. Esterase polymorphism in muscle and brain of four fresh water fishes belonging to the family cyprinidae. Journal of Applied Ichthyology 5: 88-95.

Lakshmipathi, V. and T.M. Reddy. 1990. Comparative study of esterases in brains of the vertebrates. Brain Research 521: 321-324.

Markert, C.L., J.B. Shaklee and G.S. Whitt. 1975. Evolution of a gene. Science 189: 102-114.

Masters, C.J. and R.S. Holmes. 1975. Isoenzymes and phylogeny. In: Haemoglobins, isoenzymes and tissue differentiation (eds. A. Neuberger and E.L. Tatum), pp. 61-128. North Holland Research Monographs, Frontiers of Biology Vol. 42. North Holland, Amsterdam.

Medda, S. and R.T. Swank. 1985. Egasyn, a protein which determines the subcellular distribution of B-glucuronidase has esterase activity. Journal of Biological Chemistry 266: 15802-15808.

Metcalf, R., G.S. Whitt, W. Childers and R. Metcalf. 1972. A comparative analysis of the tissue esterases of the white crappie (*Pomoxis annularis* Refinesque) and black crappie (*Pomoxis nigromaculatus* lesveur) by electrophoresis and selective inhibitors. Comparative Biochemistry and Physiology 41: 27-38.

Nelson, J.S. 1984. Fishes of the world. Wiley Interscience Publication (John Wiley & Sons), New York.

Rao, M.K., B.K. Padhi and A.R. Bakshi. 1989. Lactate dehydrogenase isozymes in fifty two species of teleostean fishes. Taxonomic significance of LDH-C gene expression. Biochemical Systematics and Ecology 17: 69-76.

Reddy, T.M. and V. Lakshmipathi. 1988. Esterases in *Amblypharyngodon mola*. Current Science 57: 24-27.

Rosen, D.E. and C. Patterson. 1969. The structure and relationship of the paracanthopterygian fishes. Bulletin of the American Museum of Natural History 141(3): 357-474.

Shaklee, J.B., M.J. Champion and G.S. Whitt. 1974. Developmental genetics of teleosts. A biochemical analysis of lake chubsucker ontogeny. Developmental Biology 38: 356-382.

Tsin, A.T.C. and F.R. Santos. 1985. The 3,4-didehydroretinal chromophore of gold fish porphyropsin. Journal of Experimental Zoology 235: 181-186.

Tsin, A.T.C. 1986. Enzymatic esterification of exogenous retinal and 3,4-didehydroretinol in the retinal pigment epithelium. Journal of Experimental Zoology 238: 311-317.

Verma, A.K. and J.S. Frankel. 1980. A comparison of tissue esterases in the genus *Barbus* by vertical gel electrophoresis. Comparative Biochemistry and Physiology 65B: 267-239.

Wald, G. 1967. Molecular basis of visual excitation - Nobel Prize address. Science 162: 230-239.

Whitmore, E. and L.I. Gilbert. 1972. Haemolymph lipoprotein transport of juvenile hormones. Journal of Insect Physiology 18: 1153-1154.

Whit, G.S. 1984. Genetic, developmental and evolutionary aspects of the lactate dehydrogenase isozyme system. Cell Biochemistry and Function 2: 134-139.

Manuscript received 30 October 1995; accepted 29 November 1996.