ELECTROPHORETIC PATTERNS OF ESTERASES OF SURFACE MUCUS OF THIRTEEN FRESH WATER FISHES

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Abstract

Electrophoretic patterns of esterases of skin surface secretions of thirteen freshwater fish species were studied by subjecting them to thin layer polyacrylamide gel electrophoresis. Sensitivity of individual esterase zones to three inhibitors, viz., pCMB, paraoxon and physostigmine, was tested. The patterns were found to be species-specific as well as group-specific. The possible origin of esterases in mucus is discussed.

Fish skin is overlaid by a slimy mucus secretion, which disspates slowly but constantly into the surrounding medium. The viscosity of mucus secretion protects the fish from mechanical damage as well as from parasitic invasion (Wilson 1976). Mucus contains mainly the glycoproteins (Fletcher & Grant 1969). A host of other components like phospholipids (Mittal & Nigam 1986), antimicrobial agents (Skarnes & Watson 1967) and enzymes like lysozymes (Fletcher & White 1973) were shown also reported to be present in mucus secretions. The mucus components were shown to be species-specific (Barry & O'Rourkee 1959, Herzberg 1978). Electrophoretic patterns of esterases of surface mucus were shown to be useful in identifying the *Tilapia* species (Herzberg, 1978) and in establishing their genetic relatedness (Wu & Wu, 1983). The species-specific character of fish surface mucus assumes importance in identifying the genetic stocks without actually sacrificing the fish or damaging it, as it happens when tissue samples or serum samples are chosen for such identification. In spite of this advantage, work on fish mucus is scanty, especially in wild species. The present paper describes the esterase patterns of surface mucus of thirteen freshwater fish species.

Materials and Methods

Fishes were collected from tanks (ponds) located within the radius of 40 km from Kakatiya University, Warangal (India). They were immobilized on ice and the mucus was collected from the surface of the skin with the help of sterile cotton swabs of uniform dimension. After saturation with mucus, cotton swabs were placed in ice-jacketed centrifuge tubes. An aliquot, 0.5 ml of 0.1 M Tris-HCI buffer (pH 7.4) containing 0.9% NaCl, was added to the tubes and the contents were mixed on a vortex mixer at high speed for one minute. The cotton swabs were squeezed out of the mucus by pressing them against the walls of the centrifuge tubes with the help of a glass rod. The mixture, after removing the cotton swabs, was centrifuged for 20 minutes at 2000 rpm in a clinical centrifuge to remove the suspended particles and scales. The clear supernatant was mixed with equal volumes of 20% sucrose solution containing 0.01% bromophenol blue as a tracking dye. An aliquot of 0.1 ml of this mixture was loaded directly onto the separating gel for electrophoresis.

The thin layer (1.5 mm thick) polyacrylamide slab gels (7.5%) were used for electrophoresis. The gel mixture was prepared in accordance with the procedure of Clarke (1964) and poured into the glass plates separated by 1.5 mm thick spacers. The gelling process was allowed for about 45 minutes. As the stacking gel gave no added advantage to the separation of the esterase zones, the step was avoided. After loading the samples onto the separating gel, the remaining space was filled with electrode buffer and the gel plates were connected to a vertical electrophoresis chamber. Tris (0.05 M) - Glycine (0.38) buffer (pH 8.3) was used as the gel buffer. The same buffer diluted (1:9) with distilled water was used as electrode buffer. A constant current of 20 mA was supplied during the first fifteen minutes, after which the current was raised to 40 mA for the rest of the run. The current supply was terminated when the tracking dye reached a distance of 12 cms from the origin. 1-Napthyl acetate was used as the substrate; pCMB (para chloromercuribenzoate, 10^{-3}M), paraoxon [0,0-Diethyl-(4-nitrophenyl)phosphate, 2×10^{-5}M] and physostigmine (10^{-4}M) were used as inhibitors. Staining procedures were the same as given by Lakshmipathi & Reddy (1989).

Inhibitors were purchased from Sigma Chemical Company (USA).

All other reagents were of analytical grade, purchased locally either from Loba Chemicals, S.D. Fine Chemicals or from Qualigens.

Results and Discussion

Results obtained on the pattern of esterases are given in Fig. 1. The relative mobilities of individual zones and their classification are given in Table 1. Detailed discussion on the classification of esterases was given earlier (Lakshmipathi & Reddy 1989). In summary, esterase zones inhibited by the organophosphate compound, paraoxon, were classified as carboxyl esterases (CE); those inhibited by paraoxon and physostigmine as choline esterases (ChE), and the enzymes which were not affected by any of the inhibitors were classified as ER esterases. Those which were inhibited by all the three inhibitors were classified as CHsp (cholines esterases like enzymes). The fishes chosen belonged to nine families and four orders (Jayaram 1981).

The electrophoretic patterns of esterases of the mucus secretions of the fishes examined (Table 1) indicated both species-specific and group-specific patterns. Fishes belonging to order siluriformes exhibited only slow moving zones (RM $\leq$.50). Two of the three fishes, i.e., *Mystus* and *Clarias*, each exhibited two slow-moving zones (Rm < 0.45). In *Heteropneustes fossilis* a third zone with Rm 0.52 was observed. The slow-moving zone of each of these fishes is an ER esterase. Carboxyl esterase was found only in *Mystus,* but the other two fishes contained the CHsp esterases. *Strongylura strongylura,* the only species examined under the order Atheriniformes, exhibited only one zone which was a carboxyl esterase. It is a moderately fast-moving zone (Rm 0.54). Among the four fishes examined under the order Channiformes, *Channa marulius* has two fast-moving zones, a choline esterase-like enzyme (Rm 0.70), a carboxyl esterase (Rm 0.60) and one slow-moving choline esterase (Rm 0.42). *Channa orientalis* has three zones in its mucus, a fast-moving carboxyl esterase (Rm 0.60) and two choline esterases (Rm 0.52 and 0.42). *Channa punctatus* has two slow-moving zones, a carboxyl esterase (Rm 0.48) and a choline esterase (Rm 0.42). *Channa striatus* has only one zone, the fast-moving carboxyl esterase (Rm 0.60). The fast-moving carboxyl esterase (Rm 0.60) exhibits high activity in all the three *Channa* species in which it is present. With the exception of one zone (Rm 0.60) in *Chanda nama*, mucus esterases of all the fishes

under the order Perciformes are slow-moving (Rm values < 0.50). *Chanda nama* has four zones in its mucus, one CE (Rm 0.60), one ER (Rm 0.46) and two ChE (Rm 0.29 and 0.25). *Etroplus maculatus* contains three zones: one CHsp (Rm 0.48), one ER (Rm 0.38) and one CE (Rm 0.32). *Oreochromis moassambicus*, the tilapia, exhibits three zones, two carboxyl esterases (Rm 0.46 and 0.38) and one ER esterase (RM 0.42). *Glossogobius giuris* has three zones (Rm 0.46, 0.42 and 0.38), all of which are resistant to inhibition by the three inhibitors (i.e., ER esterases). There is only one zone (Rm 0.32) in *Colisa fasciatus*, which is a carboxyl esterase. The slow-moving zones (Rm 0.38) found in *Etroplus, Oreochromis, Glossogobius* and the single zone found in *Colisa* exhibit high activity amongst the esterase zones found in the fishes of Perciformes.

A survey of distribution of different classes of esterases in the four orders indicates that in Siluriformes ER esterases and choline esterase-like enzymes are present. Only one zone in Mystus is a carboxyl esterase. Channiformes are characterized by the presence of caboxyl and choline esterase (like) enzymes. Carboxyl esterase, which is a fast-moving zone (Rm 0.60) in three of the fourn species of Channiformes, exhibits high activity, while the slow-moving choline esterases exhibit low activity. Fishes belonging to the order Perciformes exhibit predominantly carboxyl and ER esterases. Only two fishes, *Chanda nama* and Etroplus maculatus have choline esterase (like) zones in their mucus. These zones exhibit low activity. The slow-moving carboxyl or ER esterases found in *Etroplus, Oreochromis, Glossogobius* and *Colisa* exhibit high activity. The single species studied under Atheriniformes exhibits a single zone of carboxyl esterase. The patterns of esterases thus exhibit not only species-specific but also order-specific patterns.

The physiological function of non-specific esterases found in almost all tissues of various animal groups is not very clear. Although synaptic functions of choline esterases are well-known, these enzymes are also known to hydrolyze amide bonds, peptide bonds, and may have wider functions, hithero unknown (Balasubramanian & Bhanumurthy 1993). Carboxyl esterases were shown to be associated with juvenile hormone hydrolysis in insects (Whitmore et al. 1972). Lipoproteins associated with or transporting lipids have been found to demonstrate esterolytic activity in insects (Whitmore & Gilbert 1972), as well as in vertebrates (Grafius et al. 1971, Kaminski &

Dubois, 1972). The unicellular glands present in the skin of fishes, viz., the goblet cells, the club cells, the sacciform cells and ionocytes, are known to contribute to the secretion of surface mucus (Mittal & Nigam 1986). Secretions from these cells are usually carried to the surface of the skin in the form of mebrane bound vesicles, whiche are then extruded on to the surface of the skin. The membrane segments of these vesicles, voided onto the surface of skin, contribute to the lipoprotein fractions (Whitear 1976, 1977), as well as to the lipid fractions found in mucus (Mittal & Nigam 1986). Esterases present in the surface mucus of the skin of fishes may thus represent either the lipoprotein fragments of these vesicles exhibiting esterolytic activity or may be part of the enzyme complexes released by the gland cells for the purpose of defense against parasitic invasions. Lysozymes (Fletcher & Grant 1969), enzymes exhibiting chitinase activity (Fletcher & White 1973) as well as interferons (Dekinkelin & Dorson 1973) were shown to be released into mucus in response to bacterial and viral invasions (Wilson 1976).

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Table 1. Electrophoretic mobility and classification of esterases found in the mucus of thirteen fresh water fish species.

Fish species	Zones	1	2	3	4	5	6	7	8	9	10	11
	Rm	0.70	0.60	0.54	0.52	0.48	0.46	0.42	0.38	0.32	0.29	0.25
Order:	Siluriformes											
Family I:	Bagridae											
1.	Mystus vittatus								+++ CE			++ ER
Family II:	Clariidae											
2.	Clarias batrachus							+ CHsp			+ ER	
Family III:	Heteropneustidae											
3.	Heteropneustes fossilis				+ CHsp				+ CHsp	++ ER		
Order:	Atheriniformes											
Family IV:	Belonidae											
4.	Strongylura strongylura			++ CE								
Order:	Channiformes											
Family V:	Channidae											
5.	Channa marulius	++ CHsp	+++ CE					+ ChE				
6.	Channa orientalis		+++ CE		+ ChE			+ ChE				

Table 1 (continued)

Fish Species	Zones	1	2	3	4	5	6	7	8	9	10	11
	Rm	0.70	0.60	0.54	0.52	0.48	0.46	0.42	0.38	0.32	0.29	0.25
7. Channa punctatus						+ CE		+ ChE				
8. Channa striatus			+++ CE									
Order: Perciformes												
Family VI: Chandidae												
9. Chanda nama			++ CE				+ ER			+ ChE	+ ChE	
Family VII: Cichlidae												
10. Etroplus maculatus						+ CHsp			+++ ER	++ CE		
11. Oreochromis mossambicus							+ CE	+ ER	+++ CE			
Family VIII: Gobiidae												
12. Glossogobius giuris							+ ER	+ ER	+++ ER			
Family IX: Belontidae												
13. Colisa fasciatus										+++ CE		

NOTE:

1. Activity (Visual observation of stain deposited on the gel) is shown as (+++) high activity, (++) moderate activity, (+) low activity.

2. ER (Esterases resistant to inhibitors), ChE (Chlorine esterases), CE (Carboxyl esterases), CHsp (Chlorine esterase like enzymes).

3. Arrangement of the fish species under different orders is in accordance with the arrangement given in Jayaram (1981).

4. Rm (Relative mobility) is given as a fraction of the distance migrated (from the origin) by the individual zones in comparison to that of the tracking dye.

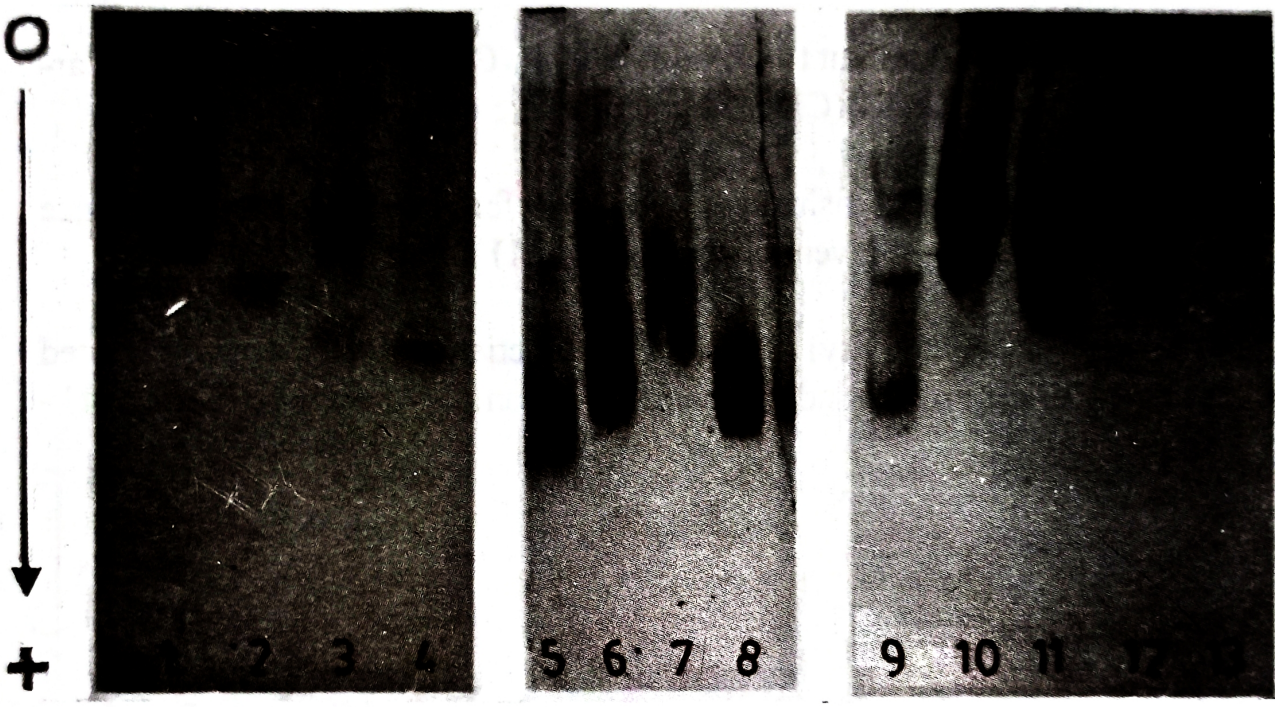

Fig. 1. Electrophoretic patterns of mucus of thirteen freshwater fishes.

1. *Mystus vittatus*, 2. *Clarias batrachus*, 3. *Heteropneustes fossilis*, 4. *Strongylura strongylura*, 5. *Channa marulius*, 6. *C. orientals*, 7. *C. punctatus*, 8. *C. striatus*, 9. *Chanda nama*, 10. *Etroplus maculatus*, 11. *Oreochromis mossambicus*, 12. *Glossogobius giuris* and 13. *Colisa fasciatus*.

(O = Origan; + = Anode; ↓ = Direction of current flow)

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