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STERASE VARATIONS IN THE *ARIAN ATER* (blackslug) PHYLUM: MOLLUSCA

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ABSTRACT

Esterases from the six tissues (viz; Ctenidia, Hepatopancreas, Intestine, Mantle, Foot and tentacles) of the slug *Arionater* collected from the fields of komatipally village, extracted and analyzed using Polyacrylamide gel electrophoresis (PAGE) and nine esterase bands were detected in six tissues. The esterase bands were distributed in five main zones which could be classified as CE, AcE, ArE, Esdp and ER depending on the inhibition properties and relative mobility of esterase bands.

KEYWORD:- Esterases, Ctenidia, Hepatopancreas.

INTRODUCTION

Molluscan esterases have been studied by several investigators, e.g., Pulmonata (Malek and File, 1971; Haites et al., 1972; Oxford, 1973a-c; Wahren and Tegelstrom, 1973; Selander and Foltz, 1981; Mulvey et al., 1987), Bivalvia (Reid, 1968; Reid and Dunnill, 1969), and Prosobranchia (Talesa et al., 1990; Wang,1994).

As far as we know no previous electrophoretic work has been carried out esterases of slug (*Arionater*). *Arion ater* was used as grease to lubricate wooden axle-trees or carts in Sweden. His use is documented since at least the 18th century. Although Black slugs are edible (if somewhat unappetising), their consumption is inadvisable partly due to the poisons that are used to control their population in urban areas where they are considered pests, but also as they are carriers of French heartworm. Valbonesi *et al.*, (2003) worked on the Characterization of cholinesterase activity in three bivalves inhabiting the North Adriatic Sea and their possible use as sentinel organisms for biosurveillance programmes. Sunil and Ajay

(2010) noticed the Molluscicidal activity and enzyme inhibition of *Cryptostagi grandiflora* plant to nervous tissue of snail *Lymnaea acuminata.* Cuna *et al*., (2011) studied the extensive microgeographical shell polymorphism in a planktotrophic marine intertidal snail. Dunithan *et al*., (2012) studied the morphology of *Elimia livescens* (Mollusca: Pleuroceridae). Schilthuizen *et al*., (2012) observed the ecology of shell shape difference in chirally dimorphic snails. Sepulveda and Ibanez (2012) reported the clinal variation in the shell morphology of intertidal snail *Acanthina monodon* in the southeastern Pacific ocean. Torres *et al*., (2011) studied the geographic phenetic variation in the golden apple snail, *Pomacea canaliculata* (Ampullariidae) based on geometric approaches to morphometrics.

MATERIALS AND METHODS

Aron ater was collected from fields of komatipally village, located about 30km from Kakatiya University campus and dissected the tissues and processing Ctenidia-5%, Hepatopancreas-2% , Intestine-2% Mantle-10% and Foot -30%, Tentacles-5%. Homogenates were kept over ice for 30 minuts and centrifuged at 2000rpm for 10 minuts at room temperature and supernatant used for electrophoresis. Vertical slab gel electrophoresis (14x14cm separated by 2mm thick spacers) was carried out using 7.5% polyacrylamide gel (containing Glycine-28gr/1Lit and Tris-Hcl 6gr/1 Lit) pH 8.3 was used as gel buffer and a 1:9 dilution of the same was used as tank (electrode) buffer. Aqueous bromophenol blue (Final concentration 0.05%) was used as traking dye. The run was carried out a constant current of 20m Amps was supplied for the first fifteen minutes, after which the current was raised to 40m Amps and terminated after 45 minuts. The gels was stained at room temperature following the procedure of Redfield and Salini (1980). One naphthyl esters of acetate, was used for substrate study, pCMB (parachloro mercuribenzoate) (10^{-4}M), Paraoxon (0.0 – diethyl - (4-nitrophenol) Phosphate ($2x10^{-5}$M), Physostigmine (10^{-4}M), EDTA (10^{-3}M) and $AgNO_3$ (Silver nitrate, 10^{-2}M) were used in inhibitor sensitivity studies. The gels were preincubated in the buffer containing the above concentrations of inhibitors for half an hour, following which they were stained for esterase activity using 1-naphthylacetate as the substrate. To prevent reversal of inhibition .Since the tissue samples of fresh water mussel were electrophoresed in separating gels under identical conditions in the zymogram, bands were serially numbered with the fastest migrating fraction getting the first number and lowest the last. Taking Rm value and proximity of bands into consideration .The enzyme activity areas were broadly categorized into different zones.

RESULTS AND DISCUSSION

The pattern of esterases were observed in the six tissues of *Arion ater*. Their relative mobility and inhibitor sensitivity are given in the Table 3.1 which summarize different classes of esterases that were found in each of the six tissues of the *Arion ater*. The relative proportion of different classes of esterase enzymes are contributing to the total esterase activity.

Ctenidia: There are four esterase active zones on the zymogram with Rm values .90, .83, .65, and .58. Among these, the zone with Rm value .90 and .83 both inhibited by Paraoxon and AgNO3. So, these zones are considered as CE esterases. The remaining two zones with Rm value .65, .58 were not inhibited by any inhibitors used. Hence, these two zones are considered as ER esterases.

Hepatopancreas: This tissue exhibits four zones with Rm value .83, 76, .63, .58. Among these, the zone with Rm value .63 and .58 were showing partial activity with ER esterases. The remaining zones with Rm value .83, .76 were examined as AcE and CE esterases respectively.

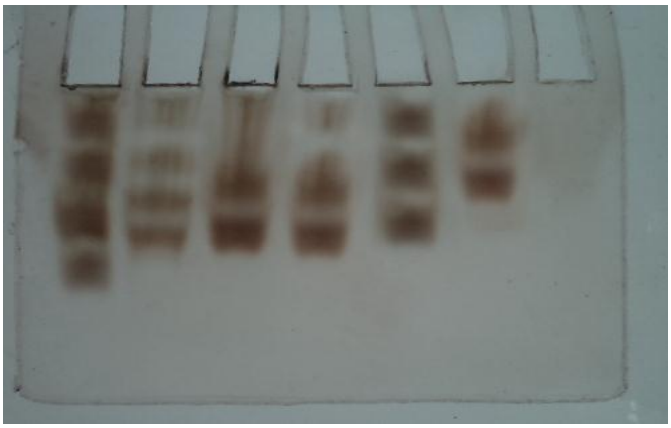
Intestine: Intestine consisting of only two zones with Rm value .76 and .68 were classified as ArE and Esdp esterases respectively.

Mantle: Mantle contains three esterase active zones on the zymogram. Among these, the zone with Rm value .76 and .65 were showing partial activity with CE esterases and another zone with Rm value .60 was exhibits ER esterase with partaial activity.

Foot: Foot exhibits three esterase active zones, the zone with Rm value .80 and .76 both inhibited by Paraoxon and $AgNO_3$. So, they were noticed as CE esterases and another zone with Rm value .60 was inhibited by pCMB and $AgNO_3$. So, this zone is considered as ArE esterase.

Tentacles: Tentacles consisting of only two esterase active zones on the zymogram. The zones with Rm value .76 and .63 both showed partial activity with CE and Esdp esterases respectively.

Esterase band patterns of *Arion ater*



1 2 3 4 5 6

1 = Ctenidia; 2 = Hepatopancreas; 3= Intestine; 4= Mantle; 5= Foot; 6= Tentacles.

The pattern of esterases observed in various tissues of *Arion ater* indicates (Table 1.1) distibution of esterases. Among the six tissues, ctenidia, hepatopnacreas contain maximum number of zones, four zones each and followed by mantle, foot with two zones each. When the esterase active zones found in various tissues are arranged according to their electrophoretic mobility, a total of nine zones can be found in this species. Out of these zones, the zone with Rm value .76 was found in all tissues except ctenidia. It is examined as CE esterase in hepatopancreas, mantle, foot and tentacles, whereas the same zone is noticed as ArE esterase in intestine. The zone with .90 was examined in ctenidia with CE esterases. The zone with Rm value .80 and .68 were found in CE, Esdp esterases with foot and intestine respectively. The zone with Rm value .83 was present in ctenidia with CE esterase, but in hepatopancreas it is noticed as AcE esterase. The zone with Rm value .65 was found in only ctenidia and mantle with ER and CE esterases respectively. The zone with Rm value .60 is examined in mantle, foot with ER and ArE esterases respectively and another zone with Rm value .58 is noticed in ctenidia and hepatopancreas and are classified as ER esterases.

Comparative study of different type of esterases contributing to tissue enzyme activity indicates that carboxylesterases (CE) are the principal contributors to the tissue enzyme activity of all tissues.

Table 1.1 Inhibitor sensitivity of individual esterase zones in *Arion ate*

Name of tissue	Ctenidia				Hepatopancreas				Ntestine		Mantle			Foot			Tentacles	
Rm Values	.90	.83	.65	.58	.83	.76	.63	58	.76	.68	.76	.65	.60	.80	.76	.60	.76	.63
Activity	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Pcmb	++	++	++	++	+	++	++	++	-	-	++	++	+ +	++	++	-	++	-
Paraoxon	-	-	++	++	++	-	++	++	++	-	-	-	++	-	-	++	-	-
Physostigmine	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
EDTA	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
$AgNO_3$	-	-	++	++	+	-	++	++	-	-	-	-	++	-	-	-	-	-
Classification	CE	CE	ER	ER	AcE	CE	ER	ER	ArE	Esdp	CE	CE	ER	E	E	ArE	CE	Esdp

CE = Carboxylesterase; ChE= Cholinesterase; ER= Esterases resistant to inhibitors; ArE = Arylesterases

Ese=Esterase sensitive to eserine; Esdp= Esterase sensitive to organophosphates and pCMB; AcE=Acetylesterase

+++ = Strong activity; ++ = Partial activity; + = Weak activity; - = Complete inhibition.

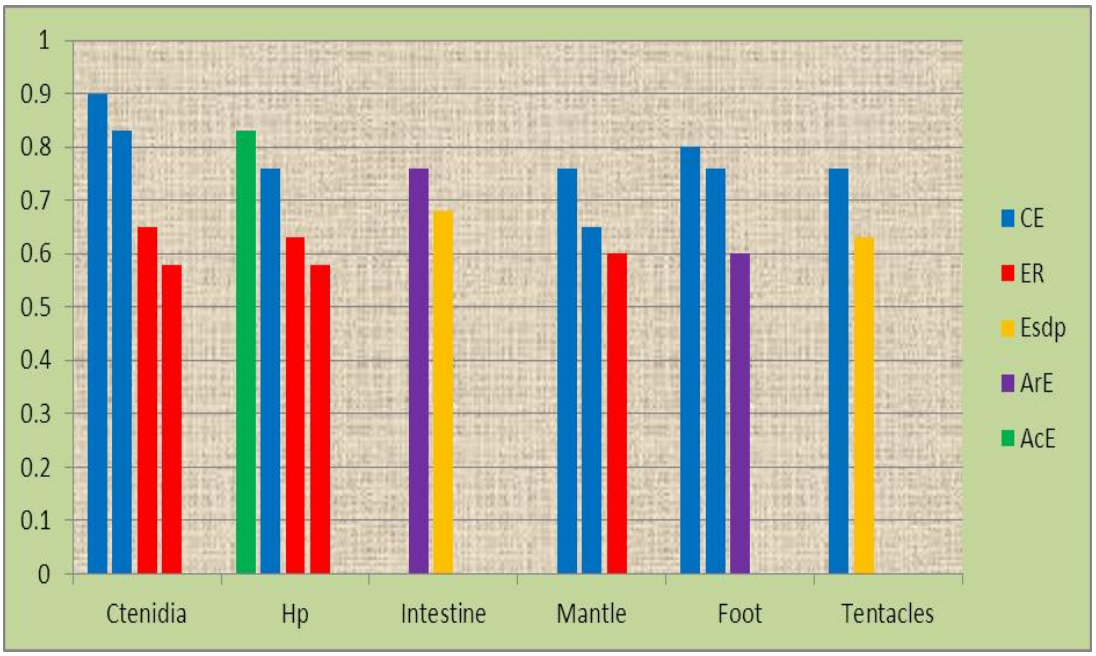
Table 1.2: Tissue specific distribution of esterase zones in *Arion ater*

Tissues / Rm values	1 .90	2 .83	3 80	4 .76	5 .68	6 .65	7 .63	8 .60	9 .58
Ctenidia	++ CE	++ CE				++ ER			++ ER
Hepatopancreas		++ AcE		++ CE			++ ER		++ ER
Intestine				++ ArE	++ Esdp				
Mantle				++ CE		++ CE		++ ER	
Foot			++ CE	++ CE				++ ArE	
Tentacles				++ CE			++ Esdp		

CE = Carboxylesterase; ChE= Cholinesterase; ER= Esterases resistant to inhibitors;

ArE = Arylesterases: Ese=Esterase sensitive to eserine; Esdp= Esterase sensitive to organophosphates and pCMB ; AcE=Acetylesterase

+++ = Strong activity; ++ = Partial activity; + = Weak activity; - = Complete inhibition



Graph: showing tissue specific distribution of esterase zones in *Arion ater*

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