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Original Article

Electrophoretic Patterns of Esterases from Different Tissues of *Arion Hortensis*

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ABSTRACT

Abstract: The present study was carried out to investigate the electrophoretic patterns of different types of esterase enzymes in different tissue extraction of *Arion hortensis* through electrophoresis. The results revealed that the activity of individual esterase was not inhibited by EDTA and physostigmine, however a partial inhibition was observed in the presence of $AgNo_3$ and a complete inhibition was observed in the presence of paraoxon and pCMB.

Key words: Arion hortensis, electrophoresis, inhibition, physostigmine, paraoxon.

1. INTRODUCTION

Snails and slugs are belongs to the phylum Mollusca, which have similar morphology except that slugs have no shell or reduced shell. Slugs are used as a zootherapeutical product for the treatment of asthma, sprains, boils and ulcer in traditional Brazilian medicine in the Northeast of Brazil and India. Snails and slugs slime based products are claimed to be the new miracle face-fixer and also used to treat acne, reduce pigmentation, scarring, combat wrinkles and treat dermatological conditions. Snails and slugs are used both as a food and as a treatment for a variety of medical conditions [1] described, the common garden slug, *Arion hortensis*, is sometimes swallowed whole as a treatment for gastritis or stomach ulcers. Slug slime is used in the treatment of wounds and warts

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Esterase enzymes are multiple forms of a single enzyme, which often have different isoelectric points and therefore can be separated by electrophoresis. Electrophoretic studies were done extensively on the different tissues of various animals from which it reveals that the enzyme exit in multi molecular forms and functions [2]. As the electrophoretic banding patterns of esterases of different tissues show species-specific variation it could be successfully used for the identification of molluscan species [3]. Esterase enzymes are one of the lipid-hydrolyzing enzymes, possess high significance in genetics and toxicology [4]. The banding pattern of esterases appears to be genetically controlled and therefore it has been used to estimate the genetic distance among different populations or the distance between populations [5]. Esterases are also used as bioindicators to measure the toxic potency of pesticide residues usually applied in agriculture. The residual effect of pesticide in aquaculture specifically in molluscans are high which in tern cause death of molluscans particularly, after the rainy seasons [6]. Considering the above facts, it is essential to understand the genetic status in terms of esterase variability. The paper deals with polymorphic pattern of esterase variations in different body tissue of the Arion hortensis.

2. MATERIAL AND METHODS

Collection of animals: Arion hortensis was collected from paddy fields of Komatipally village located about 30km from Kakatiya University campus.

Extraction and collection of samples: dissected the tissues and processing Ctenidia- 5%, Hepatopancreas-2%, Intestine-2%. Mantle-10%, Foot – 30% and tentacles-5%. The samples were homogenized in 10% 0.01M Tris-HCL buffer (pH 7.4) containing 0.9% NaCl. The homogenate was centrifuged at 2000 rpm in a clinical centrifuge at room temperature (27±30).

Experimental procedure for preparation of native gels: The supernatant was mixed with equal volumes of 20% sucrose containing 0.01% bromophenol blue as tracking dye. An aliquot of 0.1ml of this solution was loaded directly on to the separating gel. Esterase patterns were separated on thin layer (1.5mm thickness) Native Polyacrylamide gels (7.5%). The gel mixture was prepared according to Clarke. Gelling was allowed for 45 minutes. After loading on to the gel, the samples were over laid with electrode buffer and gel plates were connected to the electrophoretic tank. Tris (0.05M), Glycine (0.38M), buffer (pH 8.3) was used as the electrode buffer. A constant current of 50 volts for the first 15 minutes followed by 150 volts for the rest of the run was supplied during electrophoresis. The electrophoretic run was terminated when the tracking dye migrated to the distance of 6 cm from the origin.

Staining and inhibition studies: Esterases were visualized on the gels by adopting the staining procedures of [7] .pCMB (parachloro mercuri benzoate) (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 as inhibitors. The gel was first incubated in diluted (1:4) Tris-Hcl buffer (pH 7.4) containing appropriate concentrations or inhibitors for 30 minutes. The gel was then transferred to a staining mixture containing 1-naphthyl acetate as the substrate. Appropriate concentrations of inhibitors, as were used for the pre-incubation, were added to the staining mixture to prevent the reversal inhibitory effect of compounds.

3. RESULTS

The patterns of esterases were observed in the six tissues of *Arion hortensis*. Their relative mobility and inhibitor sensitivity are given in the Table 1.1 which summarizes different classes of esterases that were found in each of the six tissues of the *Arion hortensis.* The relative proportion of different classes of esterase enzymes are contributing to the total esterase activity.

Ctenidia: There are four esterase active zones in this tissue. Among these the zone with Rm value .40 was inhibited by paraoxon and $AgNO_3$. So, it is noticed as CE esterase and another zone with Rm value .35 is not inhibited by any inhibitors used so it is classified as ER esterase, the remaining two zones with Rm values .68 and .55 were showed as partial activity which are ArE esterases.

Hepatopancreas: This tissue exhibits three esterase active zones on the zymogram. One of the zone with Rm value .40 is showing strong activity with ER esterase and remaining zones with Rm value .60 and .55 are showed partial activity with ArE esterases.

Intestine: Intestine contains three esterase active zones with Rm value .60, .53 and .28 with AcE, ArE and CE esterases respectively and all zones were exhibits partial activity.

Mantle: Mantle consisting of only two zones with Rm value .53, .28 both inhibited by pCMB, Paraoxon and $AgNO_3$. Hence, these two zones are considered as Esdp esterase.

Foot: Foot exhibits three esterase active zones on the zymogram. The zones with Rm value .60, .55 and .50. All these zones are inhibited by Paraoxon and $AgNO_3$. So, they were classified as CE esterases and this zone are exhibited partial activity.

Tentacles: This tissue exhibits three esterolytic active zones. Among these one of the zone with Rm value .35 is not inhibited by any inhibitors used, so it is considered as ER esterase. The remaining zones with Rm value .58, .55 with ArE and CE esterases respectively.

Table 1.1: Inhibitor sensitivity of individual esterase zones in *Arion hortensis*

Name of tissue	Ctenidia				Hepatopancreas			Intestine			Mantle		Foot			Tentacles		
Rm Values	.68	.55	.40	.35	.60	.55	.40	.60	.53	.28	.53	.28	.60	.55	.50	.58	.55	.35
Activity	++	++	++	+++	++	++	+++	++	++	++	++	++	++	++	+++	+++	++	++

pCMB	-	-	++	++	-	-	++	+	-	++	-	-	+ +	++	++	-	++	++
Paraoxon	++	++	-	++	++	++	++	++	++	-	-	-	-	-	-	++	-	++
Physostigmine	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
EDTA	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
$AgNO_3$	-	-	-	++	-	-	++	+	-	-	-	-	-	-	-	-	-	++
Classification	ArE	ArE	CE	ER	ArE	ArE	ER	AcE	ArE	CE	Esdp	Esdp	CE	CE	CE	ArE	CE	ER

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 hortensis*

Tissues / Rm values	1	2	3	4	5	6	7	8	9
	.68	.60	.58	.55	.53	.50	.40	.35	.28
Ctenidia	++ ArE			++ ArE			++ CE	+++ ER	
Hepatopancreas		++ ArE		++ ArE			+++ ER		
Intestine		++ AcE			++ ArE				++ CE
Mantle					++ Esdp				++ Esdp
Foot		++ CE		++ CE		++ CE			
Tentacles			++ ArE	++ CE				++ ER	

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.

4. DISCUSSION

The pattern of esterases observed in the six tissues of *Arion hortensis* (Table 1.2) indicates tissue specific distribution of esterases. Among the six tissues, ctenidia had four zones of esterases. Hepatopancreas, intestine, foot and tentacles had three zones of esterases each and other tissues had two zones of esterases. When the esterase active zones found in various tissues are arranged according to their electrophoretic mobility, a total nine zones can be found in this species. Out of these only one zone with Rm value .55 is present in all tissues except Intestine and mantle, it is ArE esterase in ctenidia and hepatopancreas, where as foot and tentacles it is CE esterase. The zone with Rm value .58 is present in only tentacles with ArE esterase and other zones with Rm value .68 and .50 both present in ctenidia and foot with ArE, CE esterases respectively. The zone with Rm value .60 is found in hepatopancreas with ArE esterase and the same zone is considered as AcE esterase in intestine and CE esterase in foot. The zone with Rm value .53 is present in intestine and mantle with ArE, Esdp esterases respectively. The zone with Rm value .40 is found in ctenidia with CE esterase and hepatopancreas with ER esterase. The zone with Rm value .35 was examined in ctenidia and tentacles with ER esterases and it exhibits similar biochemical properties in both tissues and another zone with Rm value .28 is considered as CE esterase in intestine and Esdp esterase in mantle.

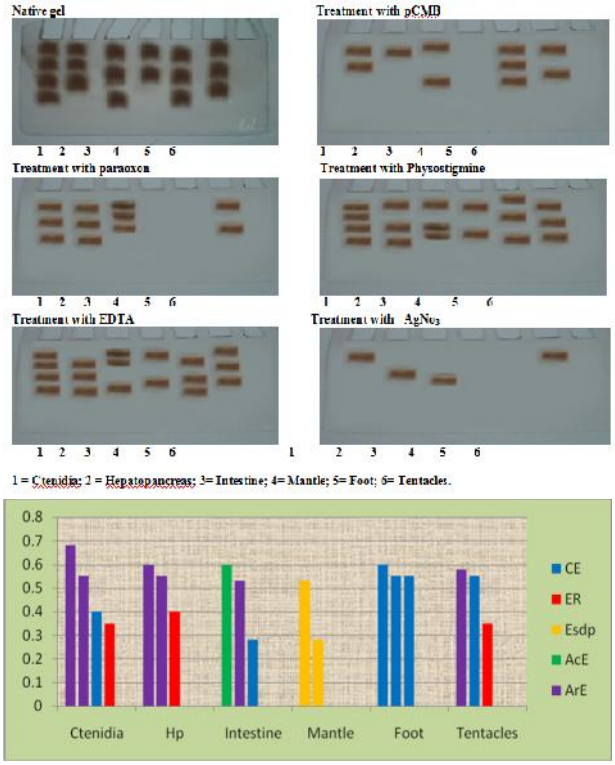

Fig 1: Tissue specific distribution of esterase zones in *Arion hortensis*

5. CONCLUSION

The present study was clearly indicates that the ctenidia, intestine, foot and tentacles esterase activity levels indicate high intensity and hepatopancreas, mantle esterase activity levels indicates moderate and low intensity in normal. The ctenidia indicate high intensity in presence of physostigmine and EDTA, moderate intensity in presence of paraoxon and pCMB and low intensity in presence of $AgNo_3$. The hepatopancreas indicates moderate intensity in presence of paraoxon, physostigmine and EDTA, low intensity in the presence of pCMB and $AgNo_3$. The intestine indicates moderate intensity in presence of paraoxon pCMB, physostigmine and EDTA, low intensity in presence of $AgNo_3$. The mantle indicates low intensity in EDTA and physostigmine, no intensity in presence of pCMB, paraoxon and AgNo3. The foot indicates moderate intensity in presence of pCMB, physostigmine, EDTA, no intensity in presence of paraoxon and $AgNo_3$ and the tentacles indicates moderate intensity in presence of pCMB, physostigmine and EDTA, low intensity in presence of paraoxon and $AgNo_3$. The comparative study of various classes of esterases contributing to tissue enzyme activity indicates that ArE esterases are the major contributors to total enzyme activity in all the tissues.

In view of the above results the esterases can be used as tools in establishing the genetic relatedness among the closely related species [8], and also as a marker molecule during the evolution of new species of slugs.

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