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RESEARCH ARTICLE

Functional Classification of Esterases in *INDONAIA CAERULEUS* (Phylum: Mollusca)

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ABSTRACT

Polyacrylamide gel electrophoresis system (PAGE) and inhibition tests used to obtain a functional classification of esterases in *Indonaia caeruleus* of five tissues. i.e., Ctenidia, Hepatopancreas, Intestine, Mantle, Foot and circulatory fluid (Hemolymph). Esterases have potential uses over a broad range of applications in the agri-food industries. In recent years, the number of esterase activities reported has increased and in parallel, even more related protein sequences may be discerned in the growing genome databases and the esterases are classified as based upon the inhibitor effect such inhibitors as pCMB, Paraoxon, Eserine, EDTA, $AgNO_3$.

KEYWORDS

Inhibition, Esterase, Hemolymph, pCMB, *Indonaia caeruleus*

INTRODUCTION

Esterase, acting on both general and specific type of esters, affects the rates of reversible reactions depending on the thermodynamics, i.e., the organic phase favors ester formation that can be hydrolyzed in the aqueous phase by the same enzyme (Dodrick 1989). However, esterases differ from lipases mainly on the basis of substrate specificity and interfacial activation (Long 1971). Lipases, which have a hydrophobic domain covering the active site, prefer triglycerides of long chain fatty acids, and thus have different properties than esterases, which have an acyl binding pocket (Pleiss et. al. 1998). As applications for esterases are found in various fields, and due to growing interest in this enzyme, various aspects of esterases have been reported, mainly in the avenues of distribution, quantitation, production, targeted synthesis, purification, and molecular biology.

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The band pattern also exhibits profound variation with varying electrophoretic conditions (Reid *et. al.* 1969). As a consequence of these problems, use of inhibitor studies becomes inevitable of characterization and genetic interpretation of esterase zymograms. This paper aim is classification and tissue comparison of different classes of esterases in bivalve.

MATERIALS AND METHOD

Indonaia caeruleus was collected from lakes, located about 30km from Kakatiya University campus and dissected the tissues and processing Ctenidia-30%, Hepatopancreas-5% Intestine-20%, Mantle-30%, and Foot-30% and the haemolymph is collected from the anterior adductor muscle according to the procedure of Ford 1986, Yanick & Heath 2000. Homogenates were kept over ice for 30 minutes and centrifuged at 2000rpm for 10 minutes 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 tracking 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 minutes. The gel was stained at room temperature following the procedure of Redfield and Salini (1980). 1- naphthyl esters of acetate, was used for substrate study, Paraoxon-$2x10^{-5}$, Eserine-10^{-4} pCMB-10^{-4}, EDTA-10^{-3}, $AgNO_3$-10^{-2}, 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

Table 1: Inhibitor sensitivity of individual esterase zones in ***Indonaia caeruleus***

Name of tissue	**Ctenidia**				**Hepatopancreas**			**Haemolymph**			**Intestine**			**Mantle**		**Foot**	
Rm Values	.80	.75	.55	.33	.83	.50	.43	.58	.43	.33	.83	.43	.33	.55	.43	.65	50
Activity	++	++	+++	+++	++	++	++	++	+++	++	++	++	++	++	++	++	++
pCMB	++	++	+	++	-	++	-	++	++	++	++	++	++	++	-	-	-
Paraoxon	-	-	++	-	++	++	++	-	++	-	-	-	-	-	-	++	-
Physostigmine	++	++	++	-	++	++	++	-	++	-	++	++	-	++	++	++	++
EDTA	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
$AgNO_3$	-	-	+	++	-	++	-	++	++	++	-	-	++	-	-	-	-
Classification	CE	CE	AcE	ChE	ArE	ER	ArE	ChE	ER	ChE	CE	CE	ChE	CE	Esdp	ArE	Esdp

CE = Carboxylesterase; ChE= Cholinesterase; ER= Esterases resistant to inhibitors; ArE = Arylesterase. Ese = Esterase sensitive to eserine; Esdp= Esterase sensitive to organophosphates and pCMB; AcE = Acetylesterase.

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

Table 2: Tissue specific distribution of esterase zones in ***Indonaia caeruleus***

Tissues / Rm values	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
	.83	.80	.75	.65	.58	.55	.50	.43	.33
Ctenidia		++ CE	++ CE			+++ AcE			+++ ChE
Hepatopancreas	++ ArE						++ ER	++ ArE	
Haemolymph					++ ChE			+++ ER	++ ChE
Intestine	++ CE							++ CE	++ ChE
Mantle						++ CE		++ Esdp	
Foot				++ ArE			++ Esdp		

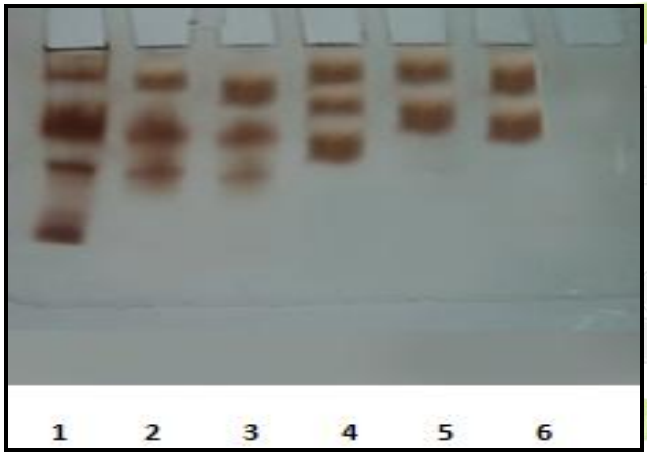
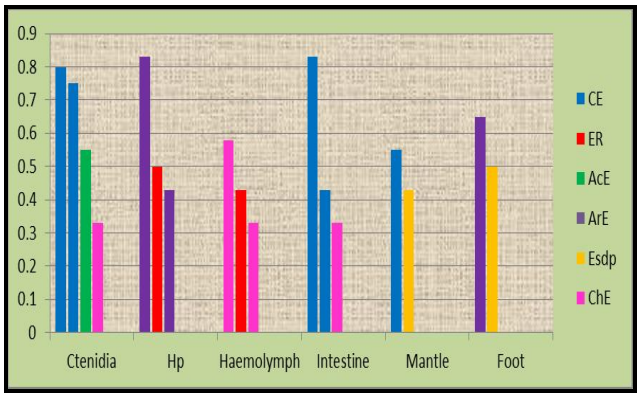


Plate 1: Indonaia caeruleus

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



Graph 1: Tissue specific distribution of esterase zones in *Indonaia caeruleus*

Ctenidia

There are four esterase active zones in this tissue; the zones with Rm value .80 and .75 were showing partial activity. They are completely inhibited by Paraoxon and $AgNO_3$. Hence, both classified as CE esterase. The zone with Rm value .55 was not inhibited by any inhibitors used, but showed the weak activity with pCMB and $AgNO_3$. Hence, it is noticed as AcE esterase and another zone with Rm value .33 was showing strong activity with ChE esterase, this zone is completely inhibited by Paraoxon and Physostigmine, but not inhibited by other inhibitors used.

Hepatopancreas

Hepatopancreas exhibits three esterase active zones on the zymogram. The zones with Rm value 83 and .43 are inhibited by pCMB and $AgNO_3$. So, it is noticed as ArE esterase. The remaining zone with Rm value .50 was not inhibited by any inhibitors used so it is examined as ER esterase.

Haemolymph

Hemolymph contains two classes of esterases ChE (2), ER (1). Relative proportion of these two classes of esterases present in this tissue suggests that ChE esterases are the major contributors of this tissue esterase activity. Rest

of enzyme activity is contributed by ER esterases. The zones with Rm value .58 and .33 were showing partial activity with ChE esterase. On the other hand the zone with Rm value .43 was showing strong activity with ER esterase.

Intestine

Intestine exhibits three esterase active zones on the zymogram. The zone with Rm value .83 and .43 were classified as CE esterase, these two zones are inhibited by Paraoxon and $AgNO_3$ and another zone with Rm value .33 is inhibited by Paraoxon and Physostigmine. So, it is classified as ChE esterase.

Mantle

Mantle contains only two zones with Rm value .55 and .43 both showing partial activity. One of these, the zone with Rm value .55 is completely inhibited by Paraoxon and $AgNO_3$. So, it is considered as CE esterase and another zone with Rm value .43 is inhibited by pCMB, Paraoxon and $AgNO_3$. Hence, it is noticed as Esdp esterase.

Foot

Tissue esterase pattern of foot shows the presence of two zones of esterases, both zones are partial activity. The zone with Rm value .65 was classified as ArE esterase, this zone is completely inhibited by pCMB and $AgNO_3$ and another zone with Rm value .50 is noticed as Esdp esterase.

The pattern of esterases observed in various tissues of *Indonaia caeruleus* (Table 2 and graph 1) indicates a highly tissue specific distribution of esterases. Among the six tissues, ctenidia exhibit the maximum number of zones four. Hepatopancreas, haemolymph and intestine exhibits three zones each. Mantle and foot had two zones each. When the esterase active zones found in various tissues are arranged according to their electrophoretic mobility, a total of eight zones found in this snail. Out of these, the zone with Rm value .80 and .75 were found in ctenidia only. The zone with Rm value .65 is present in foot with ArE esterase and another zone with Rm value .58 was examined in haemolymph with ChE esterase, it is inhibited by Paraoxon and Physostigmine. The zone with Rm value .55 is found in ctenidia and mantle, this zone is not affected by any inhibitors used, but only showed the weak activity with pCMB and $AgNO_3$ and another tissue it is inhibited by paraoxon and $AgNO_3$. Hence, it is noticed as carboxyl esterase (CE). The zone with Rm value .50 is found in hepatopancreas and foot with ER and Esdp esterase respectively. The zone with Rm value .43 is found in all tissues except ctenidia and foot. It differs in classification in hepatopancreas and haemolymph with ArE and ER esterases respectively. On the other hand the same band is CE esterase in intestine and Esdp in mantle. The zone with Rm value .33 is exhibits in ctenidia, haemolymph and intestine, this zone exhibits similar properties of inhibitor sensitivity. It is inhibited by Paraoxon and Physostigmine. Hence, in three tissues it is considered as ChE esterase.

The study of functional classes of various esterases contributing to tissue enzyme activity indicates that CE esterases are principal contributors to different tissues of this bivalve.

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