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ESTERASE ACTIVITY AND DETECTION OF DIFFERENT ESTERASES FROM *POMACEA CUMINGI* IN RELATION TO TISSUES AND AGES

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ABSTRACT

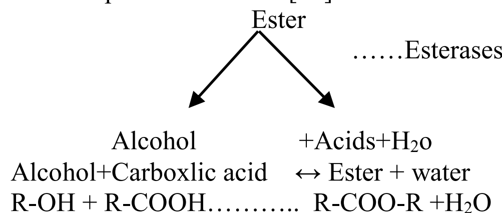
General esterase activity in the different body tissues of adult females of 5-day and 15-day old pomacea cumingi were measured spectrophotometrically and different esterases (chE, CE, AcE, ArE, Aryl and Esdp) were characterized by electrophoresis method. Esterase activity was studied in the tissues of ctenidia, Hepatopancreas, intestine, mantle, foot and tentacles of the two ages in the presence of α- naphthyl acetate as a substrate and paraoxon, pCMB, physostigmine, AgNo3 and EDTA as inhibitors. Spectrophotometric analysis indicated that the esterase activity in adult p. cumingi tissues was significantly higher in the tissues of ctenidia, hepatopancreas of 5-day old females than those of 15-day old respectively. Mean while the activity was greater in the intestine, foot, and mantle tissues of 15-day old females than in 5-day old ones. One to eight esterase bands were detected in the different body tissues of the two ages of adult females. These bands were characterized as CE, Esdp, chE, ArE, ER and AcE esterases by using the five inhibitors. Age and tissue specific differences were observed in some of the regions. The tentacles of both were classified into esterase resistant to inhibitors (ER) and Acetyl esterases (AcE).

KEY WORDS: *esterase activity, ages, inhibitors, pomacea cumingi, electrophoretic*.

INTRODUCTION

An esterases are the hydrolyze enzymes that splits esters into an acid and an alcohol. Two categories of such enzymes were recognized first by [1], enzymes, which hydrolyze the esters of short chain (C_2-C_4) fatty acids were recognized as esterases, while those which hydrolyzed the long chain fatty acid esters (>C8) were recognized as lipases[2]. Esterase enzymes are involved in important physiological process such as nervous impulse control, Reproduction, Developmental process, Detoxification and tolerance of xenobiotics besides being good biomarkers to predict environmental pollution. They have been used as gene markers in a mid variety of organisms. These enzymes also attracted the action of industry in past

few decades due to their application in food, detergent, fine chemical, waste water treatments, Bio-diesel production, and pharmaceutical industries and in Bio-remediation. [3], [4], [5], [6], [7], [8], [9]. The high region and spacio specificity of these enzymes has applications in the Kinetic resolution of optical isomers for synthesis of optically pure substances in pharmaceutical and chemical industries. Their ability was to catalyze a variety of esterase without the aid of cofactors is an additional advantage (Bornscheuer, 2002). Esterases play vital roles in the metamorphosis of insects [10].



Alcohol+Carboxlic acid ↔ Ester + water

R-OH + R-COOH……….. R-COO-R +H_2O

A great advance in the study of esterases was made when organophosphates were recognized as inhibitors of these enzymes [11]. [12] distinguished the enzymes into two, 'A' and 'B' esterases, by using the organophosphate inhibitors E-600 (Diethyl-paranitrophenyl phosphate). 'B' esterases were reported to be sensitive to the compound where as the 'A' esterases hydrolyzed it. A third enzyme, 'C' esterase [13] was shown to be neither sensitive to organophosphates nor was it capable of hydrolyzing them. Low concentrations of pCMB (parachloromercuribenzoate) slightly activated these enzymes. Early studies on non-specific esterases in various groups of animals by different authors produced a diversified nomenclature in literature. But they were essentially the synonyms of the three types, A, B and C esterases [14]. The terminologies employed are as hereunder:

A – Esterase = Aromesterase = Arylesterase – Organophosphate resistant esterase = E-600 esterase = DFPase.

B – Esterase = Aliesterase – Carboxylesterases = Organophosphate sensitive esterase.

C – Esterase = Acetylesterase = Organophosphate resistant, sulphydryl resistant esterase.

Cholinesterases were distinguished from these esterases by their sensitivity to eserine (Physostigmine) [15]. Attempts were also made [16] to classify esterases on the basis of substrate specificity. But this approach was found to be inadequate as several of the enzyme forms demonstrated broad substrate specificity [17],[18], [19]. Information on sensitivity of esterases to various inhibitors enabled to classify esterases into four categories [20], [21], [22].

1.Carboxylesterases (E.C. 3.1.1.1):-These enzymes are sensitive to inhibition by organo-phosphates but are not affected by Physostigmine (eserine). They preferentially hydrolyze the short chain aliphatic and aromatic esters [23].

2.Arylesterases (E.C. 3.1.1.2):-They are sensitive to inhibition by pCMB and pHMB (Parachloro mercuri-benzoate and parahydroxymercuribenzoate). They preferentially hydrolyze aromatic esters especially the acetic esters [24], [25].

3.Acetylesterases (E.C. 3.1.1.6):-They are resistant to inhibition by organophosphates, Physostigmine and also by pCMB / pHMB, [26].

4.Cholinesterases:-This group includes both acetylcholinesterases (AChE, E.C. 3.1.1.7) and Pseudocholinesterases (E.C. 3.1.1.8). They are inhibited both by organophosphates and eserine. Acetyl-cholinesterases preferentially hydrolyze the acetyl choline and aromatic acetyl esters [27], [28], [29] while the others (E.C. 3.1.1.8) hydrolyze the butyryl choline esters and aromatic butyrate esters [30] successfully adopted this classification for distinguishing esterases of vertebrate and invertebrate tissues separated by zymogram technique. The present study deals with the biochemical characterisation of the tissues and age specific esterases in order to understand their possible role.

MATERIAL AND METHODS

Pomacea cumingi 5-day and 15-day old were collected from Vaddepally Lake located about 20km from Kakatiya University near Hanamkonda, Telangana. Animals were transported alive to the laboratory and reared until analysis. Ctenidia (150mg), Hepatopancreas, intestine (200mg each), mantle, foot (250mg each) and tentacles (150mg) taken from freshely killed specimens were homonized in 1ml of ice –cold extracting solution (10% sucrose solution for mantle foot and tentacles and 0.01% M Tris Hcl buffer containing sucrose 500mg/lit; pH 7.0 for hepatopancreas and intestine samples several other solutions were also tried for homonizing tissues but only the above ones produced sharp bands without trailing. Homogenates were kept over ice for 30min and centrifuged at 16000rpm at 4^0C for 45 minutes in a refrigerated centrifuge and supernatant used for electrophoresis. Vertical slabgel electrophoresis (1.0mm thick, 7.5cm tall) was carried out using 7.5% polyacralamide gel (containing 5mg NAD per 20ml gel mixture) at 20^OC Tris (500mm) borate (650mM) Na_2 EDTA $2H_2O$ (16mM) (TBF), pH 8.6 was used as a gel buffer and a 1:9 dilution of the same was used as tank (electrode) buffer. To increase resolution of bands, 35mg of NAD was dissolved in 150ml of buffer in upper tank. For loading the gel 3µl each of hepatopancreas and intestine and 6 µl each of intestine mantle foot and tentacles homogenates were used. Aqueous bromophenol blue (Final concentration 0.05%) was

used as tracking dye . The run was carried out at a constant current of Ma (150V) and terminated after 50min. The gels were stained at room temparature following the procedure of [31].1-naphthyl esters of acetate used as substrate. EDTA (100mM), AgNo3 (10^{-2}M), p^{CMB} (10^{-4}M), paraoxon ($2x10^{-5}$M) and physostigmine (10^{-4}M) were used in inhibitor sensitivity studies. The gels were pre-incubated in the buffer containing the above concentrations of inhibitors for half an hour, following which they were stained for esterase activity. To prevent reversal of inhibition the same concentration of inhibitor that was used for pre-incubation was added to the staining mixture also. Since the target of the major programmed was to score genetic polymorphism within the species, initially the tissue samples of 5-day old and 15-day old snails were electrophoresed in separate gels under identical conditions, but age differences were very less. Hence in the further study for the tissue comparisons, samples were always run in the same gel and under conditions exactly identical in all aspects. In the zymogram, bands were serially numbered with the fastest migrating fraction getting the first number and slowest the last. Taking the R_m value and proximity of bands into consideration, the enzyme activity areas were broadly categorized into different regions.

RESULT AND DISCUSSION

5-day old *pomacea cumingi*

Esterase is enzyme analysis with native polyacralamide gel electrophoresis of 5-day old of *pomacea cumingi* six tissues showed on the table 1.1. Among these six tissues ctenidia exhibited five esterolytic active zones on the zymogram and hepatopancreas, foot had three zones of esterases, intestine, mantle and tentacles contain two zones of esterase.When the esterase active zones found in various tissues are arranged according to their electrophoretic mobility. The zone with Rm value .33 and .28 were found in only intestine in these the zone with R_m value .33 inhibited by paraoxon and physostigmine so, it is designated as chE esterase and another zone with Rm value .28 was inhibited by p^{CMB}, paraoxon, and $AgNo_3$, so it is classified as Esdp esterase. The zones with Rm value .70 and .60 were found in only ctenidia, in these the zone with Rm value .70 was inhibited by paraoxon and $AgNo_3$, so it is considered as carboxyl esterase and other zone with Rm value .60 was inhibited by p^{CMB}, paraoxon and $AgNo_3$, considered as Esdp esterase. The zone with Rm value .68 was found in ctenidia and hepatopancreas, in ctenidia it is designated as Esdp esterase, where as CE esterase enzyme in hepatopancreas. The zone with Rm value .55 was found in ctenidia and hepatopancreas, in ctenidia it is not inhibited by any inhibitors used so it is considered as ER esterase, where as in hepatopancreas it is inhibited by paraoxon and $AgNo_3$ so it is considered as CE esterase. The zone with Rm value .48 was found in ctenidia, mantle, foot and tentacles, in ctenidia and tentacles it is considered as ER esterase and in foot it is classified as Esdp esterase and in mantle it is inhibited by p^{CMB} and $AgNo_3$ so it is classified as ArE esterase. The zone with Rm value .45 found in only hepatopancreas it is classified as ER esterase. The zone with Rm value .38 was found in only foot it showed partial activity with AcE esterase. Mantle exhibits one esterolytic active zone on the zymogram this zone with Rm value.36 was considered as partial activity with ArE esterase. The zones with Rm value .30 and .25 were found in tentacles and foot respectively.

Table 1.1 Tissue specific distribution of esterase zones in 5-day old *Pomacea cumingi*

	1	2	3	4	5	6	7	8	9	10	11	12
Tissues / Rm values	.70	.68	.60	.55	.48	.45	38	.36	.33	.30	.28	.25
Ctenidia	++ CE	++ Esdp	++ Esdp	+++ ER	++ ER							
Hepatopancreas		++ CE		++ CE		+++ ER						
Intestine									++ ChE		++ Esdp	
Mantle					++ ArE			++ ArE				
Foot					++ Esdp		++ AcE					++ AcE
Tentacles					+++ ER					++ AcE		

CE = Carboxylesterase; ChE= Cholinesterase; ER= Esterases resistant to inhibitors; ArE = Arylesterases; Ese=Esterase sensitive to eserine;
Esdp= Esterase sensitive to organophosphates and pCMB ; AcE =Acetyl esterase: +++ = Strong activity; ++ = Partial activity; + = Weak activity;

Graph: 1.1. Tissue specific distribution of esterase zones in 5-day old *Pomacea cumingi*

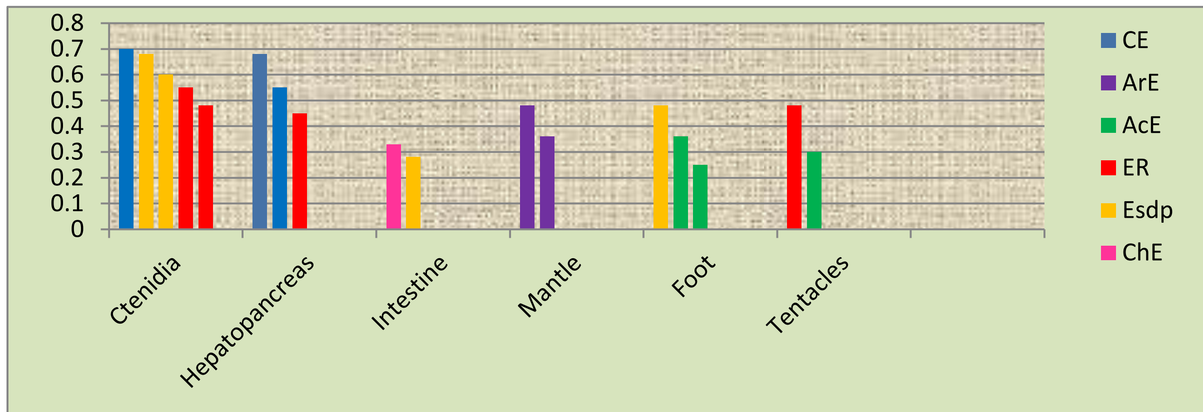
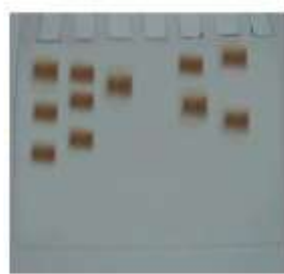


PLATE-I Electrophoretic patterns of 5-day old *Pomacea cumingi*

Native gel

1 2 3 4 5 6

treatment with pCMB



1 2 3 4 5 6

treatment with paraoxon

1 2 3 4 5 6

treatment with physostigmine

1 2 3 4 5 6

treatment with EDTA

1 2 3 4 5 6

treatment with AgNo3

1 2 3 4 5 6

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

15 day old *Pomacea cumingi*

Table 1.2 Tissue specific distribution of esterase zones in 15-day old *Pomacea cumingi*

Tissues / Rm values	1 .78	2 .63	3 .60	4 .58	5 .50	6 .48	7 .33	8 .25
Ctenidia		++ Esdp			++ Esdp	++ Esdp		+++ ER
Hepatopancreas	++ CE	+++ ChE		+++ ChE		+++ Esdp		
Intestine					++ CE	++ Esdp		++ ER
Mantle				++ ArE	++ CE			+++ Esdp
Foot			++ Esdp		++ ArE	++ Esdp	+++ ER	
Tentacles					++ ER		++ AcE	

CE = Carboxylesterase; ChE= Cholinesterase; ER= Esterases resistant to inhibitors; ArE = Arylesterases; Ese=Esterase sensitive to eserine;
Esdp= Esterase sensitive to organophosphates and pCMB ; AcE =Acetyl esterase: +++ = Strong activity; ++ = Partial activity; + = Weak activity;

The pattern of esterases observed in the six tissues of 15 day old *Pomacea cumingi* indicates a highly polymorphic distribution of esterase showed on the table 1.4. Among these six tissues ctenidia, hepatopancreas, foot contain four zones of esterases, intestine and mantle had three zones, tentacles had two zones. When the esterase active zones found in various tissues are arranged according to their electrophoretic mobility. The zone with Rm value .60 was found in only foot and .78 is found in only hepatopancreas it is inhibited by paraoxon and $AgNO_3$. The zone with Rm value .63 was only found in ctenidia and hepatopancreas, in ctenidia it is Esdp esterase where as ChE esterase enzyme in hepatopancreas. The zone with Rm value .50 is found in all tissues except hepatopancreas, it is not effected by any of the inhibitors used in tentacles, but in intestine and mantle it is inhibited by Paraoxon and $AgNO_3$, where as in foot it is inhibited by pCMB and $AgNO_3$. The Rm value .58 was found in hepatopancreas and mantle. In hepatopancreas it is inhibited by Paraoxon and Physostigmine, hence it is considered as ChE esterase and another tissue it is classified as ArE esterase. The zone with Rm value .48 was found in three tissues viz.; ctenidia, hepatopancreas and foot in these three tissues it is inhibited by pCMB, Paraoxon and $AgNO_3$. So, it is considered as Esdp esterase. The zone with Rm value .33 was found in foot with ER esterase and tentacles with AcE esterase. The zone with Rm value .25 is found in ctenidia and mantle it is considered as ER and Esdp esterase respectively.

Graph: 1.2. Tissue specific distribution of esterase zones in 15-day old *Pomacea cumingi*

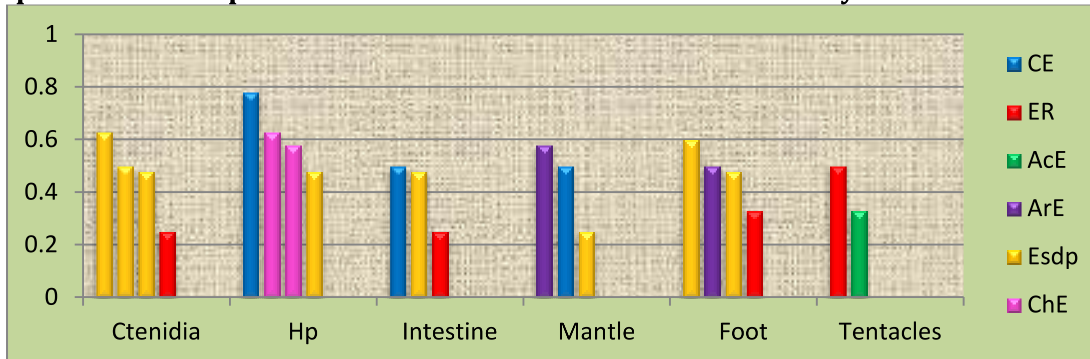
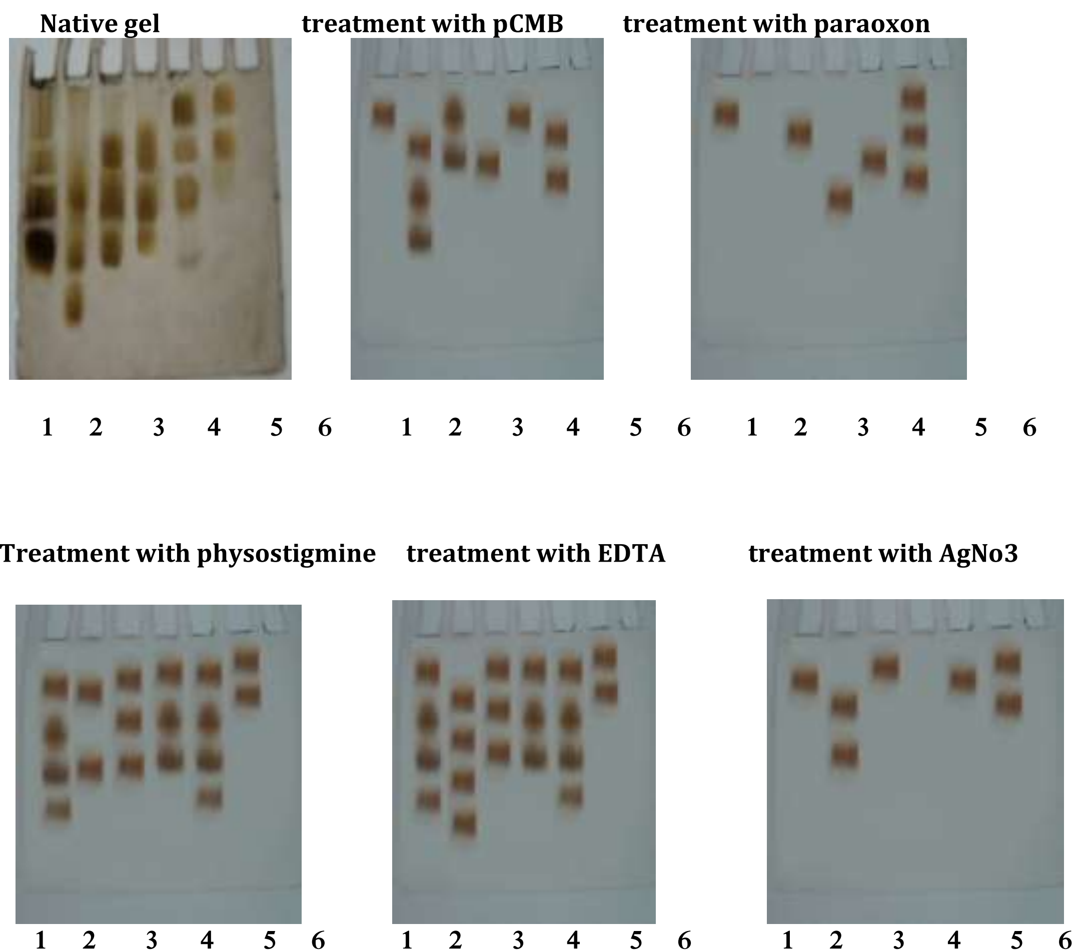


PLATE-II Electrophoretic patterns of 15-day old *Pomacea cumingi*



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

CONCLUSION

In the present study zymogram analysis indicated that the esterase activity in adult *P.cumingi* tissues were significantly higher in the ctenidia of 5-day old females than those of 15-day old respectively , mean while the activity was greater in the hepatopancreas, intestine, mantle and foot of 15-day females than in 5day old ones.

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