ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. VIII: 405-4408: 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.com

EFFECT OF EYESTALK ABLATION ON PHENOLOXIDASE ACTIVITY OF FRESHWATER CRAB, *BARYTELPHUSA CUNICULARIS* (WEST- WOOD: 1872)

Swapnaja N. Mahajan and Enciy R. Martin

KEYWORDS

ProPO system
Phenol oxidase
Eyestalk ablation
Barytelphusa cunicularis

Proceedings of National Conference on Harmony with Nature in Context of Bioresources and Environmental Health
(HARMONY - 2015)
November 23 - 25, 2015, Aurangabad,
organized by
Department of Zoology,
Dr. Babasaheb Ambedkar Marathwada University
Aurangabad (Maharashtra) 431 004
in association with
National Environmentalists Association, India
www.neaindia.org

The Ecoscan
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

SWAPNAJA N. MAHAJAN AND ENCIY R. MARTIN*
Department of Zoology,
Dr. Babasaheb Ambedkar Marathwada University, Aurangabad - 431 004, Maharashtra, INDIA
e-mail: martin_encily@yahoo.com

ABSTRACT

In the present study, we investigated the role of eyestalk ablation on phenol oxidase activity. The purification of phenol oxidase enzymes from eyestalk ablated crabs showed a statistically significant decrease in enzyme activity and specific activity compared to control crabs. Thus, the studies conclude that eyestalks appear to control phenol oxidase or ProPO system thus, may have a role in immune regulation.

***Corresponding author**

INTRODUCTION

In crustacean the X -Organ Sinus Gland Complex (XOSG) is localised in eyestalk and is the principle neuroendocrine gland. The XOSG synthesize, store and secrete hormones, which regulate several metabolic processes (Review Fingerman, 1997). In crustacea eyestalk ablation has been used since, 1970 to improve the aquaculture production in *Penaeusspp* (Bary and Lawrance, 1992). In vertebrates, the immune response is under the control of central nervous system, whereas in invertebrates it is still uncertain if neuro-endocrine glands have any role in invertebrate's immune system (Asusena *et al.*, 2012). In crustacean, one of the important enzyme belonging to humoral immunity and associated with innate immune system is Phenol oxidase (Martin, 2009; Martin, 2011; Alvarez and Chung, 2013; Fan *et al.*, 2011). In invertebrates an invading pathogen is first recognized by the Prophenoloxidase (ProPO) system. On activation of this system a series of cascade reaction occurs is which result in pathogen encapsulation by melanin formation. This is an important melanogenic pathway which involve in immune reaction by hemocytes (Burnett and Burnett, 2015).

Various workers reported that the eyestalk ablation showed metabolic or immunologic consequences in crustaceans (Martin *et al.*, 1993; Rosas *et al.*, 1993; Maggion *et al.*, 2004; Sainz-Hernandez *et al.*, 2008). Despite numerous studies on the prophenol oxidase (ProPO) activating system (Sritunyalucksana and Soderhall, 2000), there are no studies in literature on phenol oxidase after the eyestalk ablation. Perazzolo *et al.* (2002) observed a decrease in total phenoloxidase (PO) activity seven days after ablation of shrimp, but explained the decrease because of stress, instead of endocrine control.

Thus, the present study was carried out to ascertain, if bilateral eyestalk ablation has a significant effect on PO activity of the freshwater crab *Barytelphusa cunicularis* (West- Wood).

MATERIALS AND METHODS

In the present study, freshwater crab *Barytelphusa cunnicularis* (West-Wood) were procured from river Godavari, Near Kaigaon Toka, Aurangabad. After bringing to the laboratory the crabs were washed thoroughly to remove slime, dirt etc. and transferred to 10L trough containing de-chlorinated tap water with aerator. Water was changed every 2h for 4 times on the first day. Thereafter, water was changed every alternate day. Only female crabs acclimated for more than four days, were used for experiment. The crabs were maintained in water containing DO 6.54-8mg/L; Temperature 25-$\pm$1ºC, under normal D: L cycle. Female crabs weighing about 191.33 $\pm$ 0.4g with carapace length of 6.5 $\pm$ 0.1cm were only considered for the present study.

Preparation of Hemocyte lysate supernatant (HLS)

The HLS was prepared by the method described by Martin (2011), except the cell homogenate was centrifuged at 15000xg for 20 minutes and the resulting supernatant was used as a source of phenol oxidase. All experiments were carried

Table1: The Purification of PO enzyme from Eyestalked Crab

Sample	volume (ml)	Protein abs^{ns} @ 280nm	Total protein (mg/ml)	Enzyme activity abs^{ns}@ 490nm	Enzyme Activity(U)	Specific activity(U/mg)	Yield(%)	Purification (fold)
HLS	20	0.360	26.0	0.038	6.32×10^{-3}	$2.43\times10^{-4,}$	100	1
ASppt(30-40%)	16.8	0.369	10.92	0.036	5.04×10^{-3}	4.61×10^{-4}	79.74	1.89
Dialysis	14	0.208	5.04	0.029	3.37×10^{-3}	6.69×10^{-4}	53.32	2.75
Gel filtration (F-6)	3	0.073	0.039	0.017	4.25×10^{-4}	108.9×10^{-4}	6.72	44.81

Table 2: The Purification of PO enzyme from Eyestalk Ablated Crab

Sample	Volume (ml)	Protein abs^{ns} @ 280nm	Total protein (mg/ml)	Enzyme activity abs^{ns}@ 490nm	Enzyme Activity(U)	Specific activity(U/mg)	Yield (%)	Purification (fold)
HLS	20	0.492	17.8	0.025	4.16×10^{-3}	2.33×10^{-4}	100	1
ASppt(30-40%)	16	0.380	11.04	0.029	3.86×10^{-3}	3.50×10^{-4}	92.78	1.5
Dialysis	13	0.193	4.55	0.026	2.81×10^{-3}	6.17×10^{-4}	67.54	2.64
Gel filtration (F-6)	3	0.126	0.066	0.011	2.74×10^{-4}	41.6×10^{-4}	6.58	17.85

out at 4ºC.

Eyestalk ablation

Acclimated crabs were divided into two group's viz. an eyestalked (Control with eyestlked) and other group (Experimental - eyestalk ablated). Eyestalk ablation was carried out meticulously, such that crabs experienced the minimum stress. This was done by following the eyestalk ablation technique as described by Allayayi *et al.* (2011).

Enzyme Purification

The HLS was subjected to salt fractionation at 30-40% ammonium sulphate pH 7.5 and temperature 4ºC. The precipitate was removed by centrifugation and was re-dissolved in 20mM sodium cacodylate (CAC) buffer; pH 7.5.This re-dissolved solution was subjected to dialysis in a 1L, 20mM CAC buffer for overnight. The dialyzed protein solution was assayed for PO. The protein solution was centrifuged at 14000xg for 30 minutes. Only the salt free solution was passed through a Sephadex (G-75) column chromatography previously pre- equilibrated with CAC buffer, pH 7.5. The eluant volume collected was 3ml at the rate of 0.7ml/min.

Enzyme Assay

The PO activity was measured according to method of Ashida and Dohke, (1988) with modifications as suggested by Fan *et al*. (2011). PO activity was estimated as the increment in the rate of absorbance. An increase of 0.001 per minute was taken to be 1 unit (U): activityA490 $\times$ 10^{-3}/minutes.

Protein Estimation

The protein concentration of different PO preparation was measured by UV method, using BSA as the protein standard.

Statistical analysis

The data were subjected to statistical analysis by 'student t test'. P value <0.05 is considered to be statistically significant.

RESULTS AND DISCUSSION

The PO purification scheme and results are summarized in Table (with eyestalk) and Table 2 (eyestalk ablated). In crustaceans, the eyestalk is the seat of X-Organ Sinus Gland (XOSG), and is the main neuroendocrine centre that regulates physiological as well as metabolic process (Hopkins, 2012).In the present study, the PO activity in eyestalk ablated crabs showed a statistically ($p<0.05$) significant decrease compared with the control crabs. This suggests that eyestalk ablation may result in direct or indirect influence on innate immunity in crustacean. There are reports that, in *M. americanum* females, immunologic variables such as ProPO and PO activity were not affected by unilateral eyestalk ablation (Asusena *et al.,* 2012; Perazzolo, 2002; Maggioni *et al.,* 2004). However, some worker reported that bilateral eyestalk ablation resulted in alternation in immune response (Maggioni *et al.,* 2004; Varghese, 2008). Sainz-Hernández (2008) reported a decrease in PO activity in bilaterally eyestalk ablated shrimp *L. vannamei.* Our study with both eyestalk ablation is in confirmation with report of Sainz-Hernández (2008). Our finding clearly, suggests that PO activity decreased in eyestalk ablated crab. Thus, we conclude that, PO enzyme necessary for innate immunity is regulated by the eyestalk. The regulatory mechanism and how it influenced immunity in this crab is currently being elucidated in our laboratory.

ACKNOWLEDGEMENT

Authors thank the Head, Department of Zoology for providing necessary facilities.

REFERENCES

Allayie, S. A., Ravichandran, S. and Bhat, B. A. 2011. Hormonal regulatory role of eyestalk factors on growth of heart in mud crab, *Scylla serrete* Saudi *J. Biological Science.* **18:** 283-286.

Asusena, A. C. J., Carlos, S. H. J., Arturo, F. C. J., Genaro, D. P. 2012. The Effects of Eyestalk Ablation on the Reproductive and Immune Function of Female *Macrobrachium americanum. J Aquacult Res. Dev.* 3:156 doi:10.4172/2155-9546.1000156

Ashida, M. and Dohke, K. 1988. Activation of pro-phenoloxidase by the activating enzyme of the silkworm, *Bombyxmori*. Insect Biochem, **10:** 37-47.

Bray, W. A. and Lawrence, A. 1992. Reproduction in *Penaeus* species in captivity. In: Marine Shrimp culture: Principles and Practices. Fast, A.W., Lester, J. (Ed.) Elsevier, Amsterdam, pp. 93-170.

Burnett, K. G. and Burnett, L. E. 2015. Respiratory and Metabolic Impacts of Crutacean Immunity: Are there Implications for the Insects? *Integrative and Comparative Biology*. pp. 1-13.

Fan, T. J., Jiang, Z., Fan, X. Y., Yu, M. M. and Jiang, G. J. 2011. Purification and characterization of phenoloxidase from brine shrimp *Artemia sinica*, Acta Biochim Biophys Sin (2011): pp. 1-7

Fingerman, M. 1997. Crustacean Endocrinology: A Retrospective, Prospective, and Introspective Analysis. *Physiological Zoology*. **70(3):** 257-269.

Hopkins, P. M. 2012. The eye it: A brief history of crustacean neuroendocrinology. *General and Comparative Endocrinology*. **175:** 357-366.

Maggioni, D. S., Andreatta, E. R., Hermes, E. M. and Barracco, A. 2004. Evaluation of some hemato-immunological parameters in female shrimp *Litopenaeus vannamei* submitted to unilateral eyestalk ablation in association with a diet supplemented with super doses of ascorbic acid as a form of immune stimulation. *Aquaculture*. **241:** 501-515.

Martin, R. E., Sarojini, R. and Nagabhushanam, R. 1993. Species or group specificity in biological studies of crustacean hyperglycemic Hormone. *Ind. J. Comp. Animal. Physiol.* **11(1):** 47-49.

Martin, E. R. 2009. Purification of a 52kDa proteinase inhibitor of prophenoloxidase activity system from hemocyte Lysate Supernatant of Freshwater crab, *Barytelphusa cunicularis* (*West - Wood) Bioscan*. **4(2):** 227.

Martin, E. R. 2011. Purification of Enzyme Phenoloxidase from Freshwater Crab, *Barytelphusa cunicularis* and its Kinetic Study**.** *World J. Zoology*. **6(4):** 357-359, 20

Perazzolo, L. M., Gargioni, R., Ogliari, P., Barranco, M. A. A. 2002. Evaluation of some hemato-immunological parameters in the shrimp *Farfantepenaeus paulensis* submitted to environmental and physiological stress. *Aquaculture*. 214, pp. 19-33.

Rosas, C., Fernandez, I., Brito, R., Iglesias, E. D. 1993. The effect of eyestalk ablation on the energy balance of pink shrimp, *Penaeus notiales*. *Comp. Biochem. Physiol. 104A,* pp.183-187.

Sainz-Hernández, J. C., Racotta, D., Hernandez-Lopez, J. and Dumas, S. 2008. Effect of eyestalk ablation on metabolism and immune system in shrimps. *Aquaculture*. **283:** 188-193.

Sritunyalucksana, K. and Soderhall, K. 2000. The proPO and clotting system in crustaceans. *Aquaculture*. **191:** 53-69.

Verghese, B., Radhakrishnan, E. and Padhi, A. 2008. Effect of moulting, eyestalk ablation, starvation and transportation on the immune response of Indian spiny lobster, *Panulirus homarus*. *Aquaculture Research*. **39:** 1009-1013.