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Assessment of Phenoloxidase Activity in Hemolymph of Pre, Inter and Post-Molting Stages

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Abstract- Phenoloxidase (PO) is an enzyme that vitally protects arthropods both internally and externally. PO protects arthropods internally from invasions by infectious microorganisms, and protects them externally by catalyzing the sclerotization of an animal's newly formed or repaired exoskeleton In the present study determine the phenoloxidase activity in hemolymph of pre, inter and post molting stages. The phenoloxidase activity was highest in pre-molting followed by inter-molting and post-molting. The stability of the POs was very different depending on a number of factors such as temperatures, pH, substrate used, ionic strength, buffer system, and time of incubation. The results of the present study concluded that pre-molting stage has greater activity of phenol oxidase in hemolymph.

Keywords*—Phenoloxidase, Pre, Inter and Post molting stages, hemolymph.*

I. INTRODUCTION

Phenoloxidase (PO) is an enzyme that vitally protects arthropods both internally and externally. PO protects arthropods internally from invasions by infectious microorganisms, and protects them externally by catalyzing the sclerotization of an animal's newly formed or repaired exoskeleton [1]. Using oxygen as a proton acceptor, PO catalyzes the conversion of phenols, for example L-3,4 dihydroxyphenylalanine (L-DOPA), to quinones which spontaneously rearrange into the end product melanin [2,3]. Melanin is responsible for clotting hemolymph at wounds, for darkening and hardening post-molt carapace, and for minimizing bacterial and fungal infections through encapsulation [4,5,2]. PO is stored and synthesized as prophenoloxidase (PPO), the inactive form of PO [5]. In the presence of a bacterial or fungal infection, hemocytes release PPO into the hemolymph, where PPO is converted to PO by a serine protease [2]. In the laboratory, PPO can be activated using detergents, for instance sodium dodecylsulfate (SDS) [6].

II. MATERIALS AND METHODS

Sample collection: - The sample used in this study was Portunus sanguinolentus collected directly from a Nagappattinum coast, Tamil Nadu India and the hemolymph collected immediately for phenoloxidase.

Determination of Phenol oxidase activity: - Phenol oxidase activity assayed by Preston & Taylor [7]. The haemolymph was collected in a cold tissue homogenizer. The clotted haemolymph was homogenized and centrifuged at 4000 rpm to remove the particulate material for 10 minutes using a refrigerated centrifuge. Muscle homogenized using phosphate buffer. The supernatant was used as enzyme source. With 2 ml of substrate, add 0.2 ml of enzyme source. The increase in O.D. of the mixture should be noted immediately at 420 nm in a spectrophotometer. Note the O.D. of the same upto 3 minutes for every 30 seconds interval. Prepare the control with 0.2 ml of distilled water in 2 ml of substrate. The results can be expressed as average O.D. /mg protein/minute.

III. RESULTS AND DISCUSSION

The enzyme phenoloxidase (PO) is known as tyrosinase, catechol oxidase, o-diphenol oxidase, monophenol oxidase, among other (according to the type of substrate they use) is present in and under the shell of shrimp and others crustaceans, and acts as a catalyst in the reaction that causes blackspot, and is a crucial component of the arthropod proPO system and the key enzyme in the synthesis of melanin, responsible for this browning process [8, 9]. PO is a bifunctional copper containing enzyme that belongs to the type-3 copper protein family and possesses both tyrosinase/ monophenolase and catecholase/diphenoloase activities [10,

11] that in turn convert a variety of monophenols and o-diphenols into o-quinone that ultimately act as precursors for melanin production [12, 13]. Distribution of PPOs in the different body parts of crustaceans is diverse. They are located mostly in the cuticle, specifically on the internal surface, inside chromatophores, however these enzymes were also found in the hepatopancreas (where they are synthesized), muscle and hemolymph of *Penaeus monodon* [13, 14]. POs exist in crustaceans as zymogens (proPO) and are believed to be involved in both immunity and self-recognition [13] processes. ProPO activation happens because of the serine proteinase cascade triggered by microbial compounds (carbohydrates and lipopolysaccharides) and a series of other proteins [15].

In the present study determine the phenoloxidase activity in pre, inter and post molting stages. The phenoloxidase activity was highest in pre-molting followed by inter-molting and post-molting and represent in table 1. The reduced activity may be climate, temperature and pH of the environment. PO activity would be also reduced due to the significant conformational change of the protein. In a number of studies, the values for optimum pH of PO activity are reported between pH 6.0 and pH 8.0. The influence of temperature on the stability of PO from crustaceans indicates that the enzymes appeared to have similar stability regions (20-50^{o}C). The stability of the POs was very different depending on a number of factors such as temperatures, pH, substrate used, ionic strength, buffer system, and time of incubation [13].

Table 1. Determination of phenoloxidase activity in pre, inter and post molting stages

S. No.	Sample	Result (O.D./mg protein/minute)
1.	Pre-molting	1.557± 0.108
2.	Post-molting	0.186 ±0.013
3.	Inter -molting	0.208±0.014

Values are expressed as mean ± SD for six crabs

PO were considered the only enzymes involved in the oxidation of diphenols to o-quinones in the hemolymph, The mechanism of phenoloxidase (PO) catalyzes the oxidation of phenols into o-quinones that are highly reactive compounds. They can polymerize spontaneously to form high-molecular-weight compounds or brown pigments (melanin), or react with amino acids and proteins that enhance the brown color produced [13]. In crustaceans, POs are involved in cuticle sclerotization and healing of injuries, their activation has a key role in the primary immune response system. Therefore is necessary to carry out harvesting without too much truculence to avoid wounding the animals and triggering the melanosic process. During post-harvest stages, pro-PO can be activated through proteolytic enzymes leached from the crustaceans digestive tract; the hydrolysis caused by these proteases also originates substrates for active PO [16, 9].

IV. CONCLUSION

The results of the present study concluded that pre-molting stage has greater activity of phenol oxidase in haemolymph.

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