SOME INVESTIGATIONS ON THE ACTIVITY OF PHENOL OXIDASE IN THE ISOPOD *CIROLANA FLUVIATILIS*

ABSTRACT

Phenol oxidase activity in the isopod *Cirolana fluviatilis* averaged 0.0015 w.units/mg protein/min in the larger isopod samples (10-17 mg) and 0.00034 O.D. units/mg protein/min in the smaller samples (6-9 mg). The enzyme showed equal affinity for epinephrine and dopamine and >50% affinity for DOPA.

PHENOL oxidase (EC. 1.10.3.1), a widely studied enzyme plays an important role in the formation of egg capsules, cocoons, byssal threads, cement and carapace in different species of animals and has been reported to be present in most crustaceans (Smith and Seederhäell, 1992). Moreover, the enzyme shows variations in affinity for substrate in different animals, as it is known to have greater affinity for DOPA (3,4-dihydroxy phenylalanine) in platyhelminths and molluscs, while in crustaceans epinephrine has been reported as the preferred substrate. *Cirolana fluviatilis*, an isopod crustacean occurring in the Cochin backwaters, has a sturdy exoskeleton very smaller to that found in prawns. It was therefore deemed worthwhile to investigate the activity of phenol oxidase in this isopod and study its affinity for various substrates.

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The live samples were brought to the laboratory and after blotting carefully to remove excess moisture with a filter paper, each sample was weighed individually. Based upon the weights the samples were segregated into two batches, the larger size group ranging in weight from 10-17 mg and the smaller size group ranging in weight from 6-9 mg. The whole animals were homogenized with ice-cold buffer by the help of an agate pestle and mortar and suitable aliquots taken for estimation of protein and enzyme activity. Protein was determined by the method of Lowry *et al.* (1951) using Bovine serum albumin as standard. Phenol oxidase activity was estimated in the whole extracts by the method of Preston and Taylor (1970) with slight modifications using the substrates epinephrine, dopamine, DOPA and lyrosine. Activity was expressed as the amount of enzyme required to form one O.D. unit of adrenochrome in three minutes under specified conditions while specific activity was expressed as enzyme units/mg protein.

The isopod extract exhibited phenol oxidase activity. In the larger size animals having 8 per cent protein concentration (10-17 mg), an activity of 0.0015 O.D. units/mg protein/min/per animal was obtained, while in the smaller animals having a protein concentration of 6 per cent, 0.00034 O.D. units/mg protein/min/animal was obtained.

The distribution and location of regions with maximum phenol oxidase activity were studied in different species of prawns by Antony and Nair (1968). The head juice and tail extracts were reported to have very high order of enzyme activity in comparison to the cuticle and muscle. The enzyme level fluctuates during the moult cycle in exoskeleton as well as haemolymph of *P. indicus* reaching a peak during immediate moult (Sridhar and Diwan, unpublished).

However, in the present study because of the small size, the entire animal extract was assayed and not individual regions and the activity is given in Table 1, in comparison to the activity obtained in different segments of the exoskeleton in prawn *P. indicus.*

TABLE 1. *Phenol oxidase activity of Cirolana fluviatilis in comparison to that of prawn P. indicus*

Part	Enzyme Activity	Fold increase
Isopod extract	0.0015	1
Haemolymph	0.031	21
Rostrum	0.074	49
Carapace	0.093	62
Abdomen	0.082	55
Telson	0.2033	136
Uropod	0.095	63

The affinity of the isopod enzyme for various substrates was investigated and results are presented in Table 2. The enzyme exhibited strong prefrence for three substrates viz. epinephrine, dopamine and DOPA. The affinity for epinephrine and dopamine was more or less similar, a finding contradictory to that observed in the case of prawns where the affinity for dopamine is almost one fourth to that observed in the case of epinephrine (Anilkumar, 1992). However, the affinity shown by the isopod phenol oxidase for DOPA was greater than 50 per cent an observation in agreement to that obtained in all species belonging to the lower phyla.

TABLE 2. *Substrate specificity of phenol oxidase from the isopod Cirolana fluviatilis in comparison to that of the penaeid prawn P.indicus*

Substrate	% phenol oxidase activity	
	Isopod	*P.indicus*
Epinephrine	100	100
DOPA	68.42	27
Dopamine	94.73	46
Tyrosine	nil	nil

The enzyme from isopods appears to be highly versatile as it can use any substrate in comparison to that of prawns. However, there is need for further detailed studies in order to investigate the presence of a single enzyme or different forms of the same enzyme, exhibiting affinity for a number of substrates as observed in the present study.

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