STUDIES ON PHENOLASE ENZYMES IN PRAWNS
I. THE DISTRIBUTION AND PHYSICAL PROPERTIES OF PHENOLASES IN PENAEID PRAWNS

P. D. ANTONY AND M. RAJENDRANATHAN NAIR
Central Institute of Fisheries Technology, Cochin-11

The distribution of phenolases in certain species of Penaeid prawns has been studied. Attempts were made to locate the regions of maximum enzyme activity in the prawns. The relative dopase activity has been examined in extracts from head, tail, shell with cuticles and muscle. The head juice and tail extracts were found to register very high order of enzymic activity. *Metapenaeus affinis, Metapenaeus monoceros* and *Penaeus indicus* record comparatively higher enzymic activity than *Parapenaeopsis stylifera* and *Metapenaeus dobsoni.* No definite relationship has been found between the relative activity of the enzyme and size grade at least in one species examined. Experiments were done to determine the pH optima of the enzyme and the influence of pH on its deactivation. Exposure to higher temperatures upto 55C was shown to activate the crude enzyme considerably. The possible implications of the observations have been discussed.

INTRODUCTION

Melanosis is a well known phenomenon responsible for the incidence of black spots in prawns and other crustacea and is due to the activity of certain phenolase enzymes. It has been first observed by Fieger *et al.*(1950)that the discolouration usually begins in membranes which connect ends of overlapping segments. In serverely blackened shrlmp, pronounced black bands appear in the tail and head fins, shell segment, crawling legs and tips of swimming legs. Bailey *et al.* (1960 b) have reported that among the most important enzyme contributing to shrimp melanogenesis appears to be the one released from blood leucocytes. The distribution of phenolase enzymes in a few species of prawns has been investigated by those workers who found the enzymes to be localised in exoskeleton, antenna, blood and head juice. However, information on this subject is lacking in the case commercially important species of tropical waters. The

present paper gives an account of the studies made on the distribution of phenolase enzymes in certain species of Penaeid prawns and the physical poperties of the enzyme preparation with regard to its sensitivity towards pH and temperature conditions.

Materials and Methods

Marine species of prawns used for the study were obtained from fish landing places in and around Cochin and brackish water species from the adjoining backwaters. The samples were kept in polythene bags and chilled in ice pending analysis. Weighed amounts of head, shell, tail and muscle portions were blended with 2 parts of cold water (below 4C) in a waring blendor for 2 minutes and centrifuged in a Refrigerated Centrifuge at 7500 r. p. m. at 0C. The centrifugate was diluted 10 times for the assay except in the case of muscle extract, which was used as such without dilution.

Dopa oxidase activity

Dopa oxidase activity was determined by the method of Bailey *et al.* (1960a). The assay mixture contained 1 ml. of the enzyme, 2ml. of 3:4 dihydroxy phenylalanine (1.97 mg/ml) in a total volume of 11 ml. made up with 0.05 M Sodium phosphate buffer (pH 6.8). The optical density of the solution was measured at at 470 mμ after incubation for 3 hours at 35C and relative dopa oxidase activity expressed as O.D x 10^3 after deducting the endogenous oxidation of the enzyme and substrate. The specific activity is obtained by dividing the relative activity by the amount of protein in mg/ml. of the enzyme extract.

The colorimetric method of Smith & Stoltz (1949) was adopted in certain experiments employing the oxidation of leucodye of 2:6-dichlorophenol indophenol. In this method also, the relative activity was obtained by multiplying O. D. at 660 mμ by 10^3.

To study the effect of heat on the enzyme activity, lots of enzyme are taken up in phosphate buffer (pH 6.8) and maintained at temperatures 30–80C for 60 sec. and dopase action measured by the Indophenol method.

Optimum pH and acidic deactivation

Optimum pH of the enzyme activity was determined with 1 ml. of the enzyme and 2 ml. of dopa (1.97 mg/ml) in a final volume of 11 ml. in buffers of pH ranging from 5.8 to 8. The acidic deactivation was determined by incubating 1 ml. of the enzyme in different buffers at 4C for 24 hours and measuring the enzyme activity after adding dopa, a reagent blank being run with the enzyme and buffer solution and dopa immediately before the assay.

Results

The relative dopa oxidase activities of the head juice of species of Penaeid prawns of commercial size group are given in Table I. Table II represents the relative catecholase and tyrosinase activities in marine prawns *P. indicus* belonging to different size groups. The different anatomical regions viz. head, tail, shell with pellicles and muscle have been tested for the distribution of relative dopase activity (Table III). The preparation from head recorded the maximum specific activity and that from muscle, the least.

The behaviour of the crude enzyme on exposure to higher temperatures has been studied with extract from head juice of *M. affinis* at pH 6.8 with respect to dopase activity. As seen from Fig. I the enzyme is very much activated over the temperature range 30–55C.

TABLE I RELATIVE DOPASE ACTIVITY OF THE HEAD JUICE OF DIFFERENT PENAEID PRAWNS.

Species	Vernacular name	Source	Size in commercial catches (length in cms.)	Relative Dopase activity
Meta penaeus affinis	Kazhandan	Marine	12.0–13.0	702.4
Metapenaeus monoceros	Choodan	Backwater	12.5–13.5	520.5
Penaeus indicus	Naran	Backwater	13.0–14.5	400.0
Parapenaeopsis stylifera	Karikadi	Marine	8.0– 9.0	70.6
Metapenaeus dobsoni	Poovalan	Backwater	8.5– 9.5	80.9

TABLE II PHENOLASE ACTIVITY OF *P. INDICUS* (MARINE) OF DIFFERENT SIZE GRUOP

Size group (Nos/450 g)	Phenolase activity of head juice	
	Relative catecholase activity	Relative tyrosinase activity
11–15	820	310
16–20	154	73
21–25	189	119
26–30	209	187

TABLE III DISTRIBUTION OF DOPASE ACTIVITY IN *M. MONOCEROS & M. AFFINIS*

Location	*M. monoceros*		*M. affinis*	
	Rel. Activity	Specific activity	Rel. activity	Specific activity
Head	460	922	894	1794
Tail	427	820	450	1572
Shell including Pellicle	241	486	387	1279
Muscle	6.1	3.8	8.0	1.3

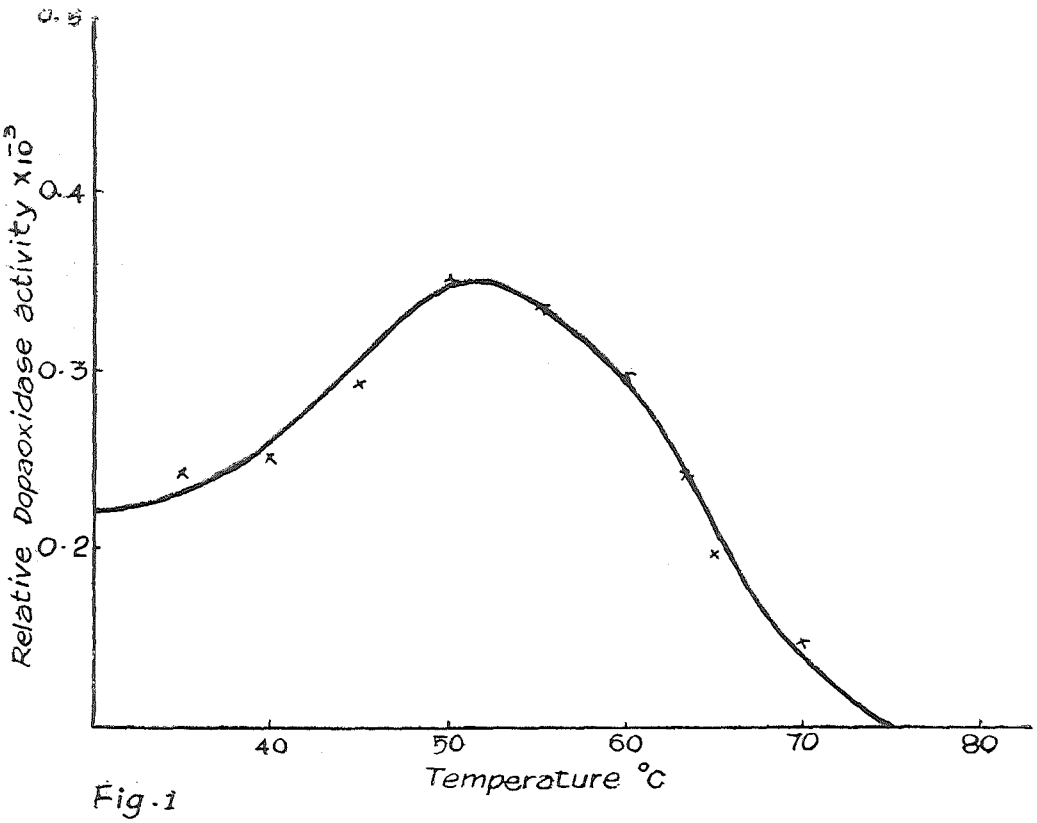

Fig. 1

Effect of temperature on Dopa oxidase activity of M. affinis

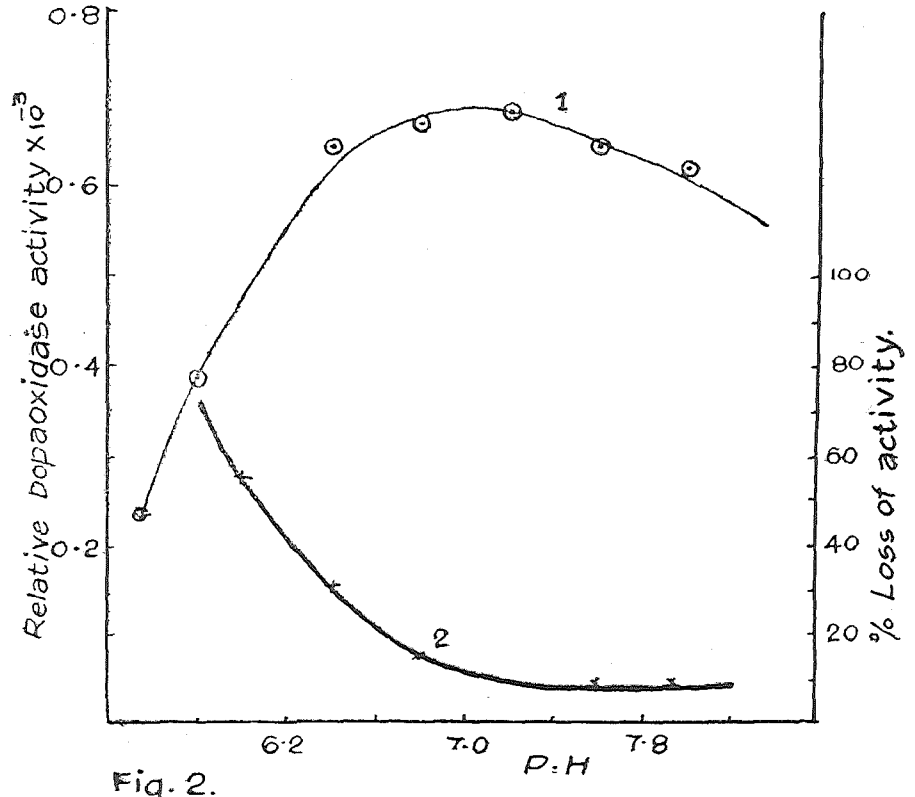

Fig. 2.

Effect of p.H on activity of Dopa oxidase

1. p.H - Activity Curve.
2. Acidic deactivation curve.

The activity of the crude enzymes from *M. monoceros* has been tested over the pH range 5.8 to 8.0. The results (Fig. 2) show that the enzyme has a very wide pH range (6.4 – 7.6) at which it exerts the maximum activity. Decrease in activity is evident below pH 6.0. The preparation showed linear relationship with substrate Dopa over the concentration 1×10^{-3}M to 8×10^{-3}M.

The acidic deactivation of the enzyme has been further examined with the head juice of *M. affinis.* The dopa oxidase activity of the enzyme recorded a rapid fall when incubated at pH in acid range. The enzyme is however found to be comparatively stable at pH range on alkaline side, as also represented in Fig. II.

Discussion

The high dopa oxidase as observed in Penaeid prawns *P. indicus, M. monoceros* and *M. affinis* would account for the high incidence of black spots in these species and their poor quality when stored in refrigerated condition as compared to *P. stylifera* and *M. dobsoni.* Although Bailey *et al.* (1960) have reported that phenolase activity concentrated in the blood of *P. azeticus* and *P. setiferus* would contribute to the activity of the head juice, the experiments reported here have shown that blood is not the only principal source of activity. It has been found, that in all the species examined, the extract from tail also showed considerable activity though next to head juice. The segmental glands may also contribute much to the dopa oxidase activity and is supported by the fact that the onset of black spots occurs at these segments. The fact that prawns subjected to long dragging periods during trawling which cause rupture of these glands, develop black spots at a faster rate, would further support the view.

The enzymes isolated from different species appear to behave in an identical manner with respect to the oxidation of dopa, catechol and tyrosine. Experiments with different size grades of prawns *P. indicus* have shown very high order of catecholase and tyrosinase activity in head juice of larger size group, but the activity does not follow any regular order in relation to the size of prawns. Although the factors responsible for the differences in relative activity could not be clearly established, it has been suggested by Bailey *et al. (loc cit.)* that absence of dihydric phenols in preparations from some species and the presence of these substrates in other preparations might account for the differences in behaviour. It was also shown that preparation from antenna which contained *O-dihydric* phenols greatly enhances the oxidation of monophenols. This is partly corroborated by *in vitro* studies with phenolase enzymes from *M. affinis.* It is observed that diluted extract from the head juice of *M. affinis* darkened even without addition of substrate on incubation at 37C whereas original shell extract and muscle extract showed activity only on addition of dopa/catechol at 10^{-3}M level.

The enzyme shows activation when held at temperature above 30C upto 55C and the increased activity during exposure to higher temperatures has been attributed to the phenolase enzyme being proenzymic in nature with its active centres getting exposed by heat (Bodin *et al.*, 1944). This is borne out by the ice storage experiments with prawns which were previously exposed to deck in fishing trawlers at normal air temperatures for longer durations and which were found to be more prone to black spot formation as compared to prawns iced immediately on catching and kept for same length of storage period.

The enzyme is found to be more active and stable at pH ranges on alkaline side although it has a wide pH maxima 6.4 — 7.6. It is possible that the bacterial action on prawn muscle and slime which could cause a shift in pH towards the alkaline range might also promote the incidence of black spot formation.

ACKNOWLEDGMENT

The authors are grateful to Dr. A. N. Bose and Dr. V. K. Pillai for their keen interest and helpful suggestions.

REFERENCES

Bailey M. E., E. A. Fieger and A. F. Novak. 1960a. *Food Res., 25,* 5, 557.

Bailey M. E., E. A. Fieger and A. F. Novak. 1960b. *Food Res.* 25, 5, 565.

Bodine J. H., T. N. Tahmisian and D. L. Hill, 1944. *Arch. Biochem. 4,* 403.

Fieger E. A., A. R. Colmer and J. A. Alford, 1950. *Southern Fisherman 10,* 12.

Smith F. G. and E. Stoltz 1949. *J. Biol. Chem., 179,* 865.