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AMYLOLYTIC ACTIVITY OF THE HEPATOPANCREAS OF *ORATOSQUILLA* NEPA

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Amylolytic activity of hepatopancreas of *Oratosquilla nepa* at different parameters like temperature, pH, enzyme concentration and substrate concentration were carried out. Enzyme activity at different enzyme concentration varied from 4.27 μg/g protein/ minute to 13.16 μg/ minute. Maximum activity was at0.4 ml enzyme concentration. Increase in substrate showed increase in enzyme activity pH even though showed two peaks, maximum activity occurred at pH 8. The temperature maximum observed in amylase activity of *O. nepa* was 33°C. These findings show that digestive amylase of *O. nepa* is well adapted to its ambient water.

Key words : Amylolytic activity, hepatopancreas, *Oratosquilla nepa*.

INTRODUCTION

Digestion in invertebrates occurs only in one area of the gut, which in Crustacea is known as hepatopancreas. It produces a mixture of different types of enzyme. Digestive enzymes, as other enzymes, are catalysts concerned with digestion. They are conveniently considered according to the major food items. Among carbohydrases amylases are of almost universal occurrence in animals.

Review of literature showed that the digestive enzymes of crustaceans were extensively studied only in decapods of crustaceans. No report is available on the study of the enzyme activity of stomatopods. In the present study an attempt has been made to assess amylolytic activity of *O. nepa* at different parameters like temperature, pH, enzyme concentration and substrate concentration.

MATERIALS AND METHODS

A few specimens of *O. nepa* were kept in aerated seawater without feeding for two days to avoid the effect of differential feeding on the enzyme activity. On the third day they were chilled and the hepatopancreas dissected out. Care was taken to remove the entire alimentary canal which was enclosed by it and the adjoining tissues. Known weight of the tissue was homogenized in ice-cold distilled water in a glass homogenizer. The homogenized solution was centrifuged for ten minutes at 3000 rpm. The supernatant served as the enzyme extract. In the present study quantitative estimation of amylase activity were carried out by sacchrogenic assay of Henry & Chiamori (1960).

RESULTS AND DISCUSSION

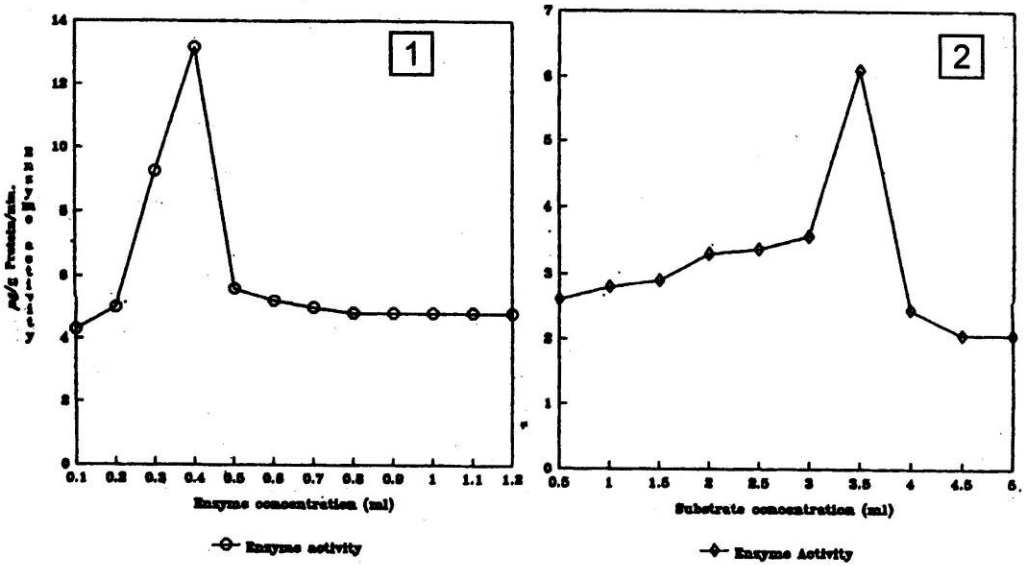
Effect of enzyme conentration : Enzyme activity at different enxyme concentrations were studied and it varied from 4.27 μg/ g protein. Minute to 13.16 μg/g protein/ minute. The maximum activity was showed at 0.4 ml enzyme concentration. After the

optimum concentration an inhibition was observed (Fig. 1).

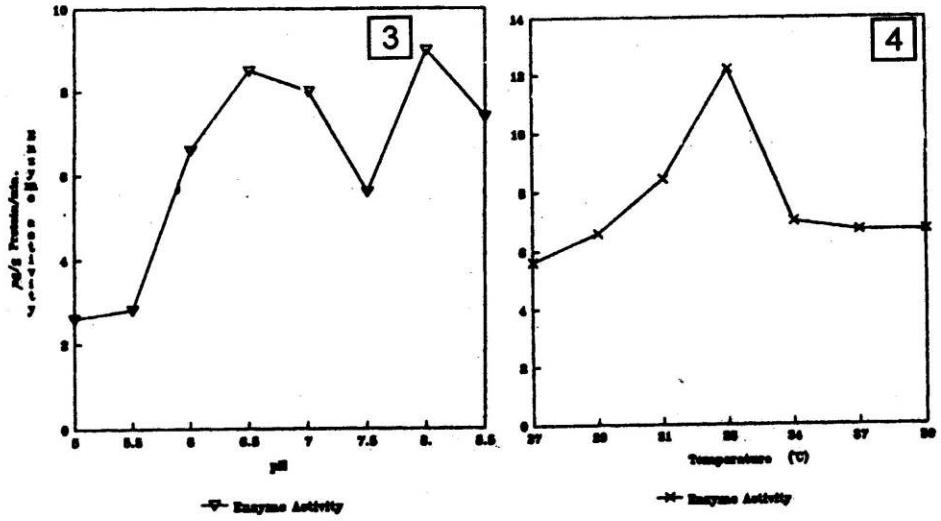
The rate of enzymatic reaction increases with the increase in enzyme concentration. However, at a certain level the addition of the enzyme concentration decreases the enzyme activity. This may be mainly due to the free enzyme reversibly combine with substrate to form enzyme substrate complex and prevent the formation of products (Lehninger, 1993). These results corroborates with the results of Agrawal (1962) for *Orchestia gammarella*. At higher enzyme concentration the amount of product formed was lower. This may be due to the binding of co-enzyme present in the amylases which prevents it from reacting with the starch and produces a considerable inhibition of the overall reaction (Dixon & Webb, 1962).

Effect of substrate concentration : The starch solutions of different concentrations were studied (0.5 ml to 0.5 ml). The activity was maximum for 3.5 ml of starch solution containing 0.0385 g of starch (Fig. 2).

The rate of hydrolysis of starch increases with the increasing substrate concentration up to 3.5 ml of substrate containing 0.385 g of starch. At this stage substrate concentration is so high that all the available catalytic sites on the enzymes are occupied by the substrate molecules or at this velocity enzyme activity is proportional to the substrate concentration (Nagabhushanam & Kodarkar, 1978). Once this point has been reached, further increase in substrate concentration has no effect on the reaction rate and moreover such increase may slow down the rate of reaction and even inhibit the formation of product. Karlson (1969) reported that this inhibition is due to the stronger strength of substrate that possibly convert the total enzyme into enzyme-substrate complex.



Figs. 1-2 : Enzyme activity at different concentrations. **1**. Enzyme, **2**. Substrate.



Figs. 3-4 : Enzyme activity at different, 3. pH; 4. Temperature.

In the present study increase in substrate concentration from 0.5 ml to 3.5 ml (0.0055 g to 0.0385 g of starch) showed the increase in enzyme activity (2.6 to 6.1 μg/ g protein minute). After reaching the maximum, the reaction rate becomes independent of substrate concentration. Although there is an increase in substrate concentration the enzyme activity decreased. This may be due to the indenpendency of amylase from the substrate concentration (Lehninger, 1993).

Effect of pH : The results using phosphate buffer series (5.0-8.5) showed that two amylases peaks were present in the hepatopancreatic juice of *O. nepa* (Fig. 3). It is quite active from pH 5.0 to 8.5 and its optimum activity occurred at pH 6.5 and 8.0.

The pH activity of any enzyme depends on the acid base behaviour of enzyme and substrate. The activity relationship of an enzyme may be a factor in intracellular control of its activity. In acid medium, the amylase of hepatopancreatic juice of *O. nepa* is active between the pH of 5.0-6.5 with an optimum at 6.5. The optimum pH of crustacean amylase ranges from pH 4.5 to 7.5 (Brun & Wojtouricz, 1976).

Effect of temperature : Amylase activity was investigated at different temperature ranging from 270C to 40)C and the optimum activity was found at 33^{0}C (Fig.4). Enzyme activity was found to be increased with increase in temperature. This may be due to the activation of the molecules capable of entering into transition state. Transition stae is the activated condition at which it possesses the maximum energy. Thus it accelerates the rate of chemical reaction (Lehniger, 1993). This observation in *O. nepa* is in agreement with the studies of Divakaran & Pillai (1947) in *Parhyale hawaiensis*. Karunakaran & Dhage (1977) observed that the maximum amylase activity was at temperature 35^{0}C and 44^{0}C in *Penaeus indicus* and *Metapenaeus monoceros*.

The benthic temperature in the sea was never recorded above 35°C pH observed was 8 or more than 8. The temperature maximum observed in amylase activity of *O. nepa* was 33°C. pH even though showed two peaks maximum activity occurred at pH 8. These findings show that the digestive amylase of *O. nepa* is well adapted to its ambient water.

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