Trypsin activity in the hepatopancreas of *Macrobrachium lamarrei* (Crustacea: Decapoda)

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Summary. Trypsin from the hepatopancreas of *Macrobrachium lamarrei* showed optimum activity at pH 7.5 and temperature 45 °C. The enzyme activity increased with the increase in incubation period and enzyme concentration. Michaelis constant of the enzyme was 2.38×10^{-2} M.

The enzymatic components for protein hydrolysis in crustaceans are very much similar to that of digestive proteases in vertebrates, except for the lack of a pepsin-like enzyme[2]. Very few studies have been made regarding the digestive proteases in crustaceans, and these few are limited to the determination of optimum pH and temperature of the enzymes. The present study deals with the nature of the hepatopancreatic trypsin of *Macrobrachium lamarrei.*

Materials and methods. Animals were procured from the local river Gomati. Hepatopancreas was dissected out, weighed immediately and homogenized in ice-cold distilled water using all-glass homogenizer and the homogenate centrifuged at 3000 × g for 15 min at 4 °C. The supernatant was used as the enzyme source. For enzyme assay the reaction mixture containing 0.2 ml of 0.1 M substrate (p-tosyl-L-arginine methyl ester HCl), 0.8 ml of appropriate buffer and 0.1 ml of enzyme extract was incubated at 37 °C for 1 h. After incubation, the enzyme activity was stopped by adding 0.5 ml of 10% trichloroacetic acid (TCA) and the mixture was centrifuged at 2500 rpm for 10 min. Trypsin activity was measured by the colorimetric method of Yang and Davies[3].

The pH for the optimum activity of the enzyme was determined first using different buffer systems (0.1 M sodium citrate-HCl buffer for pH 4.5–5.5; 0.1 M Sörensen's phosphate buffer for pH 5.5–8.0; 0.1 M veronal sodium-HCl buffer for pH 8.0–9.5) and then all other experiments were performed at optimum pH. The enzyme activity was studied at temperatures of 10–80 °C. To see the effect of the incubation period, a pool of reaction mixture was incubated at 37 °C, and samples were withdrawn at different time

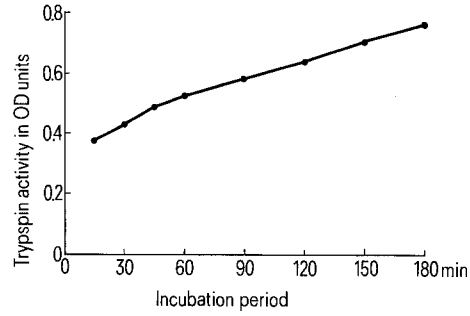

Fig. 3. Effect of incubation period on the activity of hepatopancreatic trypsin of *M. lamarrei.*

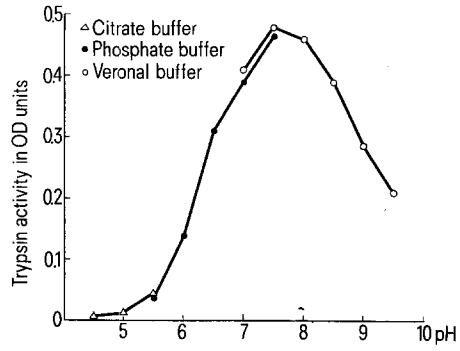

Fig. 1. Effect of pH on the activity of hepatopancreatic trypsin of *M. lamarrei.*

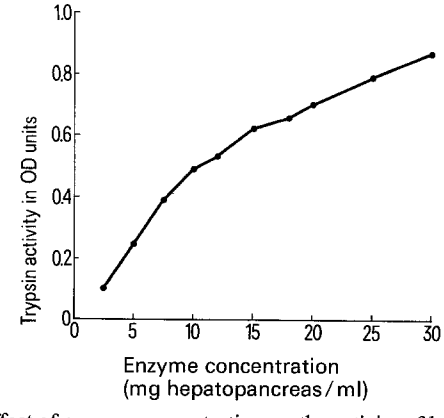

Fig. 4. Effect of enzyme concentration on the activity of hepatopancreatic trypsin of *M. lamarrei.*

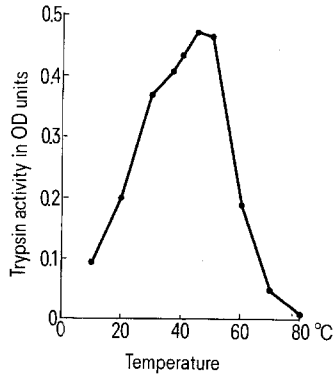

Fig. 2. Effect of temperature on the activity of hepatopancreatic trypsin of *M. lamarrei.*

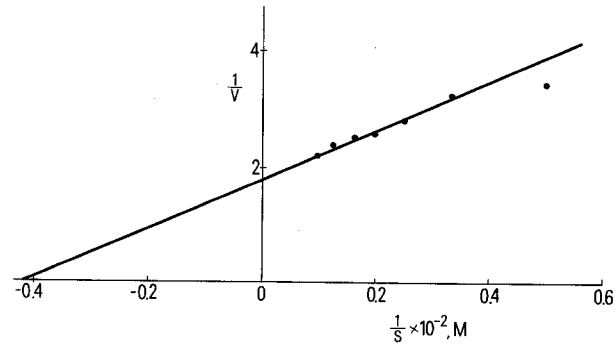

Fig. 5. Lineweaver-Burk plot for Michaelis constant of hepatopancreatic trypsin of *M. lamarrei.*

intervals for enzyme assay. The effect of enzyme concentration was observed at 2.5–30 mg hepatopancreas/ml extract. For substrate concentration experiments, the concentration of TAME varied between 0.005–0.10 M.

Results and discussion. The hepatopancreatic trypsin of *M. lamarrei* showed optimum activity at pH 7.5 (figure 1). As the pH range of the digestive tract of *M. lamarrei* is 6.4–6.7[4], at this pH range the enzyme activity will be quite high, i.e. about 60% of the optimum. The pH for the optimum activity of various crustacean proteases ranged from 7.0–8.53 (table) which closely resembles that found in *M. lamarrei.*

Trypsin from the hepatopancreas of *M. lamarrei* showed optimum activity at 45 °C (figure 2); and more than half-activity at 25–30 °C, which is the temperature of the ambient, showing that the enzyme will be quite active in natural conditions. Same temperature optimum was reported for *Panulirus japonicus*[13] and slightly higher (49 °C) optima for *Orconectes virilis*[7] and *Penaeus setiferus*[14].

pH for the optimum activity of proteases from various crustaceans

Crustacean	Source	pH
Thalamita crenata[5]	Gastric juice	8.53
Orchestia gammarella[6]	Midgut caeca	8.0–8.5
Orconectes virilis[7]	Gastric juice	8.0
Diogenes bicristimanus[8]	Digestive gland	7.0–7.8
Podophthalmus sp.[9]	Midgut gland	7.9
Homarus americanus[10]	Gastric juice	8.0
Streptocephalus dichotomus[11]	Hepatopancreas	7.4–8.0
Cancer borealis and C. irroratus[12]	Hepatopancreas	8.0

The hydrolysis of the substrate increased linearly with the increase in incubation period (figure 3) and enzyme concentration (figure 4), showing that the enzyme activity was not affected by the concentration of hydrolytic products of the substrate as has also been reported in certain insects[3,15,16]. The data on the effect of substrate concentration on the trypsin activity, when plotted in a Lineweaver-Burk plot gave a straight line (figure 5). Michaelis constant (K_m) of the enzyme was found to be 2.38×10^{-2} M.

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1 Acknowledgments. The authors are thankful to University Grants Commission, India for the award of a Junior Research Fellowship.

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The activity of sorbitol dehydrogenase in some mammalian erythrocytes

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Summary. The activity of sorbitol dehydrogenase was found to be high in the red blood cells of man, dog, guinea-pig and mouse and comparatively lower in those of goat, sheep, rabbit, cat and rat.

The enzyme sorbitol dehydrogenase (SDH) is a part of the polyol pathway in which glucose is metabolised to fructose according to the following reactions:

$$\text{glucose} + \text{NADPH} + \text{H}^+ \xrightarrow[\text{reductase}]{\text{aldose}} \text{sorbitol} + \text{NADP}^+$$

$$\text{sorbitol} + \text{NAD}^+ \xrightarrow{\text{SDH}} \text{fructose} + \text{NADH} + \text{H}^+$$

Although this pathway was originally described in seminal vesicles[1], human red cells have recently been shown to metabolize approximately 3% of their glucose in this way under normal conditions[2,3]. NADH produced as a consequence of the conversion of sorbitol to fructose provides reduced pyridine nucleotide for methaemoglobin reduction[3]. The enzyme from human red blood cells has been partially purified and characterized[4]. The present study was undertaken in order to establish whether this enzyme is present in the red blood cells of some domestic and laboratory animals.

Materials and methods. Blood was collected from the anticubital vein in man; the jugular vein in sheep, goat, dog and cat; by cardiac puncture in guinea-pig, rat and mouse and from the marginal ear vein in rabbit. Blood samples were collected in tubes containing dried sodium heparin. The red blood cells were washed 3 times in cold saline, frozen and thawed once, and haemolysates were prepared by adding 3 volumes of water. The enzyme activity of SDH was measured at 37 °C in a Gilford recording spectrophotometer according to the method of Torrance[5] and was expressed as μmoles/min/g Hb.

Activity of sorbitol dehydrogenase in the red blood cells of various mammalian species

Species	Number	Sorbitol dehydrogenase activity (μmoles/min/g Hb) Mean ± SEM
Man	10	1.490 ± 0.068
Mouse	6	2.360 ± 0.289
Dog	6	1.910 ± 0.376
Guinea-pig	6	1.769 ± 0.112
Rat	6	0.753 ± 0.066
Cat	6	0.643 ± 0.064
Rabbit	6	0.496 ± 0.038
Goat	4	0.119 ± 0.043
Sheep	6	0.109 ± 0.021