Original Article

Effect of temperature variation and determination of optimum temperature of alpha amylase activity in *Telescopium telescopium*.

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Abstract

The changes in enzyme activity of α- amylase from hepatopancreas of *Telescopium telescopium* from the Chorao Island of Goa State were investigated during year 2004 to 2006. The enzyme activity was observed highest at 20 minutes of incubation time and was 30.0 ± 10.83 µ mols/mg protein/min. The lowest enzyme activity was observed at zero minute of incubation and was 19.17 ± 1.23 µ mols/mg protein/min. At 40 ^{0}C of incubation temperature the highest enzymatic activity was observed and was 32.05 ± 3.307 µ mols/mg protein/min which is recorded as the optimum temperature. The enzyme activities were also noted for one week starved *Telescopium telescopium* in laboratory.

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Introduction

Telescopium telescopium (Linnaeus 1758) is herbivorous and detritus feeder. This species is the last remaining survivor of a genus that had several fossil records. It occurs in different size ranges during their development and growth. Maximum size recorded was 110 mm in length. It has tolerance to wide range of salinities and temperature as it is an estuarine gastropod and it remain exposed to sun during low tides for several hours. Large variations in salinity and temperature occur in the intertidal region[1],*Telescopium telescopium* must be highly adapted to avoid being dried out. At the optimum temperature range the rate of reaction rises because of the increase in the number of molecules with the required energy of activation. After exceeding the optimum temperature range in a specific set of conditions the rate of reaction decreases constantly and with further temperature rise it falls off to zero.

Materials and Methods

Specimens of *Telescopium telescopium* of mean shell length of 9.1 cm (range 8.9 to 11.1 cm) were obtained from Mandovi estuary, Chorao islands, Goa. The gastropod was carefully opened with the help of bone cutter. The intestinal region was located and hepatopancrea was isolated aseptically. The epithelial sheath that covers the tissue was carefully removed with the help of forceps. Wet weight of each animal was measured and recorded. Samples were collected with the same procedure after one week of starvation. The samples were homogenized in chilled mortar and pestle in 10 ml phosphate buffer of pH 7 and centrifuged in a cooling centrifuge at 2000RPM for 20 mins. Supernatant was collected and stored at 4 ^{0}C and further used for the enzyme extraction, purification and assay. Purified enzyme solution obtained from purification steps is used to determine the amylase enzyme activity. Ammonium sulphate precipitations were performed as described by Deutscher.[2] Supernatant produced through crude homogenate centrifugation was brought to 25% saturation with ammonium sulphate and subsequently centrifuged at 20,000 rpm for 30 mins at 0 ^{0}C. The resulting pellets were pooled and stored at 4 ^{0}C. Each pellet was resuspended in deionised water; subsequently the process was repeated to obtained pellet with 50% and 75% ammonium sulphate saturation. For the removal of salts from protein solution, dialysis was performed. Regenerated cellulose dialysis tubing 3500 MWCO was used for dialysis. The ammonium sulphate precipitated fractions including all supernatants and pellet solutions were dialysed twice for 24 hours dialysis against distilled water at 4 ^{0}C. The dialyzed solution was pipette out of the tubing and stored. The volume of each fraction was measured and recorded. Fractions of pellet solutions and supernatant solutions were collected separately and tested separately for alpha amylase activity. The fifth to seventh fraction of pellet solution exhibited maximum enzyme activity (90-95 %) and these fractions were used subsequently for column chromatography.

Column chromatography was performed.[3] Amylase from crude extract dialysate was purified using DEAE cellulose. Flow rate was 1ml/min. All fractions were 1ml in volume and collected in size exclusion chromatography fraction tube. Using peristaltic pump the six times diluted solution with binding and washing buffer of fraction was loaded on to Hi Trap Benzamidine column. Finally 10 fractions were collected and tested for enzymatic activity and were then pooled for use in subsequent analysis.[4]

The specific activity was determined by estimating protein from the tissue extract following the method of Lowry et al [5]and was expressed as units per mg soluble protein(µ mg^{-1}) For the appropriate incubation time for amylase activity of hepatopancreas of *Telescopiumtelescopium* the reaction mixture was incubated at different time intervals starting from zero minute to 5,10,15,20,25,30 and 40 mins. Tubes were setup containing reaction mixture of (0.5%) starch, phosphate buffer (pH 7) and sodium chloride (1.0%). Tubes were placed in water bath at 37 ^{0}C and enzyme solution equilibrated at same temperature was added. Tubes then incubated at different time intervals as mentioned above. Reaction was stopped using 2M NaOH. Tubes then boiled for 5mins. Extinction was read at 540 nm against blank in spectrophotometer and enzyme activity was calculated using formula-

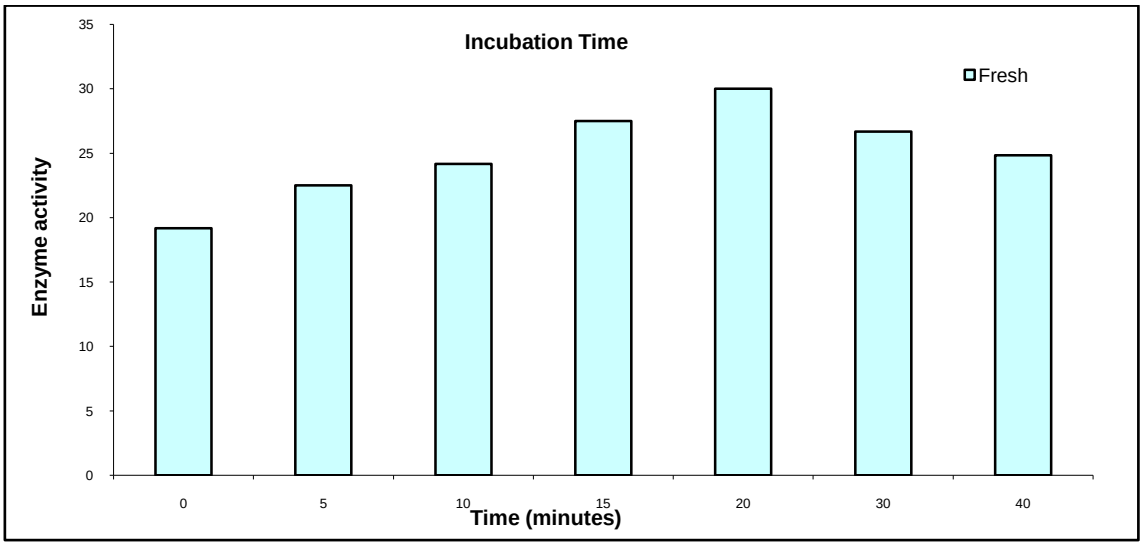
$$\text{Mass of Maltose in mg/gm protein} = \frac{O.D.}{Slope of std grap\ \ h} \times 100$$

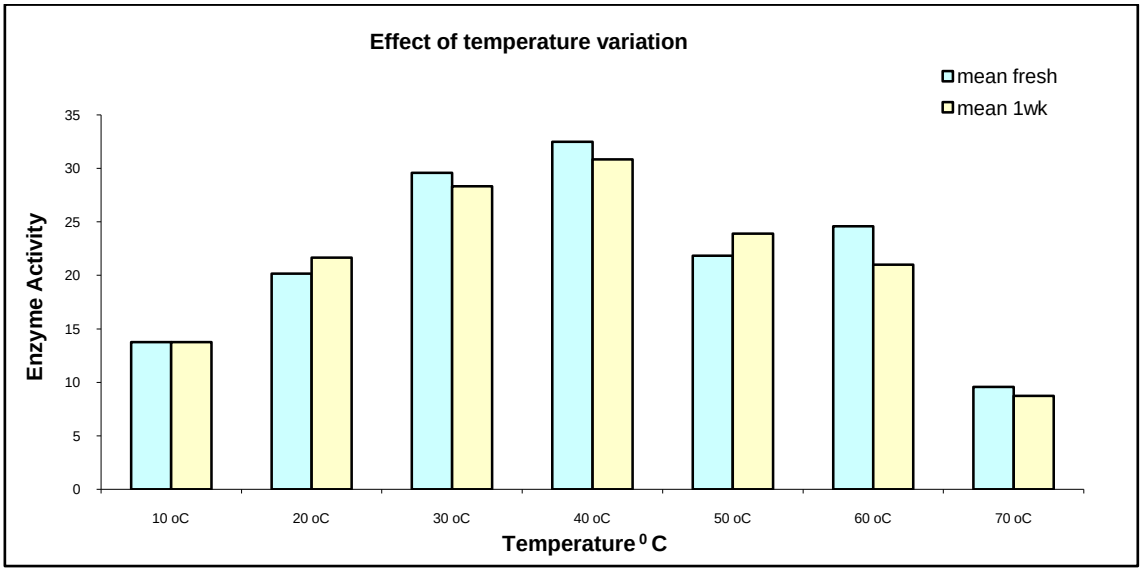
The optimum temperature for maximum enzyme activity as determined by varying incubation temperature of the reaction mixture as 10, 20, 30, 40, 50, 60, 70 ^{0}C. All enzymes start to denature at temperatures above their optimum temperature. The reaction mixture was same. The tubes were incubated for 20 mins in a water bath at the desired

temperature regimen. The enzyme activity was calculated as described earlier.

Result

Graph no. 1 shows the enzyme activities at various incubation times. The amylase activity was minimum at zero min of incubation, later the enzyme activity elevated with increase in incubation time to 20 mins. The highest enzyme activity was observed at 20 mins of incubation time and it was 30.0 ±10.83 µ mols/mgprotein/min. The lowest enzyme activity was for zero minute of incubation and was 19.17 ± 1.23 µ mols/mgprotein/min. Effect of variable incubation temperature on the amylase enzyme activity can be revealed from graph no. 2. The amylase activity at 10 ^{0}C was 13.75 ±1.25 µ mols/mgprotein/min. Later the activity showed elevation with increase in temperature (32.05 ± 3.307 µ mols/mgprotein/min). After this enzyme activity showed decrements. The lowest enzyme activity was observed for 70 ^{0}C of incubation temperature (9.58 ± 5.636 µ mols/mgprotein/min). Subsequently the amylase activity can be observed in one week starved animals.





Discussion

Many researchers have exposed the molluscan amylases to variety of substrates.[6], [7]These researchers used glycogen, amylase, amylopecten, agar, meltotriose, maltotetraose, etc. They have found that amylase I and II could digest amylopectin to maltotraose and maltopentose.Broekhuysen (1941) determined the lethal temperatures and the survival timesat high temperatures in some intertidal marine gastropods.[8] Evans (1948) investigated the mean lethal temperature varied from 46.3 ^{0}C to 36.2 ^{0}C for some littoral gastropods.[9] The starch used in the present study was a combination of amylose and amylopecten. Besides α- amylases of Abalone is known to digest glycogen particularly amylase II. In the present study effect of incubation temperature variation was observed. Since temperature is a measure of molecular agitation it controls the rate of chemical reaction and is one factor limiting energy liberation and organismic growth. Present investigation shows the Optimum temperature for amylase activity in hepatopancreas of *Telescopiumtelescopium*is 40 ^{0}C. The Maximum activity observed at 20 mins of incubation time. In nature the chances of telescopium reaching a temperature of 40 ^{0}C are rare however; the body temperature on mudflats could reach to 34 to 36 ^{0}C during summer. Thus in natural conditions the amylase activity though may not reach to a peak level, it still would be substantially high enough to promote carbohydrate digestion at a relatively faster rate.

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Competing Interests

None declared.

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