EFFECT OF pH AND TEMPERATURE VARIATION ON THE AMYLASE ACTIVITY OF THE FISH CLARIAS BATRACHUS

B.S. Rahile[1], K.B. Nagarnaik[1], M.K. Bangadkar[2] and P.P. Ingle[3]
[1]Science College Pauni Dist. Bhandara
[2]S.S. Jaiswal College Arjuni/Morgaon, Dist. Gondia
[3]Saibaba College, Parsevni
pratya87@gmail.com

ABSTRACT

Amylase is a key enzyme for carbohydrate digestion and is a limiting factor in absorption efficiency in bivalves. The present study was undertaken to study the effect of temperature and pH on the amylase activity of the fish Clarias batrachus. The liver, pyloric cacae and intestine was considered to study the amylase activity. The live fishes was acquired from the river, acclimatized in laboratory and sacrificed to acquire the liver, pyloric cacae and intestine. The results of the present study depicted that, amylase activity showed optimum pH at 7 for liver and pyloric caecal extract where as at pH 7.5 maximum activity shown by intestinal extract. The enzyme activity showed a proportional increased up to the optimum pH, decreasing thereafter proportionally towards more alkalinity. As regard the effect of temperature on amylase activity it was observed that maximum activity was found to be at 35oC for liver, 40oC for pyloric caecae and 45 oC for intestinal extract the, activity failing down temperature.

Keywords: *Amylase, Enzyme activity, Clarias batrachus, Liver, Pyloric cacae, Intestine.*

Introduction

The animal living between tide marks are obliged to adapt themselves to circumstances to an even more marked degree than the land of purely aquatic forms because they are exposed to regular alteration between submergence and emergence. Inter tidal organism appear to be well adopted to resist the stress and two of the most obvious of such stresses are those of temperature and decision.

Considerable amount of literature is available showing relationship between respiratory activity and pollution stress in aquatic animals (Robberts, 1972). Several workers have noted alternation of physiological process in bivalves and gastropods due to pollutants. Aquatic animals have to pass large quantities of water over their respiratory surface and are subjected to a relatively greater risk of exposure to the toxic substance. These compounds might act as physiological stress for non target animals.

The diversity in feeding habit is reflected in the structural adaptations in the alimentary canal. The study of digestive enzyme shows correlation with the feeding habit. Temperature tolerance as an experimental criteria for the demonstration of physiological change has found many uses. Much of the experimental literature was found on fishes and very little on invertebrates (Gowanlocj and Hayes, 1926). Work on the animals and found out lethal point at which the death occurs. It was found that the upper limit of thermal tolerance may vary according to the season or conditioning temperature (Vernberg et al., 1963).

α-Amylase (α-1,4 glucan-4-glucanohydrolase) is a key enzyme for carbohydrate digestion and is a limiting factor in absorption efficiency in bivalves (Moal et al 2000; Sellos et al 2003). In general, α-amylase can represent individual energy status, as observed in Drosophila when starch is the only carbohydrate source in the substrate (Powell & Andjelkovic 1983). α-Amylase catalyses the hydrolysis of internal (α-1,4) glucoside bonds in starch or related poly- and oligosaccharides. External factors affect the regulation processes of digestive enzymes such as the amylase in crustaceans (Guarna & Borowsky 1995; Le Moullac et al 1997). In Brine shrimpArtemia salina, adaptation of amylase enzyme varies according to particular starch concentration in the diet (Samain et al 1980). This observation could probably extend to the existing high carbohydrate content of the brine shrimp right after hatching. Influence of different pH and temperature on amylase activity in liver pyloric caecae and intestine of C. batrachus was studied. The optimum pH for a mylase activity in liver and pyloric caecae was studied and it has been found that the digestion depends upon the physical state of food as well as kind and

quality, quantity of enzyme secreted. Fishes are more specific in the digestion of different kinds of foods. The determination optimum pH and temperature in the various parts of digestive track for different digestive enzyme is the most important aspects in the study of digestion because different enzyme act optimally under different a biotic factors. The study of digestive enzyme would be useful in the understanding the adjustment of feed of fishes in the piscicultural practices.

Though C. batrachus is one of the important, protenaceous and delicious food fish, no work has been done on this aspects. The literature on diagram in fishes shows a need for further investigation is specially by systematic comparison of the digestive enzyme in representative throughut the vertebrates series. It would be of value to know if differences in digestive enzyme have occurred in the course of evolution of groups of higher vertebrates from more primitive ones, with the migration of aquatic vertebrates to land habitats, and with the metabolic changes necessitated by the transformation from aquatic life on land. Present communication relates to study effect of various pesticides pollutions on fish C. batrachus the quantitative estimation of amylase in liver, pyloric caecae and intestine of fish C.batrachus at various pH and temperature.

Materials and Methods

For the present investigation Clarius batrachus weighing about 25 to 50 gm is used. The live fishes were procured from the Wainganga river and brought to the laboratory. In laboratory fishes were acclimatize for seven days in well aerated aquarium. Before proceeding to the experiment specimens were starved for 24 hours. Starved specimens were dissected out to acquire liver, pyloric caecae and intensive. The isolated tissues were washed with cold distilled water. The mucosal lining of each part was then scraped off and homogenized in a homogenizer.

1% aqueous enzyme extracts were prepared and centrifuged. The supernatants were use immediately after the addition of toluene to prevent bacterial growth.

The amylase activity was then studied following the procedure of DNS method (Bernfeld 1955). The amylase activity was studies at different temperatures and different pH to studied the optimum temperature and pH for the activity of amylase in fish *C.batrachus.*

The data was statistically analyzed using statistical software for social sciences (SPSS). The analyzed data was presented in table 1 and 2.

Observations and Results

The observations of the effect of different pH and temperature on the amylase activity in liver pyloric caecae and intensitine of *C.batrachus* was depicted in table 1 and 2. It is revealed that amylase activity showed optimum pH at 7 for liver and pyloric caecal extract where as at pH 7.5 maximum activity was shown by intestinal extract of *C.batrachus*.

The enzyme activity was showed a proportional increased up to the optimum pH, decreasing thereafter proportionally towards more alakalinity.

As regard the effect of temperature on amylase activity it was observed that maximum activity was found to be at 35°C. For liver, 40°C, for pyloric caecae and 45 °C. for intestinal extract the, activity failing down these temperature.

Table 1: Rate of reaction of amylase under varying pH at 37^0 C in liver and different regions of alimentary canal of the *C.batrachus*

pH	Amylase activity in mg/of maltose/100mg of wet tissue per 30 minutes		
	Liver	Pyloric caecae	Intestine
5.0	3.983±0.374	2.732±0.189	5.833±0.658
5.5	7.016±0.903	5.133±0.431	9.999±0.658
6.0	10.683±0.676	6.933±0.360	12.399±0.616
6.5	11.716±0.874	10.533±0.361	14.999±0.657
7.0	17.206±0.767	15.299±0.491	16.499±0.474
7.5	15.884±0.761	13.515±0.487	17.916±0.658
8.0	9.866±0.590	10.376±0.541	11.736±0.353
8.5	8.133±0.535	7.083±0.300	8.749±0.658
9.0	5.449 ±0.412	3.616±0.266	5.999±0.725

Values are Mean±SD, N=5 for each group

Table 2: Rate of reaction of activity under different temperature but at constant optimum pH in liver and different regions of alimentary canal of the *C.batrachus*

Temperature	Amylase activity expressed in mg of maltose/100 mg of wet tissue per 30 minutes		
	Liver	Pyloric caecae	Intestine
15°c	7.949±0.320	6.985±0.346	5.633±0.466
20 °c	11.299±0.566	7.149±0.494	7.133±0.456
25 °c	13.283±0.332	8.992±0.498	8.234±0.545
30 °c	16.249±0.344	12.016±0.314	11.884±0.335
35 °c	19.540±0.450	13.483±0.355	13.560±0.331
40 °c	18.599±0.490	16.149±0.375	15.834±0.429
45 °c	16.109±0.311	15.931±0.648	11.630±0.314
50 °c	12.079±0.340	10.185±0.502	14.758±0.309
55 °c	12.079±0.340	10.185±0.502	14.756± 0.343
60 °c	0.288±0.501	8.043±0.348	11.385±0.386

Values are Mean±SD, N=5 for each group

Discussion

There is a definite correlation between the diet consumed and the type of relative strength of digestive enzyme (Young 1937). Amylase activity is stronger in herbivorous fishes than in carnivorous and omnivorous fishes (A1-Hussaini, 1949) reported that the pancreas whether compact of diffused was the main site of enzyme production.

Amylase activity was certainly due to the enzyme produced in pancreas of telecast (Barrington, 1957). Preliminary studies in *C.batrachus* confirmed that the amylase is strongly present in liver, pyloric caecae and intestine.

The pH and temperature have an important role in the activities of enzymes in the aquatic animals. A particular enzymes shows its maximum activity at a particular pH and temperature.

The amylase activity is considerably less at pH 7.4 in certain marine fishes. In the intestine of *Pleluronectus* sp. it is active at pH 7.5 and 8 (Bayliss, 1935). Cockson and Bourne (1972) found optimum pH for amylase 7.5 and 7.0 for anterior and posterior intestine of *Barbus palidiniosus.*

Amylase activity is maximum at pH 7.5 in the intestine of *Oreochromis niloticus* (Tengjroenkul et al 2000). Agrawal et al, (1975) recorded optimum pH for amylas activity from 5 to 6 in *Wallago attu*, and *Labeo rohita*. Maximum digestion of starch takes place at pH 7.4 due to liver extract and at pH 7.2 due to intestinal extract of *Periophthalmus koelreuteri* (Dhage and Mohammad, 1977).

The extract of liver and pyloric caecae of C.batrachus demonstrated the optimum activity at pH 7.0 and in the intestine at pH 7.5 There are slight variations from the above author's findings in the optimum pH. Variations are due to species variations (Agrawal et al, 1975).

Table 1 clearly indicates that the amylase activity is highest in intestine followed by that in liver and pyloric caecae. Pancreas, which is main source of enzyme secretion, is diffused in liver lobes mesenteric tissue and alimentary canal and hence intestine shows the maximum activity where actually the process of digestion takes place where slightly alkaline medium is required, whereas pyloric caecae supplements the digestion.

It is generally agreed that the enzymes of fishes are similar to those of warm blooded animals. Fishes being poikilothermic animals are liable to show changes in the digestive capabilities with thermal fluctuations. In the present investigation, the amylase activity was found to be maximum at 35°C in liver at 40°C in pyloric caecae and at 45°C in intestine. Later the activity decreases proportionally up to 60°c (Table 2)

The present findings are in concurrence with those of Dhage and Mohammad (1977). The velocity of the reaction catalyzed by enzyme is accelerated in relation to the increase in temperature within a particular level. The increase in temperature brings down the velocity of reaction.

References

1. Agrawal, V.P., Sastry, K.V. and Kaushab, S.K., (1975). Digestive enzymes of three teleost fishes. Acta Physiologica Academiae Scientiarum Hungaricae, 462:93-98.
2. AL-Hussaini, A.H. (1949). On the functional orphology of the alimentary tract of some fishes in relation to their feeding habit. Q.J1 Microc.Sci. 90 (4) : 323-3564
3. Barrington, E.J.W. (1957). The alimentary canal and digestion in "The Physiology of fishes" edited by M.E. Brown, Acad. Press, New York. Vol I. pp. 109-161
4. Bayliss. L.E. (1935). Digestion in, place Pleuronectus platessa, J Mar. Biol A.S.U.K. 20 (1) : 73-91.
5. Bernfeld, P. (1955). Amylase α and ß.In Methods in Enzymology (Colowick SP, Kaplan NO, ed.) Academic Press Inc, New York 1:149-158.
6. Cockson, A, and Bournes, D, (1972). Enzymes in the digestive tract of the two Euryhaline fish, Comp Biochem. Physiol. , 41 (A) : 715-718.
7. Dhage, K.P., Mohammed, O.M.M.J.(1977). Study of amylase activity in Mudskipper *Periophthalmus koelreuteri* (PaWas). Indian J. Fish., 24: 129-134
8. Gownaloch, J.N. and Hayes, F.B. (1926). Contributions to the study of marine gastropods I the physical factor, behavior and interidal life of Littorina. Centr. Candad. Biol. N.S.3 : 133-166.
9. Guarna M. M., Borowsky R. L., (1995). Biochemical properties of amylase isozymes from Gammarus palustris. A comparative study. Comp Biochem Physiol B 112:619-628.
10. Le Moullac G., Klein B., Sellos D., Van Wormhoudt A., (1997). Adaptation of trypsin, chymotrypsin and alpha-amylase to casein level and protein source in Penaus vannamei (Crustacea Decapoda). J Exp Mar Biol Ecol 208:107-125.
11. Moal J., Daniel J. Y., Sellos D., Van Wormhoudt A., Samain J. F. (2000). Amylase mRNA expression in Crassostrea gigas during feeding cycles. J Comp Physiol B 170:21-26.
12. Powell J. R., Andjelkovic M., (1983). Population genetics of Drosophila amylase IV. Selection in laboratory populations maintained on different carbohydrates. Genetics 103:675-689.
13. Roberts R.J.(1972).Ulcerative dermal necrosis(UDN) of salmon (Salmo salar L.)Symposium of Zoological Society, London 30,53-81
14. Samain J. F., Moal J., Daniel J. Y., Le Coz J. R., Jezequel M., (1980). The digestive enzymes amylase and trypsin during the development of Artemia: effect of food conditions. In: The Brine Shrimp Artemia. Vol. 2. Physiology, Biochemistry, Molecular Biology (Persoone G., Sorgeloos P., Roels O., Jaspers E. eds.). Universa Press, Wtteren, Belgium. p. 147-443.
15. Sellos D., Moal J., Dégremont L., Huvet A., Daniel J. Y., Nicoulaud S., Boudry P., Samain J. F., Van Wormhoudt A., (2003). Structure of amylase genes in populations of Pacific cupped oyster (Crassostrea gigas): tissue expression and allelic polymorphism. Mar Biotechnol 5:360-372.
16. Tengjroenkul, B., Smith, B.J. Caceci T. and Smith, S.A.(2000)Distribution of intestinal enzyme activities along the intestinal tract of cultured Nile tilapia, *Oreochromis niloticus* L.Acquaculture 182(3):317-327.
17. Vernberg ,F.J. (1963). Acclimation of intertidal crabe. Am. Zool.9 : 333-341.
18. Young C.M. (1937). Evaluation and adaption in the digestive system of metazoan Biol. Revs. 12(1) : 87-115.