CHAPTER - V

NON-SPECIFIC PHOSPHOMONOESTERASES IN EMBRYONIC TISSUES OF CHANNA STRIATUS (BLOCH)

INTRODUCTION

Lysosome concept(DeDuve, 1963) of lipoprotein bound particles containing acid hydrolases which are released progressively by procedures which alter or damage the membrane, must be regarded as completely established (Brachet, 1960; Pearse, 1968). Filburn and Vandale (1963), Wilson & Allenspach (1974) and Rinaldi et al (1978) considered acid phosphatase as an important lysosomal enzyme in adults and in embryos and function shortly after fertilization in the rupture of cortical granules found in peripheral cytoplasm of the ripe eggs of many

species(Saunders, 1966; Patel, 1970; Ling & Lyerla, 1976). These granules fuse with plasma membrane upon activation of the egg, they rupture and typically discharge their contents at egg surface (Ando, 1961; Eddy & Shapiro, 1976; Hart et al, 1977; Vacquier, 1975).

Hart and Pontier (1979) thought acid phosphatase activity not detectable in cortical granules of zebra fish, Brachydanio rerio eggs; instead, they were found to be largely localized in yolk vesicles, yolk platelets and their precursors; in fact stored in yolk platelets, subsequently to be used to initiate and/or promote the utilization of yolk during embryogenesis.

Yolk phosphoproteins may serve as reserves of energy rich phosphoryl groups. ATP can be generated directly from phosphoproteins (Williams, 1967). Nonspecific phosphatases, besides specific phosphoprotein phosphatases, can transfer phosphorus of phosphoproteins to acceptors such as hexose or glycerol producing useful organic phosphates (Williams, 1967; Barth and Barth, 1954). Phosphoprotein phosphatase activity is due to concerted activity of a variety of phosphatases including phosphomonoesterase, phosphodiesterase and pyrophosphatases (Yamagami, 1961). Noda (1967), in Salmo

Gairderii irideus, reported two fold increase in activities of alkaline and acid phosphatases in fertilized as compared from unfertilized eggs, and later a gradual increase attaining peak in eyed stage. Gustafson and Hasselberg (1951) found activity of acid phosphatase to be several hundred times than that of alkaline phosphatase in eggs of sea urchin.

Alkaline phosphatase is reported abundant in tissue concerned with transport of nutrients; often found in secretary organs and developing tissues and incriminated in transport of nutrients across epithelial membranes. Like acid phosphatases, they too are concerned in transfer of a terminal phosphoryl group and hydrolyze ATP by stepwise production of inorganic phosphate, but in contrast to acid phosphatases, they are inhibited by metal complexing agents (Fernley, 1971; Hollander, 1971).

Successful ontogenesis of fish embryos results from conversion of yolk materials to tissues of embryo (Zeitoun et al, 1977) and from utilization of phosphoprotein (Yamagami, 1960 a,b) and phospholipid (Smith, 1958) constituent of yolk globule (Nakagawa and Isuchiya, 1972). The importance of role of acid and alkaline phosphatases in degradation of these constituents and their eventual utilization during embryogenesis thus cannot be undermined. This

Chapter includes an account of activities of these two non specific phosphomonoesterases during embryogenesis of C. carpio.

METHODS

ACID AND ALKALINE PHOSPHATASES:

(Orthophosphoric monoester phosphohydrolase E.C. 3.1.3.2 and 3.1.3.1)

Enzyme extract was prepared by homogenizing fresh samples of each embryonic stage in icecold 0.7% saline to 10% w/v; centrifuged at 3000 rpm at 4°C for 15 minutes; supernatant used for determination of acid and alkaline phosphatase activities by the methods of King & Jegatheesan (1959) and King & King (1954) respectively, as described by Wootton (1974).

PHENOLS:

Phenol released from phenyl phosphate, following hydrolysis by phosphatases under defined conditions of time, temperature and pH, reacts with 4-amino antipyrine and potassium ferricyanide to form a coloured complex that is estimated colorimetrically.

REAGENTS:

1. Carbonate buffer (for alkaline phosphate pH 9.9):

3.35 g of anhydrous sodium carbonate and

3.36 g of sodium bicarbonate dissolved in

distilled water; pH adjusted to 9.9 and diluted to 1 litre with distilled water. stored at 4°C.

2. Citrate buffer (for acid phosphatase; pH 5.0): 42 g of crystalline citric acid dissolved in distilled water, 376 ml of 1 N NaOH added and volume made to 1 litre with distilled water with prior adjustment of pH to 5.0. Stored at 4°C.

3. 0.01 M disodium phenyl phosphate substrate: 2.18 g of disodium phenyl phosphate dissolved in 1 litre of distilled water; boiled for few minutes to kill the microorganisms; cooled; preserved with chloroform (4 ml/ litre) and stored at 4°C.

4. Stock phenol standard: 1 g pure crystalline phenol dissolved in 1 litre of 0.1 N hydrochloric acid; stored in brown bottle at 4°C. This solution gave 1 mg phenol/ml.

5. Working phenol standard (0.01 mg/ml): 1 ml of the stock standard (4) diluted to 100 ml with distilled water; preserved with few drops of chloroform and stored at 4°C in a brown bottle.

6. 0.5 N Sodium hydroxide solution: 20 g of NaOH dissolved in 1 litre of distilled water.

7. 0.5 N Sodium bicarbonate solution: 42 g of sodium bicarbonate dissolved in 1 litre of distilled water.

8. 4-Aminoantipyrene reagent: 6 g of 4-aminoantipyrene dissolved in 1 litre of distilled water; stored in brown bottle.

9. Potassium ferricyanide solution: 24 g of $K_4Fe(CN)_6$ dissolved in 1 litre of distilled water; stored in a brown bottle.

Procedure:

Test: 1 ml of carbonate (for alkaline phosphatase) or citrate (for acid phosphatase) buffer and 1 ml of substrate taken in a tube; 0.2 ml of enzyme extract added and the reaction mixture incubated at 37°C for 60 minutes; reaction stopped by adding 0.8 ml of 0.5 N NaOH solution.

Control: 1 ml of carbonate/citrate buffer, 1 ml of substrate and 0.8 ml of 0.5 N NaOH were taken and mixed thoroughly in a tube. 0.2 ml of enzyme extract was then added to it.

Standard: 1.2 ml of carbonate/citrate buffer, 1 ml of working phenol standard and 0.8 ml of 0.5 N NaOH mixed in a tube.

Blank: 1.2 ml of carbonate/citrate buffer, 1 ml of distilled water and 0.8 ml 0.5 N NaOH were taken and mixed.

1.2 ml of 0.5 N sodium bicarbonate, 1 ml of 4-aminoantipyrene and 1 ml of potassium ferricyanide were added to each tube, in that order, thoroughly mixing the solutions after each addition.

Optical densities were determined by setting zero with blank at 520 nm in a spekol. Care was taken to avoid exposure of tubes to direct sunlight.

Activity of both enzymes was expressed as μ_g phenol released/mg protein/hour. Protein was measured by the method of Lowry et al (1951) as given on page 34.

RESULTS

Activity of acid phosphatases was found higher than alkaline phosphatases in unfertilized eggs and throughout the embryonic development of C. carpio. Acid phosphatase showed two phases of increased activity, namely, soon after fertilization and during late morula stage; its activity decreased almost steadily at early morula and later, from blastula onwards until hatching (Table 21; Fig. 43).

Activity of alkaline phosphatase was minimal throughout the development, except following fertilization, upto blastodisc stage and later during comma,

Table - 21

Acid phosphatase activity (µg phenol released/mg protein/hr) in unfertilized eggs and in different embryonic stages of Cyprinus carpio.

S.No.	Stages									
	Unfertilized egg	Blastodisc	Early morula	Late morula	Blastula	Gastrula	Closing of blastopore	Coma	Eyed	Prior to hatching
1	14.00	25.60	21.70	3[illegible].30	34.30	27.30	19.90	20.40	9.30	8.50
2	14.00	25.60	22.40	41.10	36.20	21.90	20.40	19.70	9.80	10.00
3	14.40	25.60	22.40	4[illegible].20	21.60	30.70	21.40	19.70	9.80	9.50
4	13.20	28.20	22.00	40.20	31.50	33.70	18.80	19.70	10.90	9.70
5	12.30	27.60	21.70	-	31.00	30.10	18.90	21.00	10.10	10.00
Mean ± SD	13.58 ±0.84	26.52 ±1.27	22.04 ±0.35	39.95 ±1.18	30.92 ±5.62	28.74 ±4.45	19.70 ±1.33	20.10 ±0.58	10.08 ±0.47	9.48 ±0.58

TABLE - 22

Alkaline phosphatase activity (µg phenol released/mg protein/hr) in unfertilized eggs and in different embryonic stages of Cyprinus carpio

S.No.	Stages									
	Unfertilized egg	Blastodisc	Early morula	Late morula	Blastula	Gastrula	Closing of blastopore	Comma	Eyed	Prior to hatching
1	0.30	4.60	0.32	0.60	0.20	0.70	0.90	2.10	3.50	3.45
2	0.50	4.10	0.37	0.60	0.20	0.30	0.45	1.50	4.00	3.75
3	0.48	4.30	0.38	0.70	0.20	-	0.45	1.50	4.00	3.45
4	0.70	4.50	0.38	0.80	0.20	-	0.90	1.50	4.50	3.45
5	0.50	4.20	0.22	0.70	0.20	-	-	0.75	4.50	3.75
Mean ± SD	0.496 ±0.14	4.34 ±0.20	0.39 ±0.16	0.68 ±0.08	0.20 -	0.50 ±0.28	0.67 ±0.25	1.47 ±0.48	4.12 ±0.38	3.57 ±0.16

eye and prior to hatching stages, when it showed some rise (Table 22; Fig. 43).

DISCUSSION

Such higher activity of acid phosphatase, than alkaline phosphatase, during embryogenesis of C. carpio, as observed by the present author and also reported earlier (Chetty et al, 1982b), is in general accord with Gustafson and Wasselberg's (1951) observation in eggs of sea urchin, and further, increase in activity of both these enzymes after fertilization and of acid phosphatase again at late morula, fully conform with the present author's earlier report (Chapter IV, p. 111) of a steep decline in the levels of phospholipids and total lipids soon after fertilization and until late morula. Williams (1967) argues correctly that specific phosphoprotein phosphatases are not the only enzymes capable of releasing phosphate from yolk phosphoproteins, non specific phosphomonoesterases are also active.

In fact, Yamagami (1961) considered phosphoprotein phosphatase activity to be due to concerted activity of a variety of phosphatases, including phosphomonoesterase, phosphodiesterase and pyrophosphatases. Increase in activity of non specific phosphomonoesterases soon after fertilization is related to degradation of phospholipids of the yolk

globule (Nakagawa and Tsuchiya, 1972) with consequent release of phosphates which would eventually be utilized in energy metabolism, nucleic acid biosynthesis, cell membrane assembly and so on. Phospholipids acting as a source of energy in early embryonic stages, until late morula, has already been emphasized by this author earlier (Chapter IV; p.118) and finds strong support by the increase in the activity of these phosphomonoesterases. Hart and Fontier (1979) too reported their localization in yolk platelets in zebra fish, Brachydenio rerio and their subsequent utilization to initiate or promote utilization of yolk during embryogenesis. Williams (1967), Barth and Barth (1954), all agreed on the transfer of phosphoryl groups to an acceptor hexose, glycerol or protein in connection with yolk platelets.

The gradual decline in acid phosphatase activity at blastula and all subsequent stages is thoroughly understandable, considering the present author's earlier report of a steep decline in total lipids (Chapter IV; p. 111) in marked contrast with the observed accumulation of phospholipids with onset of gastrulation. The glycerolipids take over as principal energy source with onset of gastrulation and other enzyme systems related to gluconeogenesis must naturally have become active. Increased activity of alkaline phosphates immediately following fertilization may be due to increased uptake of certain metabolites and ions, as

these enzymes are reported to be involved in this process (Simkiss, 1964; Fernley, 1971). This contention gets further support in that permeability of the fish egg membrane increases during this period (Mori, 1958).

The slight rise in alkaline phosphatase activity, however, with onset of gastrulation and relatively sharper rise with organogenesis is obviously on account of active uptake of nutrients and metabolites, so much needed during this period of enhanced protein synthesis in extensive differentiation and morphogenetic activities of the growing embryo (Fernley, 1971).