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Effect of temperature and metamorphic index on acid phosphatase activity of *Rana tigrina*

J MUTHUKRISHNAN, M SENTHAMIZHSELVAN and S MOHAN
Department of Zoology, APA College, Palni 624 602, India

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Abstract. Activity of tail, gut and muscle acid phosphatase of *Rana tigrina* tadpoles in relation to metamorphic index and temperature was studied. Optimum pH of the enzyme was between 4·0 and 4·5. K_m for the muscle phosphatase was low, indicating its greater affinity to the substrate. With advancing stage of metamorphosis or increasing temperature, activity of the enzyme increased. At metamorphic climax the activity was low. Tail acid phosphatase displayed higher activity than the gut or muscle phosphatase, indicating the role of the enzyme in autolysis of the tail during metamorphosis.

Keywords. Acid phosphatase; metamorphic index; *Rana tigrina*.

1. Introduction

Tadpoles of *Rana tigrina* are subjected to variations in temperature, availability of food and depth of water column, which significantly alter the rate of metamorphosis (Marian and Pandian 1985). Acid phosphatase which is a lysosomal enzyme, plays an important role in autolytic degradation of tissues such as the regression of tail during metamorphosis of tadpoles (Nath and Butler 1971). Locke and Collins (1968) and Collins (1975) suggested that acid phosphatase can be used as an index of lysosomal activity. Acid phosphatase is also associated with growth and differentiation of tissues (Barker and Alexander 1958). Muthukrishnan and Senthamizhselvan (1985) showed that acid phosphatase plays an important role in the utilization of yolk during the embryonic development of the dragonfly *Mesogomphus lineatus*. Marian and Pandian (1985) concluded that temperature is a potent factor which significantly influences the rate of metamorphosis of *R. tigrina* tadpoles. Therefore, temperature is likely to influence acid phosphatase activity. The present paper reports on acid phosphatase activity of *R. tigrina* tadpoles as a function of metamorphic index (MI) and temperature.

2. Materials and methods

Acid phosphatase activity of the chosen tissues was estimated following the method described by Bergmeyer (1963). *p*-Nitrophenol (PNP) phosphate was used as the substrate. Three series of experiments were carried out. In the first series, optimum pH, and K_m for the tail, gut and muscle acid phosphatase of the tadpole (fore-limb bud stage, reared at 27°C) were determined. In the second series, activity of the enzyme from the tail, gut and muscle of the tadpoles of different MI reared at 27, 32 and 35°C was estimated. In the third series, multiplicity of the enzyme was tested electrophoretically. For the estimation of acid phosphatase activity, an incubation mixture consisting of 0·1 ml of the enzyme extract (the supernatant of 10%

homogenate (w/v) of the chosen tissue in distilled water, centrifuged at 1000 g for 10 min), 0·5 ml of substrate ($6{\cdot}25 \times 10^{-4}$ M) and 0·5 ml of acetate buffer (0·2 M) was used. The reaction mixture was incubated for 30 min at the temperature (27, 32 or 35°C) at which the tadpoles were acclimated. Activity of the enzyme was expressed in units of mg PNP released/mg protein/hr. Protein content of the enzyme was estimated following the method of Lowry *et al* (1951). For the electrophoretic separation of the enzyme, a polyacrylamide disc gel (7%) was used. 0·03 M Tris-HCl (pH 8·9) and 0·02 M Tris-glycine (pH 8·4) were used as gel and tank buffers, respectively. About 0·5 ml (approx. 250 μg) protein of the enzyme extract was loaded into the gel tubes. A constant current of 3 mA was supplied. The gel was stained in l-naphthyl phosphate and fast blue RR at the optimum pH (4·0 or 4·5) and incubated at 37°C for 15 min.

3. Results

3.1 *Optimum pH and* K_m

Acid phosphatase of *R. tigrina* displayed the highest activity at 4·5 pH (tail) or 4·0 pH (gut and muscle) (figure 1). K_m for the enzyme from tail, gut and muscle were $2{\cdot}78 \times 10^{-5}$ M, $3{\cdot}75 \times 10^{-5}$ M and $2{\cdot}38 \times 10^{-5}$ M, respectively. Therefore, the muscle acid phosphatase appears to have more affinity to the substrate than either the tail or gut phosphatase.

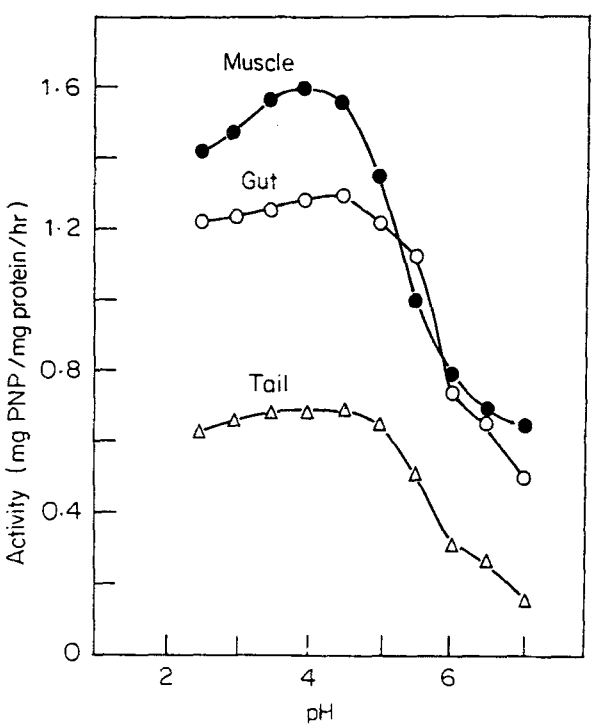

Figure 1. Activity of tail, gut and muscle acid phosphatase of *R. tigrina* tadpole as a function of pH.

3.2 *Effect of MI*

After reaching the fore-limb bud stage, the tadpoles reared at 27, 32 and 35°C required 8·0, 5·0 and 3·0 days, respectively to complete metamorphosis (figure 2). With advancing metamorphic period, activity of the enzyme from the tested tissues increased and at the metamorphic climax, it declined slightly. For instance, at 27°C, activity of the tail phosphatase increased from 0·562 mg PNP/mg/hr for the tadpoles of 2·523 MI to 1·197 unit for those of 1·133 MI; it decreased to 1·02 unit in the tadpoles at metamorphic climax. Similar trends were obtained for the gut and muscle phosphatase (figure 3). The stage at which the enzyme displayed maximum activity varied with temperature. For instance, while the peak activity of the tail acid phosphatase was observed in the tadpoles of 1·333 MI at 27°C, at 32 and 35°C it was observed at 1·13 MI (figure 3). At 32°C, tail, gut and muscle phosphatase activity was highest in the tadpoles belonging to 1·13 and 1·04 MI and freshly metamorphosed adult, respectively (figure 3B). At 35°C gut and muscle phosphatase displayed peak activity at a very early stage (1·909 MI), while the tail phosphatase displayed maximum activity only at a later stage (1·13 MI) (figure 3C).

3.3 *Effect of temperature*

Irrespective of the source of the enzyme, the activity increased with increasing temperature. Increase in temperature from 27–32 and 35°C, increased the activity of

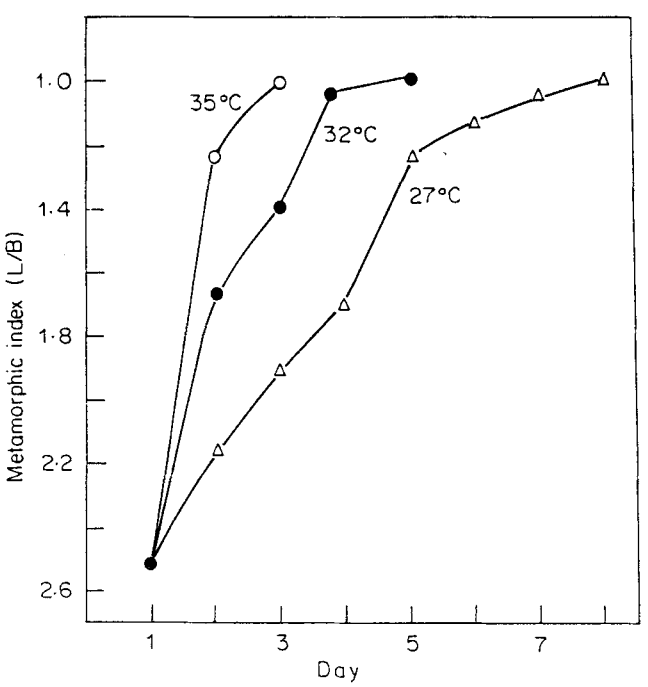

Figure 2. Metamorphic index of *R. tigrina* tadpole reared at the tested temperature as a function of age.

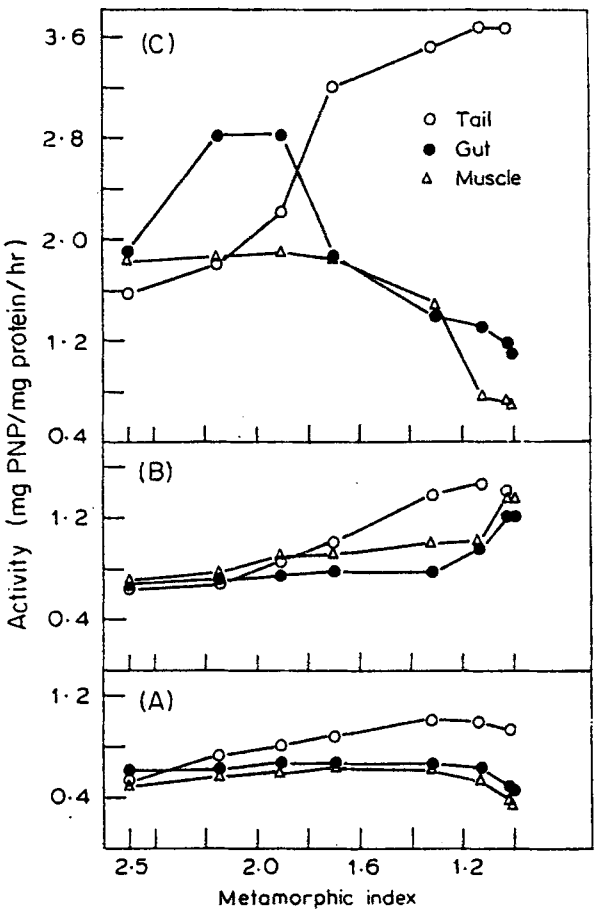

Figure 3. Activity of tail, gut and muscle acid phosphatase of *R. tigrina* tadpole as a function of MI. Panels **A, B** and **C** pertain to the activity at 27, 32 and 35°C, respectively. Each value is the mean of 3 estimations.

the tail phosphatase of 1·33 MI tadpoles from 1·197–1·42 and 3·56 units (figure 3). Influence of temperature of gut and muscle acid phosphatase was slightly different from that of the tail phosphatase. For instance, increase in the gut phosphatase activity of 1·33, 1·13 and 1·04 MI tadpoles due to increase in the temperature from 27–35°C was less than that for the other stages (figure 3). The response of muscle acid phosphatase to increase in temperature was quite different from that of the tail or gut phosphatase. In tadpoles of 1·13 and 1·04 MI, the enzyme activity decreased at 35°C, while, for all the other stages the activity increased (figure 3).

3.4 *Enzyme multiplicity*

Electrophoretic separation of the enzyme revealed the presence of 5, 4 and 3 fractions in the extracts from the muscle, tail and gut, respectively (figure 4).

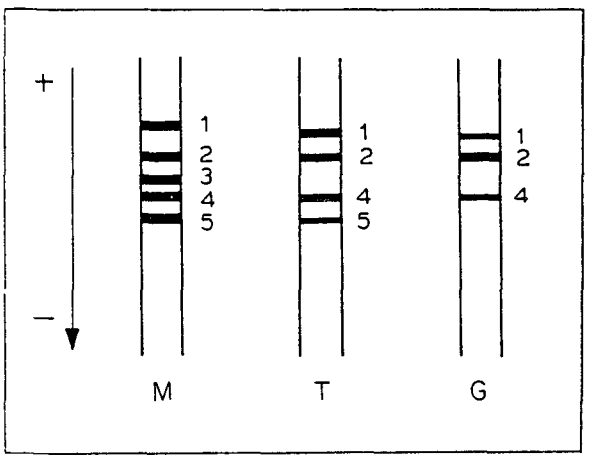

Figure 4. Electrophoretograms of muscle (M), tail (T) and gut (G) acid phosphatase of *R. tigrina* tadpole.

4. Discussion

The optimum pH of (4·0 and 4·5) reported in the present study agrees with that for the isopod *Porcellio leavis* (Saleem and Alikhan 1974). Generally the enzyme is reported to display maximum activity between 15–30°C but in the present study, temperature accelerated the activity of the enzyme to different levels. The finding that tail phosphatase activity was extremely high at 35°C may be attributed to the shortening of the metamorphic period to 4 days at the temperature. With advancing stage of metamorphosis, activity of the enzyme increased. Verkuil (1978) also demonstrated the increase in the activity of the enzyme with increasing age of the pupa of *Calliphora erythrocephala*. The rate of metamorphosis obviously depends on the rate of autolytic degradation of tissues which is determined by the activity of acid phosphatase. The tail of *R. tigrina* tadpole undergoes maximum autolysis during metamorphosis. Correspondingly, the tail phosphatase activity has been found to be higher than the other two. Acid phosphatase has been reported to exist in multiple forms in the gut of *P. leavis* (Saleem and Alikhan 1974) and egg of *M. lineatus* (Muthukrishnan and Senthamizhselvan 1985). Certain key enzymes occur in multiple forms, so that inhibition of one form of the enzyme by any factor does not seriously affect the physiological process as it is taken over by the other form(s) of the enzyme (Markert 1975). Metamorphosis, an important event in the life history of *R. tigrina* is controlled by multiple forms of acid phosphatase. It is possible that each fraction of the enzyme has a specific period of peak activity.

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