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ARYLSULFATASE ACTIVITY IN MARINE GASTROPODS

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ABSTRACT

Arylsulfatase activity was studied in foot, visceral hump and crystalline style of some gastropod molluscs like *Cerithidea fluviatilis* and *Telescopium telescopium* of the Vellar estuary, and *Cerithium rubrum*, *Melampus ceilonicus*, *Cassidula mustelina*, *Dostia indica* and *Littorina tigrina* inhabiting the Pitchavaram mangroves. Enzyme kinetics such as pH, optimal temperature and period of incubation were also studied. Enzyme activity showed two pH optima, a primary one at 5.6 and a secondary peak at 7.1, possibly indicating the occurrence of two types of arylsulfatase. In *D. indica*, *L. tigrina*, and *C. rubrum* maximum activity was at 37°C, while in other forms it was at 29°C. In *C. rubrum* and *T. telescopium*, the crystalline style showed greater enzyme activity than the visceral hump or foot. In *L. tigrina* and *Cassidula mustelina*, maximum enzyme activity was seen in the foot region. In starved *T. telescopium*, greater enzyme activity was seen in mantle and foot but not in the visceral hump.

INTRODUCTION

It is suggested that the enzyme arylsulfatase, hydrolyzes the sulfate group present in the diet though the natural substrate on which it acts is still not clear (Dodgson, Lewis and Spencer, 1953; Dodgson and Powell, 1959; Suzuki, Takahashi and Egami, 1959; Wortman and Schneider, 1960; Corner, Leon and Bulbrook, 1960; Jarrige, 1963; Agogbua, Anosike and Ugockukwu, 1978). Dodgson, Lewis and Spencer (1953) reported an increase in the activity of this enzyme with starvation. Shimony and Nigrelli (1972), who studied arylsulfatase activity in mantle, gills, digestive tract, muscle, cirri and testes of *Balanus eburneus*, suggested a probable relationship between the mantle arylsulfatase activity and the cyclic fromation and hardening of the exoskeleton in the species. Recently Dhevendaran, Kannupandi and Natarajan (1978) have found variations in the arylsulfatase activity of foot, visceral hump and crystalline style in some marine gastropod molluscs from different habitats. The present study on this enzyme activity from various organs of marine gastropods inhabiting different coastal biotopes was undertaken to gather more information about this enzyme and its functions in invertebrates.

MATERIALS AND METHODS

C. fluviatilis and *T. telescopium* were collected from intertidal mud of the Vellar estuary, while *C. rubrum*, *M. ceilonicus*, *D. indica*, *C. mustelina* and *L. tigrina* were from Pitchavaram mangroves. Of these *D. indica* was a typical rock clinging

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type found at the intertidal level in the estuarine region. *M. ceilonicus* was found attached to the aerial roots of *Avicennia officinalis* in the intertidal area, whereas *C. rubrum, C. mustelina* and *L. tigrina* were found above water on the prop roots of *Rhizophora mucronata*. The shells of molluscs were cracked carefully and the visceral humps were then removed. The crystalline style, mantle and foot were then separated and weighed. The weighed material was diced into small pieces and homogenized in 5.0 ml of distilled water. In the case of very small *M. ceilonicus* the entire animal was analysed.

Arylsulfatase activity was measured by spectrophotometric estimation of the anionic form of 4-nitrocatechol (4-NC) liberated during enzyme hydrolysis following the method of Shimony and Nigrelli (1972). Reaction mixture contained one ml of enzyme solution; two ml of 0.2M acetate buffer (pH 5.6) and one ml of 0.002M Nitrocatechol sulfate (NCS) and was incubated at room temperature ($28 \pm 2^{\circ}C$). for 12 hrs unless otherwise stated. The reaction was terminated by the addition of 4ml of 1 N-NaOH. This resulted in the red anionic form of 4-nitrocatechol (4-NC) chelating strongly with the metal hydroxide present in the enzyme solution and the precipitate settled to the bottom of the tube. To free the anionic form from metal chelation the following modified procedure was employed. Two drops of EDTA(0.25M) were added to the reaction mixture before the addition of NaOH and the mixture was allowed to stand for 10 minutes before it was centrifuged at 15,000xg for 15 minutes. One ml of the supernatant was then diluted with 4 ml of distilled water and the red anionic form of 4-NC was measured in a UNICAM Sp. 500 spectrophotometer at a wavelength of 515 mμ against a reagent blank, prepared by incubating the enzyme and the substrate separately and mixing them before the addition of NaOH to the enzyme solution. A standard curve was prepared using 4-NC in the similar manner and the enzyme activity was expressed as 4-NC liberated/mg tissue.

RESULTS AND DISCUSSION

Effects of pH on arylsulfatase activity of *C. fluviatilis* was studied using Nitrocatechol sulfate (NCS) as substrate. Two maxima-a primary peak at pH 5.6 and a secondary one at 7.1 could be observed (Fig. 1). A similar primary peak was reported in the limpet *Patella vulgata* (Dodgson, Spencer and Corner, 1963) and in *Aspergillus oryzae* (Robinson, Smith, Spencer and Williams, 1952) using acetate buffer at pH 5.8. It has been reported that ammonia, chloride and acetate ions have a marked (activation) influence on the hydrolysis of substrate by bacterial arylsulfatase (Dodgson, 1959; Harada and Spencer, 1964). Since chlorides and ammonia are found to be present in the marine environment, acetate buffer was used in the present study. Maximum activity of this enzyme at pH 5.6 confirms the earlier observation (Dhevendaran, Kannupandi and Natarajan, 1978). The secondary peak at pH 7.1 may be due to the enzyme elaborated by the micorbes associated with the

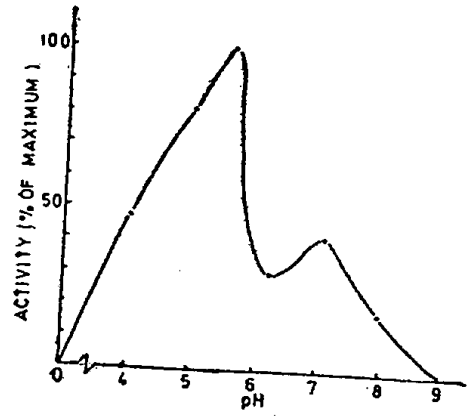

Fig. 1. pH activity curve for arylsulfatase in *Cerithidium fluviatilis*.

molluscs as suggested by Dodgson, Lewis and Spencer (1953). This additional secondary peak suggests the presence of more than one type of arylsulfatase for the first time in marine gastropods. Similar second peak at that pH was found in *Aerobacter aerogenes* (Rammler, Grado and Flower, 1964). Chandramohan, Dhevendaran and Natarajan (1974) reported arylsulfatase with two peaks of activity (at pH 6·2 and 9·0) using tripotassium phenolphthalein disulfate as substrate in marine sediments. In the present study it has been found that at pH 8·0 the enzyme activity was 45% of that of the second peak (at pH 7·1). *Alcaligines metalcaligenes*, isolated from intertidal mud, showed optimal hydrolysis (Dodgson, Melvillie, Spencer and Williams, 1954) at pH 8·0. The gastropod (*C. fluviatilis*) inhabits the intertidal mud in the Vellar estuary at Porto Novo and as already mentioned earlier, the secondary peak at pH 7·1 could well be due to the microbes associated with mud as found by Dodgson, Lewis and Spencer (1953). Enzyme activity estimated in tissues in the presence of certain endogenous inhibitors, sensitive to pH, which might exert some influence, may cause changes in the activity curve is also a possibility that cannot be overlooked as suggested by Shimony and Nigrelli (1972).

The optimum temperature for arylsulfatase activity was found to be 29°C. At 40°C nearly 30% of activity was lost and at 50°C the loss was as much as 70% (Fig. 2). Milanesi and Bird (1972) also found decreased arylsulfatase activity when the temperature increased above 38°C and suggested that it might be due either to proteolysis or to enzyme inactivation. *Proteus rettgeri* (Milazzo and Fitzgerald, 1967) exhibited maximum growth and highest arylsulfatase activity at 28°C. Dhevendaran (1978) recorded maximum growth and highest enzyme activity at 29°C in *Eschirechia coli* and noticed a decreased trend in growth and a loss of activity when temperature increased suggesting the inactivation of enzyme activity both during growth, and in the harvested bacterial cells, by the rise of temperature. Perhaps the same is true in the case of gastropods also.

The influence of incubation period on the enzyme activity is shown in Fig. 3. When NCS was used as a substrate, maximum hydrolysis was noticed after 12 hours of incubation. Nearly 80% of activity could be observed after 8 hours of incubation. No change in the activity could be observed by extending the period of incubation.

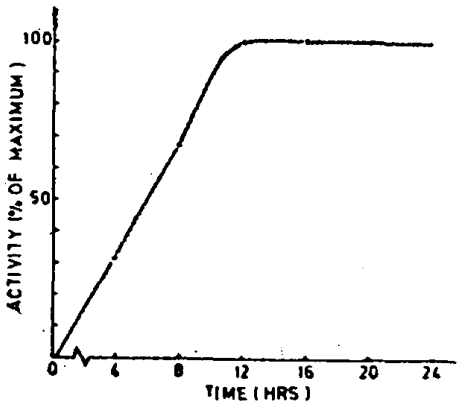

Fig. 2. Effect of temperature on arylsulfatase activity in *C. fluviatilis*.

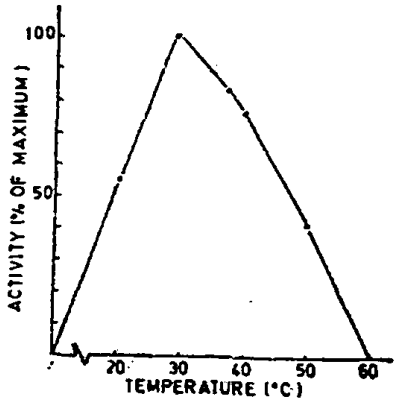

Fig. 3. Effect of incubation period on arylsulfatase activity in *C. fluviatilis*.

Dhevendaran (1978) however, observed maximum activity (92%) after 12 hours of incubation in the case of marine sediments. Tabstabai and Bremner (1970) also reported that prolonging the period of incubation increased the activity in the soil. But a declining trend in activity with longer period of incubation was reported in *Helix pomatia* (Dodgson and Powell, 1959).

Enzyme activity in different marine gastropods in various organs is shown in Table I. It could be recorded in visceral humps of all the molluscs studied. *C. ruburum* showed high activity (0.368 µg NC/mg) and *M. ceilonicus* recorded the lowest (0.153 µg NC/mg). These are herbivorous, inter-tidal in their habitat and are always found attached to the prop roots of *R. mucronata* and aerial roots of *A. officinalis* respectively, above the water column. Dodgson, Lewis and Spencer (1953) also found higher arylsulfatase activity in herbivorous molluscs than in carnivorous forms. The foot of *C. mustelina* and *L. tigrina* exhibited higher enzyme activity than in other organisms. Greater activity in foot indicates that the enzyme is not probably associated only with digestion as in other invertebrates (Roy, 1960). Interestingly these two molluscs showed greater enzyme activity at 37°C as in terrestrial snails. The crystalline style of *C. rubrum* showed higher activity (0.727 µg NC/mg) and in other molluscs this style could not be separated due to their small size. The activity of arylsulfatase in the crystalline style is reported only now and further studies may throw more light on the possible digestive functions of this enzyme in the crystalline style of molluscs.

Table II shows arysulfatase activity in various organs of *T. telescopium* under different experimental conditions in crystalline style, digestive system, foot and mantle. Crystalline style and digestive system without this style showed maximum activity (1.067 and 0.860 µg NC/mg) and the foot recorded the lowest (0.028 µg NC/mg). *T. telescopium* is undoubtedly the most potent source for this enzyme. The activity as observed in the present investigation is considerably higher than in other molluscs (Dodgson and Powell, 1959). It is possible that the enzyme might help in the transfer of sulfate from arylsulfate to polysaccharides as suggested already (Suzuki, Takahashi and Egami, 1959; Dodgson and Powell, 1959). Dhevendaran (1978) found the above to be true in marine sediments. Since *T. telescopium* feeds on sediments and detrital particles, the digestive system of this organism will certainly contain appreciable amounts of sulfated polysaccharide material. When kept for 5 days in unfiltered and filtered estuarine water, its mantle and foot regions registered higher activities. Shimony and Nigrelli (1972) who found that the mantle

Table I. Arylsulfatase activity of different marine gastropods (activity is expressed as µg NC liberated/mg tissue).

S. No	Organisms	Organs experimented		
		Visceral humps	Foot	Crystalline style
1.	*Cerithium rubrum*	0·368	0·187	0·727
2.	*Melampus ceilonicus*	0·153	—	—
3.	*Dostia indica*	0·307	0·291	—
4.	*Cassidula mustilena*	0·174	0·279	—
5.	*Littorina tigrina*	0·281	0·413	—

Table II. Arylsufatase activity of *Telescopium telescopium* growing in different conditions (μg NC/mg tissue).

Organs	Organisms acclamatized		
	Fresh organism	In unfiltered estuarine water	Filtered estuarine water
1. Crystalline style	1·067	0·660	0·300
2. Digestive system	0·867	0·428	0·131
3. Foot	0·028	0·058	0·107
4. Mantle	0·056	0·083	0·189

of the starved *B. eburneus* had higher activity than digestive tract, suggested that the enzyme may also be involved in the secretion of the exoskeleton. They also compared the enzyme activity in daily fed and unfed barnacles and registered higher activity in the latter. All these observations indicate the possibility of various roles for this enzyme, again in different forms (as indicated by the peaks of activity at various pH). Separation of the enzyme fractions by improved techniques as suggested by Agogbus, Anosiki and Ugochukwu (1978) is therefore, necessary for further study. Although it is as yet not possible to differentiate the arylsulfatases into A and B as in mammalian tissues, the properties of this enzyme from molluscs resemble the Type-II arylsulfatase (Dodgson and Spencer, 1957) of mammals.

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