Arylsulfatase Activity in Marine Polychaetes

K. DHEVENDARAN
School of Marine Sciences, University of Cochin, Cochin - 682 016

Marine polychaetes, collected from the Vellar Estuary exhibited arylsulfatase activity. *Lumbriconeries* sp. *Polydora* sp. *Monofis* sp. and *Heteromastus* sp. were selected for this study. Of these, *Heteromastus* sp. showed maximum enzymatic activity and it has been chosen for the enzyme kinetic studies such as pH, optimal temperature, period of incubation and the effect of DDT. Enzyme activity showed single peak at pH 6.2 possibly indicating the presence of one type of arylsulfatase. Maximum activity was attained after 12 h of incubation at 29°C. DDT has an inhibiting effect on the arylsulfatase activity even at the concentration of 10 p.p.m. and the activity was completely lost at 100 p.p.m.

Arylsufatase (arylsulfate sulfohydrolase E. C. 3.1.6.1) is a lysozyme that partcipates in the hydrolysis of sulfuric acid esters of aromatic compounds. Since the natural substrate is still obscure, it stimulates the detailed investigation of this enzyme in both vertebrates and invertebrates (Dodgson & Spencer, 1957; Wortman & Schneider, 1960; Conner, *et al.*, 1960; Jarrige, 1963; Agogbua *et al.*, (1978) suggested that molluscs were the potential sources and in continuance of this line various other invertebrates were considered. Quite interestingly, the marine environment is a vast untapped resource and this has been considered to be the best suitable biotope for the easy accessible exploitation of this enzyme.

The bacterium, *Alcaligenes metalcaligenes* exhibited arylsulfatase activity and is inhabiting in the marine intertidal mud (Dodgson, *et al.*, 1953). Shimony & Nigrelli (1972) correlated this enzyme activity with the cyclic formation and hardening of the exoskeleton of *Balanus eburneus,* whereas Cornet & Jangoux (1974) noted the functional relationship between this enzyme activity and the feeding habits in some echinoderms. The enzyme kinetics of the arylsulfatase in marine sediments and salt marshes showed variety of the components of this enzyme (Dhevendaran, 1978; Oshrain & Weibe, 1979). Dhevendaran *et al.* (1980) have found variations in the activity of this enzyme in foot, visceral hump and crystalline style of some marine gastropods, inhabiting ecologically different biotopes. Corner *et al.* (1960) reported the occurrence of this enzyme in the polychaete *Chaetopterus variopedatus.* The present investigation is the first attempt of enzyme kinetics of arylsulfatase in marine polychaetes and gives an added information about its functional significance.

Materials and Methods

Lumbriconeries sp. and *Monifis* sp. were collected from sandy beaches, whereas *Polydora* sp. and *Heteromastus* sp. were taken from the station, situated in the Vellar Estuary, just opposite to the Biological Station (Fig. 1).

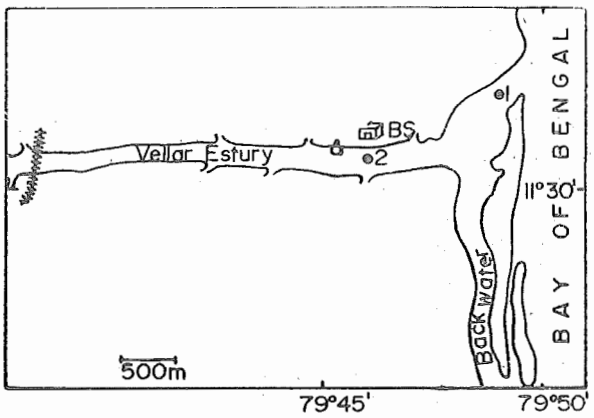

Fig. 1. Vellar estuary showing sampling stations

The sandy beach is directly under the influence of seawater and the sediment is mainly coarse and also the organic matter is negligible. But the sediment nature in the station just opposite to the Biological Station is clayey and is always rich in organic matter. It is

often flushed with saline water except during the monsoon season.

The organisms were cut open to remove all the sediments and sand grains and they were thoroughly washed with 50% seawater. The polychaetes were weighed separately. The weighed material was homogenised in 3.0 ml of distilled water.

Arylsulfatase activity was estimated spectrophotometrically in the red anionic form of 4-Nitrocatechol (4.NC), released during the enzyme hydrolysis (Shimony & Nigrelli, 1972). The reaction mixture consisted of 1 ml of enzyme solution, 2 ml of 0.2 M acetate buffer at pH 6.2 and 1 ml of 0.002 M Nitrocatechol sulfate (NCS) as substrate and was incubated at room temperature (28 ± 2°C) for 12 h unless otherwise stated. At the end of the incubation period the reaction was stopped by the addition of 4 ml of 1N NaOH. The liberated red anionic form of 4·NC was measured following the modified procedure employed by Dhevendaran *et al.* (1980) at a wave-length of 515 nm against a reagent blank. A standard curve was prepared using 4.NC in the similar manner and the enzyme activity was expressed as 4.NC liberated/mg of organism.

Results and Discussion

Arylsulfatase activity was recorded in all the four selected polychaetes. Of these, *Heteromastus* sp. displayed higher activity than that of others and hence this organism has been used for enzyme kinetic studies. It is understood from the results (Fig. 2) that the maximum activity was observed at pH 6.2 thereby confirming the earlier findings of Chandramohan *et al.* (1974) in the marine sediments and the possibility for the contribution towards the sedimentary enzyme pool and also supporting the previous reports in *bacterium, Mycobacterium piscium* and the mollusc, *Achatina achatina* (Whitehead *et al.*, 1952; Agogbua *et al.*, 1978). Conner *et al.* (1960) tested the arylsulfatase activity at pH 5.5 in *Chaetopterus variopedatus* but the optimum pH of this enzyme is still obscure. Hence an attempt was made to study the pH optimum of this enzyme in *Heteromastus* sp. and further study may throw more

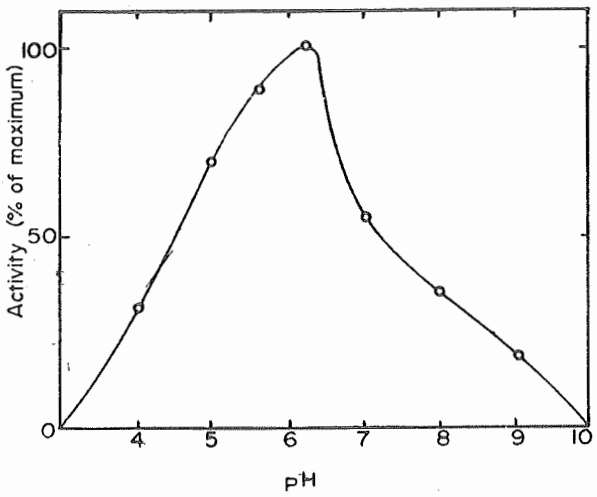

Fig. 2. pH activity curve for arylsulfatase activity in *Heteromastus* sp.

light on the possible function of this enzyme in marine polychaetes. Quite surprisingly when the pH was raised towards the alkaline side such as pH 8 and 9 the activity was very much reduced to 35% and 19% of maximum activity respectively. This indicates that the arylsulfatase under natural conditions must be at a very low level both qualitatively and quantitatively since the pH of the seawater and sediment ranged from 7.9 to 8.3. Dhevendaran (1978) confirmed this by his study in the marine sediments. At pH 5.6, 88% of the maximum activity was observed which is very close to the pH optimum in the limpet, *Patella vulgata* and in the fungus, *Aspergillus oryzae* (Dodgson, *et al.*, 1953; Robinson *et al.*, 1952) but Oshrain & Wiebe (1979) noticed this enzyme activity at the wider range of pH between 5 and 9 in salt marshes. The earlier report in marine molluscs at the same pH optimum (pH 5.6) supports this (Dhevendaran *et al.*, 1980). At pH 7.1 about 54% of the maximum activity was recorded and this may be attributed to the microbial association with polychaetes as suggested by Dodgson *et al.* (1953) in molluscs. Ammonia, chloride, citrate and acetate ions have a marked activation effect on the hydrolysis of the substrate by bacterial arylsulfatase (Dodgson, 1959; Harada & Spencer, 1964) and these ions may also have some influence on the arylsulfatase in polychaetes and it needs further study and work on this line is in progress. However, the presence of activators

or inhibitors in the tissue of polychaetes, which are sensitive to pH may also cause some considerable changes in the enzyme activity curve as suggested by Shimony & Nigrelli (1972).

Fig. 3 shows the influence of period of incubation on the enzyme activity. Maximum hydrolysis of nitrocatechol sulfate was

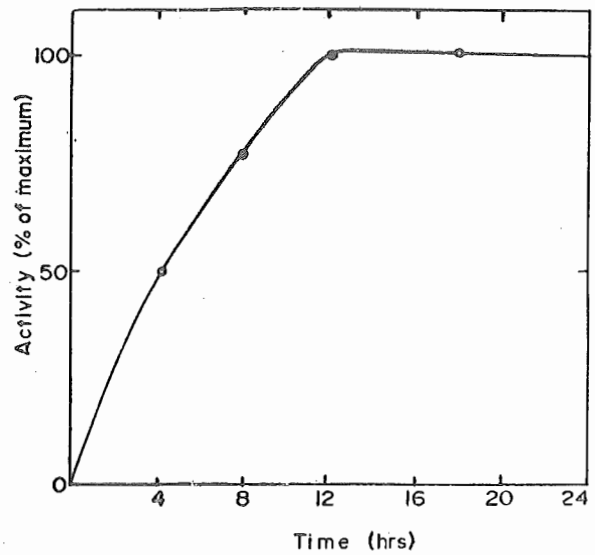

Fig. 3. Effect of incubation period on arylsulfatase activity in *Heteromastus* sp.

obtained after 12 h of incubation, similar to the observation made by Dhevendaran *et al.* (1980) in marine molluscs. Nearly 77% of maximum activity could be recorded after 8 h of incubation. No change in the activity could be observed by extending the period of incubation. Dhevendaran (1978), however, observed maximum activity (92%) after 12 h of incubation in marine sediments. Tabatabai & Bremner (1970) reported that prolonging the period of incubation increased the activity in the soil. But a declined trend in activity with longer period of incubation was reported in *Helix pomatia* (Dodgson & Powell, 1959).

The optimum temperature for arylsulfatase activity in polychaetes was noticed at 29° C (Fig. 4). It is obvious that it is an aquatic organism and showed maximum activity at this temperature, which is almost similar to the temperature of the environment where the organism is inhabiting. This result fully supports the earlier observations

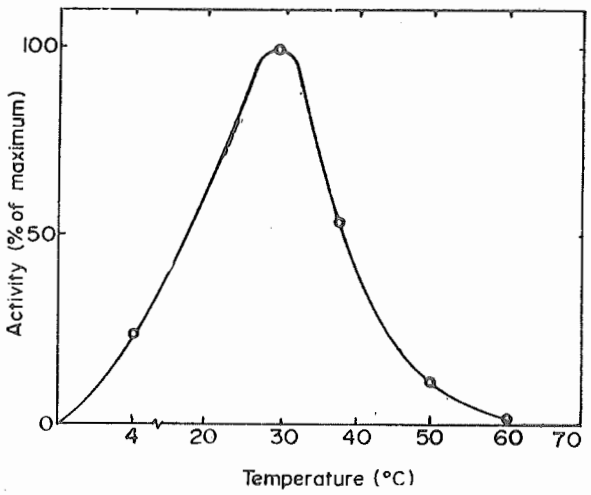

Fig. 4. Effect of temperature on arylsulfatase activity in *Heteromastus* sp.

made by Dhevendaran (1978) and Dhevendaran *et al.* (1980) in marine sediments and marine gastropods respectively. For the first time we could observe that the 23% of the maximum activity was retained at 4°C. At 40°C only 60% of the activity was lost and further increase in temperature to 65° C, there was a complete loss of enzyme activity. This may be either due to the proteolysis or to the inactivation of the enzyme by rise of temperature as suggested by Milanesi & Bird (1972).

The stations from where polychaetes collected, were frequently influenced by the discharge of nearby agricultural field and there is every possibility for the influence of various herbicides and pesticides used in the agricultural field, over the polychaetes, which were discharged into the estuarine water. So an attempt has been made to study the effect of DDT on arylsulfatase activity in marine polychaetes (Table 1). The minimum concentration of (10 p.p.m.) DDT has got inhibiting effect on the arylsulfatase activity. It is understood that the inhibitors may be one of the factors for determining the components of the arylsulfatase enzyme. Besides this, Kiss *et al.* (1975) suggested that the soil enzymes are very much influenced by differential compounds such as antibiotics, herbicides, pesticides etc. Similarly the inhibition of mercuric chloride, cyanide and phosphate on soil arlysulfatase, phosphate on the activity of arylsulfatase B in *Balanus eburneus* and of cyanide, phosphate and sulfate on the marine sediments clearly

Table 1. *Effect of DDT on arylsulfatase activity in Heteromastus* sp.

Concentration of DDT, p.p.m.	Arylsulfatase activity, µg NC/mg
10	1.177
20	1.121
50	0.188
100	0.036

indicates the possibility of occurrence of more than one type of arylsulfatase enzyme in the marine environment (Tabatabai & Bremner, 1970; Shimony & Nigrelli, 1972; Dhevendaran, 1978). Work on this line is in progress to identify the components of arylsulfatase enzyme.

Table 2 shows the arylsulfatase activity in the marine polychaetes, inhabiting the

Table 2. *Arylsulfatase activity of different marine polychaetes*

Nature of sediments	Organisms	Arylsulfatase activity, µg NC/mg
Sandy	*Monofis sp.*	0.322
Sandy	*Lumbriconeries* sp.	0.401
Clayey	*Polydora* sp.	0.480

ecologically varying niches. *Polydora* sp. exhibited considerably higher activity than other polychaetes (0.480 NC/mg). It is undoubtedly one of the potent sources for this enzyme. It is possible that the enzyme might help in the transfer of sulfate from arylsulfate to polysaccharides as suggested already (Suzuki *et al.*, 1959; Dodgson & Powell, 1959). Dhevendaran (1978) found the above to be true in marine sediments. Since *Polydora* sp. feeds on sediments and detrital particles, the digestive system of this organism will certainly contain appreciable amounts of sulfated polysaccharide material. Dodgson *et al.* (1953) and Corner *et al.* (1960) suggested that arylsulfatase activity was greater in herbivorous species possibily because of thick walled plant cells in the diet. Cornet & Jangoux (1974) also reported that arylsulfatase in some echinoderms and found higher activity in algivorous animals than in carnivores. All these observations indicate the possibility of various roles for this enzyme, in different forms (as indicated by the peaks of activity at various pH). Although it is not possible to differentiate the arylsulfatases into A and B, as in mammals, the properties of this enzyme from polychaetes resemble the Type II arylsulfatase (Dodgson & Spencer, 1957) of mammals.

Thanks are due to Prof. R. Natarajan, Director, Marine Biological Station, Porto Novo for constant encouragement and critically going through the manuscript. The facilities provided by Annamalai University, the financial assistance by UGC and Dr. B. Srikrishnadas Fisheries College, Tuticorin, Tamil Nadu for the identification of polychaetes are gratefully acknowledged. I am also thankful to Prof. N. R. Menon, Head of the Division of Marine Biology, Microbiology and Biochemistry, University of Cochin for his encouragement and facilities provided.

References

Agogbua, S.I.O., Anosiki, E.O. & Ugochukuru, E. N. (1978) *Comp. Biochem. Physiol.* **59B**, 169

Chandramohan, D., Dhevendaran, K. & Natarajan, R. (1974) *Mar. Biol.* **27**, 89

Corner, E.D.S., Leon, Y. A. & Bulbrook, R. D. (1960) *J. mar, Biol. Assoc. U.K.* **39**, 51

Cornet, D. & Jangoux, M. (1974) *Comp. Biochem. Physiol.* **47**, 45

Dhevendaran, K. (1978) *Studies in Arylsulfatase Activity of Marine Sediments. Ph. D. Thesis, Annamalai University.* p. 115

Dhevendaran, K., Kannupandi, T. & Natarajan, R. (1980) *Mahasagar,* **13**, 173

Dodgson, K. S. (1959) *Enzymologia,* **20**, 301

Dodgson, K. S., Lewis, J.I.M. & Spencer, B. (1953) *Biochem. J.* **55**, 253

Dodgson, K. S. & Spencer, B. (1956) *Biochem. J.* **62**, 30

Dodgson, K. S. & Powell. G. M. (1959) *Biochem. J.* **72**, 666

Dodgson, K.S. Spencer, B. & Corner, E.D.S. (1963) *Biochem. J.* **79**, 612

Harada, T. & Spencer, B. (1964) *Biochem J.* **83**, 373

Jarrige, P. (1963) *Bull. Soc. Chem. Biol.* **45**, 761

Kiss, S., Bularda, M. D. & Radulescu, D. (1975) *Adv. Agron,* **27**, 25

Milanesi, A. A. & Bird, J.W.C. (1972) *Comp. Biochem. Physiol.* **41B**, 573

Oshrain, R. L. & Wiebe, W. J. (1979) *Appl. Environ. Microbial.* **38**, 337

Robinson, D., Smith, J. W., Spencer, B. & Williams, R. T. (1952) *Biochem. J.* **51**, 202

Shimony, T. & Nigrelli, R. F. (1972) *Mar. Biol.* **14**, 349

Suzuki, S., Takahashi, N. & Egami, F. (1959) *Biochem. J.* **73**, 557

Tabatabai, M. A. & Bremner, J. M. (1970) *Proc. Soil. Soc. America.* **34**, 225

Whitehead, J.E.M., Morrison, A. R. & Young L. (1952) *Biochem. J.* **51**, 585

Wortman, B. & Schneider, A. (1960) *Anal Rec.* **137**, 403