Journal of Environmental Biology **September 2009,** 30(5) 655-658 (2009)

Activity of phosphatases in the hemocytes of estuarine edible mudcrab, *Scylla serrata* exposed to arsenic

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(Received: March 03, 2008; Revised received: September 09, 2008; Accepted: October 10, 2008)

Abstract: *Sundarbans biosphere reserve is an ecologically important area of West Bengal, which supports a wide variety of estuarine species including Scylla serrata estuarine mudcrab of economic value. In this present study, activity of phosphatases (acid and alkaline) of hemocytes was estimated under the sublethal exposure of arsenic in controlled static water environment for 1, 2, 3, 4, 7 and 15 days of exposure. Exposure to 3 ppm of sodium arsenite for 15 days, resulted a maximum inhibition of activity of acid phosphatase (ACP) as 0.016 μM mg^{-1} protein min^{-1} with intermediate activity in different spans of exposure against the control value of 1.83 μM mg^{-1} protein min^{-1}. Similar mode of maximum inhibition of activity of alkaline phosphatase (ALP) was recorded as 0.008 μM mg^{-1} protein min^{-1}, when the crabs were exposed to 3 ppm of sodium arsenite for 15 days against the control value of 0.694 μM mg^{-1} protein min^{-1}. Exposure to 1 ppm of sodium arsenite caused inhibition of activity of ACP which ranged from 1.76 to 0.70 μM mg^{-1} protein min^{-1}. For 2 and 3 ppm of exposure activity of ACP ranged from 1.12 to 0.42 and 0.67 to 0.016 μM mg^{-1} protein min^{-1} respectively. For the activity of ALP exposure to 2 and 3 ppm of sodium arsenite yielded an inhibition of activity which ranged from 0.472 to 0.279 and 0.275 to 0.008 μM mg^{-1} protein min^{-1} respectively. Arsenic exposure resulted a dose dependent decrease in the activities of both of the phosphatases and may be considered as a suitable biomarker of aquatic pollution in Sundarbans mangrove.*

Key word: *Sodium arsenite, Scylla serrata, Hemocytes, Phosphatase, Sundarbans*
PDF of full length paper is available online

Introduction

Generally all the pollutants released on land will eventually find their way to the rivers and oceans as the final repository. Since the estuaries are the links to the freshwater and marine systems, they contain a variety of anthropogenic chemicals referred to as xenobiotics and such xenobiotics have potential to affect normal physiology of crustaceans (Vijayavel and Balasubramanian, 2006).

Arsenic is a contaminant not only to the ground water but also to the surface water of selected plains of the state of West Bengal (Acharyya *et al*., 1999; Acharyya, 2002), which originates from chemical weathering by nature and preservation of timber, purifying industrial gases (removal of sulfur), electronics manufacturing (microwave devices, lasers, light-emitting bodies, photoelectric cells and semiconductor devices), Mining, smelting of non-ferrous metals and burning of fossil fuels are the major industrial processes that contribute to anthropogenic arsenic concentration of air, water and soil (Das and Roy Chowdhury, 2006). In West Bengal region almost 6 million people in 9 of 18 districts are endemically exposed to arsenic and over 1 million people consume drinking water containing up to 3.7 mg l^{-1} arising from normal geochemical processes (Das *et al.*, 1995; Tseng, 2007; Yoon *et al.*, 2008) and are at risk of cancer and other diseases (Flora *et al.*, 2007).

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Scylla serrata is an economically important species of crab due to its palatability, nutritive value, large size, high unit price and great demand in the local and export markets, which is harvested from its natural habitat as well as collected from crab growing pond of selected area for human consumption. It is widely distributed in the intertidal mud flat of Sundarbans biosphere reserve of West Bengal, India and is considered as one of the large groups of crab in Sundarbans mangrove swamp (Chakraborty, 1986; Ali, 2004; Saha and Ray, 2006). Natural habitat and growing pond of *S. serrata* bears the risk of threat due to exposure of metalloid arsenic (Krishnaja *et al*., 1987; Saha *et al*., 2007). Sundarbans support an enormous magnitude of biodiversity, which faces the risk of contamination by diverse xenobiotics including arsenic (Saha *et al*., 2008a).

Acid phosphatase (ACP) is lysosomal enzyme and cellular damage is usually accompanied by increase in the activity of this enzyme. Alkaline phosphatase (ALP) is a brush border enzyme and involves in transport and transphosphorylation reactions. Enzyme is used as a potential biomarker for a variety of different organisms due to its high sensitivity, less variability, high conservedness among species and often easier to measure as stress indices (Reddy *et al*., 1991; Vijayavel and Balasubramanian, 2006). Generally crustaceans have been found to be useful as indicators of certain contaminants due to their wide geographical distribution, dominant presence in coastal and estuarine communities, bottom-dwelling habits and ability to respond to environmental

pollutants and pathogens importance as an economic food source for humans (Vijayavel *et al*., 2006). Phosphatase activity in the granules of the blood cells was demonstrated in invertebrates by Hearing (1969) and Carballal *et al.* (1997). The present study is aimed to probe into the *in vivo* effects of arsenic on acid and alkaline phosphatase activity level in hemocyte of *S. serrata*. Alteration of phosphatase activity under sodium arsenite exposure in hemocytes of *S. serrata* is reported for the first time.

Materials and Methods

Animal collection and treatment: Healthy adult specimens of *S. serrata* weighing (70 ± 10 g) were collected from selected habitat in south 24 Parganas district of West Bengal and were transported alive in jute bags to laboratory. Average length and breadth of the carapace of specimen were 6 ± 0.5 cm and 9 ± 0.5 cm respectively. Animals were maintained in standard glass aquaria in batches and fed with fresh flesh of molluscs. The water was replenished 24 hourly and animals were acclimatized in laboratory condition for 7 days prior to experimentation (Heasman and Fielder, 1983).

A standard solution of 100 ppm of sodium arsenite (E Merck, Germany; 99% pure; CAS number 7784-46-5) was prepared in borosilicate glass containers using arsenic free distilled water. The pH of the solution was maintained as 7.4. An acute toxicity bioassay was conducted to determine median lethal (LC_{50}) value of sodium arsenite for *S. serrata* according to 'Behrens-Karber method' (Klassen, 1991; Shibu Vardhanan and Radhakrishnan, 2002). Each of the experimental set (1,2 and 3 ppm based on LC_{50} value *i.e.* 15 ppm) along with control consisted of 10 animals of same age and sex in triplicate forms. Biochemical experiments were carried out for 15 days in static water environment and fresh solutions of sodium arsenite were replenished every 24 hr. During treatment animal received uniform ration of daylight and routinely checked for mortality and morbidity. Dead crabs were removed immediately from the test medium. Temperature of water was maintained between 26 to 30°C.

Collection of hemolymph and preparation of cell lysate: Control and treated animals were cleaned by running tap water. Fresh hemolymph was collected aseptically from the base of second appendage of the three different animals in three different Borosilicate containers of same concentration after 1, 2, 3, 4, 7 and 15 days using a sterile syringe with a 23-gauge needle and was stored in prechilled glass vials with anticoagulant. The volume of collected blood of each animal did not exceed 1 ml (Yildiz and Atar, 2002). Hemocytes were sedimented by centrifugation of the hemolymph at 3000 rpm for 5 minutes. Cell density was adjusted with unit volume of phosphate buffer saline solution (PBS). Hemocytes were treated with Triton X 100 (0.1%) for 30 minutes on ice. Cells were routinely checked for completion of lyses. Lysate supernatant of hemocytes were stored at 4°C and used for biochemical analyses (Tanner *et al.*, 2006).

Estimation of acid phosphatase (ACP, EC 3.1.3.2) and alkaline phosphatase (ALP, EC 3.1.3.1): Acid and alkaline phosphatase activity was estimated according to Michell *et al.* (1970) and Estiarte *et al.* (2008). The reaction medium for acid phosphatase contained 0.7 ml sodium acetate buffer (pH 5.0), 0.25 ml p-nitrophenyl phosphate (pNPP, 5mM) as substrate and 0.05 ml of enzyme totaling to 1 ml was incubated for 30 minutes at 37°C. The reaction was stopped by adding 4 ml NaOH (0.1 N) and incubated for another 30 minutes at 37°C. The reaction medium for alkaline phosphatase contained 0.5 ml glycine buffer (pH 7.8), 0.2 ml magnesium chloride (10 mM), 0.25 ml p-nitrophenyl phosphate (pNPP, 5mM) as substrate and 0.05 ml of enzyme totaling to 1 ml was incubated for 30 minutes at 37°C. The reaction was stopped by adding 4 ml NaOH (0.02 N) and incubated for another 30 minutes at 37°C. The estimation involves measurement of yellow colour of p-nitrophenol in a spectrophotometrically assay at 420 nm (Shimadzu Japan). The molar concentration of p-nitrophenol was determined by using p-nitrophenol (pNP) as standard. Acid and alkaline phosphatase activity were expressed as μM mg^{-1} protein min^{-1}.

Protein was estimated following the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as the standard.

Statistical analysis: All experiments were repeated for three times and values are expressed as mean ± SD. The data are compared in paired t-test and symbols indicate values that are significantly different from controls. *p<0.05, **p<0.01, ***p<0.001.

Results and Discussion

Median lethal concentration (LC_{50}) value of sodium arsenite in crabs (*S. serrata*) was determined to be 15 ppm for 96 hr of exposure. Exposure of *S. serrata* to 1, 2 and 3 ppm of sodium arsenite for 1, 2, 3, 4, 7 and 15 days resulted in a dose dependent pattern of decrease of intrahemocyte ACP and ALP activity (Fig. 1,2). Phosphatases are nonspecific phosphomo-noesterases having pH specificity, which hydrolyze various phosphate esters and liberate phosphate from the substrates. The phosphatases also play major roles in the molting physiology of many crustaceans (Vijayavel and Balasubramanian, 2006). ACP hydrolyses the phosphorous ester in acidic medium and autolyses process of the cell after its death. ALP is a brush border enzyme and is involved in carbohydrate metabolism, growth and differentiation. The decreased activities of ACP and ALP indicate disturbance in the structure and integrity of cell organelles (lysosome) and physiological process (hormonal regulation) of organism, causing deleterious consequences (Joshi and Kumar, 2001). Decrease in phosphatase activity level shown in the present study might be due to the residue of arsenic in the water and its accumulation in hemocytes. However, in natural aquatic environment of West Bengal concentration of arsenic residues was reported to be 3.2 ppm (Das and Roy Chowdhury, 2006). The effect of arsenic exposure is indicative to possible perturbation of molting of the animal in its natural habitat due to decrease in the activities of phosphatases that involve in developmental physiology (Vijayavel *et al.*, 2006). Chronic exposure of arsenite may lead to decrease in the activity of

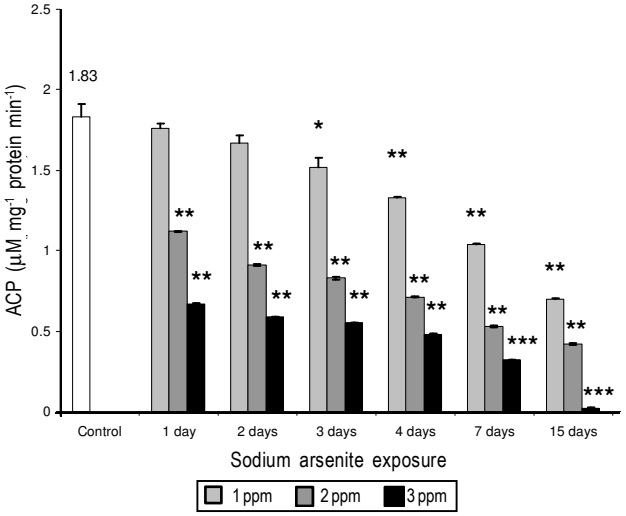

Fig. 1: Alteration of intrahemocyte acid phosphatase (ACP) activity of *S. serrata* under exposure of sodium arsenite. Data expressed as mean ± standard deviation. *p < 0.05, **p < 0.01, ***p < 0.001 (n=3)

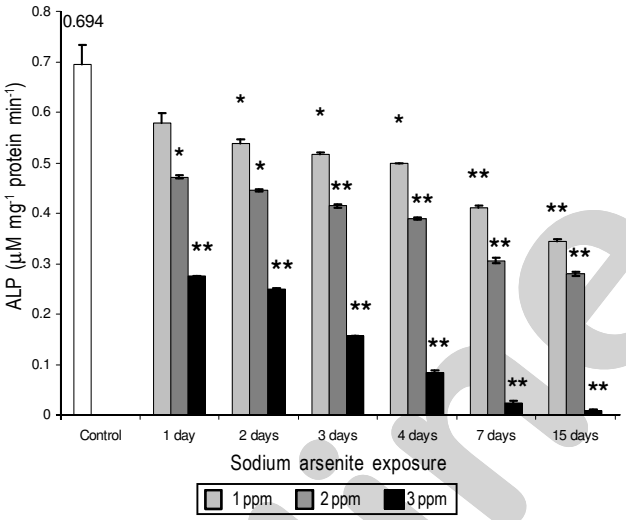

Fig. 2: Alteration of intrahemocyte alkaline phosphatase (ALP) activity of *S. serrata* under exposure of sodium arsenite. Data expressed as mean ± standard deviation. *p < 0.05, **p < 0.01, ***p < 0.001 (n=3)

phosphatases in crab in estuary. Situation would result on impairment of immunological activity and dysfunction of hemocyte (Saha *et al.*, 2007, 2008b), the chief immunological cells of crab under the continuous cellular stress resulting the crab to become immunologically weak and subject to various ailments. Crab may become susceptible to microbial infection leading to mass mortality. Situation may cause a steady decline and loss of this important biological resource from Sundarbans Biosphere Reserve.

Acknowledgments

Authors thankfully acknowledge the grant support received from UGC SAP DRSI and DST, FIST, Govt. of India.

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