Volume : 4 | Issue : 10 | October 2015 • ISSN No 2277 - 8179

Research Paper

Effect of Hypo Osmotic Stress on Phosphorylase Activity in Tiger *Prawn P. Monodon.*

Biotechnology

KEYWORDS : P.monodon, Phosporylase, Glucose, Salinity.

P N Pallavi | Research Scholar Dept of BiotechnologyS V University Tirupati-517501

ABSTRACT

The present study was aimed to know the Phosphorylase activity in Marine prawn P.monodon acclimated to low salinity levels (30, 20, 10, freshwater). The active and total phosphorylase activity levels show trends of increased in gills and decreased in muscle with decreasing salinity of acclimation medium in P. monodon. The active to total phosphorylase ratios show increased from 63.5% to 74.3% in gill and decreased from70.4 % to 60.5 % in muscle in lower salinities. The two tissues appear to utilize glycogen in higher quantities at lower salinities. Similarly carbohydrate, glycogen and hemolymph glucose levels decreased with decreasing salinities.

1. INTRODUCTION

Penaeid shrimp PLs, in particular *Penaeus monodon*, have the capacity to grow at low salinities (Davis *et al*., 2002) as occurs in estuaries, where they are subject to rapid changes in salinity and require a rapid activation of osmoregulatory mechanisms to survive. Salinity stress brings about metabolic alteration in the tissues of euryhaline animals. Glucose plays a central role in providing energy for metabolism. It is primarily stored in animal tissue as a glycogen (Roach *et al.,* 1998). Glycogen is the principal energy source in both vertebrates and invertebrate, especially during environmental fluctuations (Karlsson 1979; Oliera *et al*., 2004 and Bacca *et al*., 2005). The degradation of glycogen are mainly initiated by phosphorylase. Phosphorylase enzyme that maintains energy support for various tissues. The first step in the induction of reserve glycogen into the intracellular metabolic mill is phosphorolysis of this substrate catalyzed by phosphorylases. Gills are directly exposed to the outer aquatic environment, they have multiple functions including gas exchange, acid- base balance and ionic/ osmotic regulation (Evans *et al.,* 2005). In terms of the mechanism of ionic/ osmotic regulation, mitochondria – rich (MR) cells in gill epithelia are the main sites responsible for active ion transport functions. Which are conducted by the operations of various ion transporters and enzymes. These operations are highly energy consumptive processes (Hirose *et al.,* 2003; Evans *et al.,* 2005). Several studies have addressed the major role that carbohydrate plays in energy metabolism for osmoregulation. Energy may be mainly supplied by the oxidation of glucose and lactate obtained from the circulation as a result of carbohydrate metabolism (Perry and Walsh 1989 and Morgon *et al.,* 1997). These results imply that phosphorylase in shrimp gills may be involved in energy metabolism during acclimation to salinity changes.

In the present study the levels of active and total phosphorylase activity of shrimp gill and muscle tissue during low salinity acclimatization. *P.monodon* was selected for study due to its ability to adapt to fluctuations in salinity.

2. Material and Methods

2.1 Experimental animals and design: Tiger shrimp (*P. monodon*) were obtained from a commercial farm in Muttembakam village near Nellore(district), Andhra Pradesh (state) and were transferred to the laboratory in aerated plastic containers and maintained in laboratory holding tanks for a week in continuously aerated and filtered marine water (30 ppt) with a 12hr light-dark cycle. During this period the prawns were fed add libitum with commercial pelleted feed and then acclimated to the desired salinity by changing 5 ppt per day. After the shrimp 12g were separately acclimated from 30% to three salinity levels, i.e., 20, 10 and 5 ppt, the animals were randomly stocked into 6 tubes a density of 10 shrimp per tube with four replicates. During the periods of acclimation and 7days experiment All shrimp used in this study were individually examined for molt stage and only those at intermolt were used for biochemical analyses to minimize internal variations. Once a day changing at least 25% of the ambient medium and tap water was aerated before being added to the tube to adjust salinity. Water quality parameters including pH, salinity, temperature, dissolved oxygen and ammonia were monitored throughout the experimental period.

2.2 Sample collection: Animals were sacrificed by removing their carapace from their abdomen with a forcipes and the hepatopancreas, gills and muscle tissues were dissected out quickly. Tissues were washed in ice-cold normal saline (0.67%, w/v), blotted, flash frozen in liquid nitrogen and stored at -80°C.

2.3 Hemolymph collection: Hemolymph was collected through the arthrodial membrane at the base of a walking leg using 1ml hypodermic syringe fitted with a 25-gauge needle and prerinsed with EDTA buffer (anti coagulant is per the method of Lee *et al*., 1999).

2.4 Tissue phosphorylase activity: Phosphorylase Activity in the Muscle and Gill was estimated by colorimetric determination of inorganic phosphate released from glucose -1-phosphate (G-1-P) following the method of Cori *et al.,* 1955. Tissue homogenate (5% w/ v) was prepared in an aqueous medium containing 0.1M sodium fluoride and 0.037M ethylenediamine tetra acetic acid (EDTA) pH6.8 and centrifuged at 3,000 rpm for 10 min. The supernatant, diluted to four times (1:3) with cysteine hydrochloride (0.3M) sodium glycerophosphate (0.07 M) buffer (pH 6.8), is used as an enzyme source. Initially 0.4 ml of enzyme was incubated with 2.0mg of glycogen for 20 min at 35°C. The reaction was then nitiated by the addition of 0.2 ml of 0.016 M G-1-P to one tube (phosphorylase a) and a mixtureof 0.2 ml G-1-P and 0.004 M adenosine-5-monophosphate to an other tube (phosphorylase ab). The activity of total phosphorylase (ab) and active phosphorylase (a) was measured after incubating the reaction mixture for 15 and 30 min, respectively. Total Carbohydrate and Glycogen was estimated by the method of Carrol *et al.,* 1956. Glucose was determined by the method of Marks (1959).

3. RESULTS:

Phosphorylase activity levels of control and experimental are shown below. The gill shows the lowest levels of total phosphorylases (ab) as compared to the muscle. However, the ratio of active to total phosphorylase is highest in the gill in fresh water medium. The muscle show increasesin levels of active and total phosphorylase and decreased (60.5%)a/ab ratios through decreasing salinities of adaptation. ontrariety the gill shows a increase (74.3%) a/ab ratios in lower salinities. although the level of a and ab individually tend towards increases in lower salinities. Similarly total carbohydrate, concentration decreases from 20.5 ± 0.11 to 2.9 ± 0.11 in gill and muscle in hypo osmotic stress. Similarly total glycogen levels decreases from 56.4 ± 0.10

to 25.6 ± 0.11 in gill and muscle in low salinity stress. Hemolymph glucose levels were decreasing with decreasing salinity from 0.7 to 0.1 in 0ppt.

Statistical analysis

Results are presented as mean ± standard error of mean (SEM). Difference between the control and treatment means were compared by t-test, whereas difference among the control and Various treatment mean were analysed by one way analysis of variance (ANOVA), followed by Duncan's new multiple range test. Differences were considered statistically significant when P 0.05.

4. DISCUSSION

Phosphorylase activity in the muscle shows the highest levels of both active (a) and total phosphorylases (ab) as compared to the gill. However the ratio of total phosphorylase is highest in the gill of fresh water medium. The muscle shows increase in levels of active and total phosphorylase and decreased a/ab ratio through decreasing salinities of adaptation (Table-2). Contrarily the gill shows an increase in a/ab ratio in lower salinities although the levels of a and ab individually tend towards decrease in lower sanities. In the muscle the incidence of maximal level of a/ab ratio occurs in sea water. Since this ratio is more reliable determinant of the rate of phosphorolysis of glycogen, it follows that in sea water glycogen degradation occurs to the maximal extent in the above tissues. (Table-1) The gill exhibits a reversed trend as already mentioned above. In sea water the level of a/ab ratio happen to be the lowest in the gill. Evidently in this medium this tissue does not resort to phosphorolysis of glycogen in its energetics. In the lower salinity media the gill has been found to exhibit higher levels of a/ab ratio. It is possible that in these salinities the metabolism of tissue is geared towards utilization of glycogenolytic products. In the gill the mechanisms involved in the active uptake of ions are reportedly located. These active transport mechanisms are energized by ATPases and the glycogen pool in the tissue is probably involved in ATP generation in these salinities. In higher salinities the pressure on the active on uptake mechanism is probably reduced. It has been shown earlier that in higher salinities the heamolymph of this animal becomes isosmotic with medium. Obviously the pressure on the ion uptake mechanisms situated in gill is eased in higher salinities. This may have been causal for depression of phosphorolyse of glycogen in these salinities. Carbohydrates are the immediate source of energy in the cells. They play a major role in the cellular metabolism by serving as fuel and providing energy to the cells. Fluctuations in salinity reflect fluctuations in energy demands of the animal, changes in carbohydrate metabolism that would meet the changing energy demands may be expected to stress (Lacerda and Sawaya, 1986; Santos and Nay, 1987). When crustaceans placed in a medium of lower osmotic pressure, energy-consuming processes are required to maintain the internal osmotic pressure constant. Glucose delivers fast energy in the form of ATP via the process of glycolysis and oxidative phosphorylation and is the major circulating carbohydrate in crustaceans (Morris, 1999). Although lipid and protein utilisation is variable in crustaceans, generally, carbohydrate is used before lipid and protein as they preferred fuel for metabolic processes (Garret and Grisham, 1995; Morris, 1999).

Glucose is the major component of sugars in circulating haemolymph (Hohnke and Scheer 1970). It is central metabolic importance in virtually all organisms from microbes to man. Glucose concentrations, can be used as general stress indicators in shrimp. Generally, glucose in haemolymph increase in stressed shrimps to meet the energy demands to ward off stress. The glucose level of stressed animals might be due to the transport of glucose from hepatopancreas and muscle to hemolymph. Though an increase in glucose was observed only at 30 ppt (Figure-1), which is indicative of a rapid and selective consumption of the simple sugar at 0 ppt. Active consumption of glucose as an energy source for osmoregulation might have brought down the glucose levels in shrimps under salinity stress. Lamela et al. (2005) has observed a reduction in haemolymph glucose of *L. schmitti* exposed to lower salinities. The reduction in the glucose level due to stress and the resultant energy crisis. Racotta and Palacois (1998) reported that the glucose and lactate levels in the haemolymph were strongly affected by stress induced by manipulation in penaeid shrimp. Hall and van Ham (1998) reported that blood glucose served as a simple and reliable index for biological stress for shrimp. Telford (1968) and Lynch and Webb (1973) also reported that stress affected qualitative and quantitative nature of circulating carbohydrates.

In summary, our results show that the Phosphorylase activity under hyperregulating conditions suggests the participation of these enzyme in response to biochemical levels at varying environmental salinity. These activities in gills and muscle of *P. monodon* are involved in physiological processes of osmoregulation. We focus on establishing the exact biochemical role of these enzyme in gill and muscle euryhaline prawns responses to varying environmental conditions.

Conclusion:

The main goal of the present review is to discuss the biochemical response of the estuarine *P.monodon* to environmental saline stress. The biomoleculs involved in production of energy at low salinity levels for osmoregulation. Our result represents to produce healthy Post- larvae under low salinity conditions.

Acknowledgements The authors thank to Prof. N. Savithramma, HOD, Dept of Botany, S.V.University, Tirupati for accepting me as a guide.

Table-1 Effect of salinity on Total Carbohydrate, Glycogen concentration and phosphorylase activity in gill of *P.monodon* acclimated to different salinity levels. Data are expressed as mean ± SD of 6 observations; P>0.05.

Salinity (ppt)	TCHO (mg/gm wet wt)	Glycogen (mg/gm wet wt)	Phosphorylase (µ mol of inorganic phosphate formed/ mg protein/h) a	ab	a/ab (%)
F.W	2.9 ± 0.11	5.2 ± 0.02	24.3 ± 0.06	32.5 ± 0.14	74.3
10	8.3 ± 0.14	12.5 ± 0.03	20.5 ± 0.16	28.4 ± 0.23	71.4
20	14.8 ± 0.04	20.6 ± 0.21	16.5 ± 0.12	24.6 ± 0.18	68.4
30	20.5 ± 0.11	27.6 ± 0.11	12.6 ± 0.17	20.4 ± 0.19	63.5

Table-2 Effect of salinity on total carbohydrate, glycogen concentration and phosphorylase activity in muscle of *P.monodon*. Data are expressed as mean ± SD of 6 observations; P>0.05.

Salinity (ppt)	TCHO (mg/gm wet wt)	Glycogen (mg/gm wet wt)	Phosphorylase (µ mol of inorganic phosphate formed/ mg protein/h) a	ab	a/ab (%)
F W	25.6 ± 0.10	34.6 ± 0.11	30.4 ± 0.23	50.3 ± 0.24	60.5
10	37.4 ± 0.04	46.7 ± 0.03	38.5 ± 0.26	59.4 ± 0.20	64.5
20	45.5 ± 0.20	55.5 ± 0.06	42.5 ± 0.30	63.5 ± 0.31	66.4
30	56.4 ± 0.10	68.4 ± 0.12	47.5 ± 0.25	66.5 ± 0.22	70.4

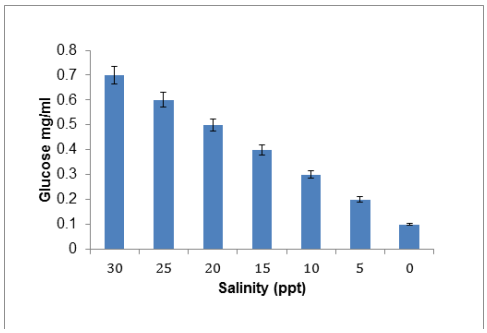


Figure -1 The influence of salinity on the hemolymph glucose levels of *P.monodon* at different salinity levels.Data are expressed as mean ± SD of 6 observations; P.<0.05.

Acknowledgements

The authors thank to Prof. N. Savithramma, HOD, Dept of Botany, S.V.University, Tirupati for accepting me as a scholar and giving me valuable suggestions.

REFERENCE

Bacca, H., Huvet, A., Fabioux, C., Daniel, J. Y., Delaporte, M., Pouvreau, S., Van Wormhoudt. A. and Moal, J. (2005). | Molecular cloning and seasonal expression of oyster glycogen phosphorylase and glycogen synthase genes. | Comp. Biochem. Physiol. 140B. 635-646. | | Carrol, N.V., Langle, R.W. and Raw, J.H. 1956. | Glycogen determination in liver and muscle by use of Anthrone reagent, | J. Biol. Chem., 22, 583. | | Cori, G.T., Illingworth, B. and Keller, P.G. 1955. | In: Method in Enzymology, Vol. I. Muscle phosphorylase. pp. 200-207, Colowick, S. P. and Kaplan, N.O. (eds.) | Academic Press, New York. | |Davis, D.A., Teichert-Coddington, D., Rouse, D.B., 2002. | Acclimation of Litopenaeus vannamei Postlarvae to Low Salinity: Influence of Age, Salinity Endpoint, and Rate of Salinity Reduction. | Journal of the World Aquaculture Society. 33, 78-84. | | Evans, D.H., Piermarini, P.M. and Choe, K.P. (2005). | The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol. Rev. 85, 97-177 | | Garret, R.H., Grisham, C.M., 1995. | Biochemistry Saunders College Publications, Forth Worth. | |Hall MR, van Ham EH (1998). | The effect of differenttypes of stress on blood glucose in the giant prawn Penaeus monodon . | J. World Aquacult. Soc. 29: 290 -299. | Hirose, S., Kaneko, Naito, N. and Takei, Y. (2003). | Molecular biology of major components o chloride cells. | Comp. Biochem. Physiol. 136B. 593-60. | | Hohnke L, Scheer BJ (1970).| Carbohydrate metabolism in crustaceans in Chemical Zoololgyi (M.Florken and B.T.Scheer Eds) Vol 5. | Academic Press, New York, pp. 147-166. | | Karlsson , J (1979) | Some features of glycogen metabolism in human skeletal muscle. | Bibl. Nutr. Dieta 27, 121-12. | |Lacerda, T. P and Swaya, P. (1986): | Effect of two hypo osmotic conditions on hemolymph glucose concentration in Callinectes danae(Crustacea). | Comp. Biochem. Physiol., 85(2): 509-512.| | Lamela, R.E.L., Coffigny, R.S., Quintana, Y.C., Martinez, M., 2005. | Phenoloxidase and peroxidase activity in the shrimp Litopenaeus schmitti, Perez-Farfante and Kensley (1997) exposed to low salinity. | Aquaculture 36, 1291-1297. | | Lee K.K., Chen, Y.L. and Lia P.C.1999. | Hemostasis of tiger prawn. Penaeu monodon affected by Vibrio harveyi, extracellular products and toxic cysteine protease. Blood cells, Molecular and Disease, 15(13): 180-192. | | Lynch MP, Webb KL (1973). | Variations in serum constituents of the blue crab Calinectus sapidus glucose. | Comp. Biochem. Physiol. A.45A: 127-139. | | Marks, V., 1959. | An improved glucose oxidase method for determining blood, C.S.F. and urineglucose levels. | Clin. Chim. Acta. 4, 395. | | Morgan, J. D., Sakamoto, T., Grau, E. G. And Iwama, G. K, (1997). Physiological and respiratory responses of the Mozambique tilapa (Oreochronus mossambicus) to salinity acclimation. Comp. Biochem. Physiol. 117A 391-398.| | Morris, S., 1999. | Integration of physiological responses of crustaceans to environmental challenge. | Afr. J.Zool. 33, 87– 106. | | Oliveira, G. T., Rossi, I. C., Kucharski, L. C. and Da Silva, R.S. (2004). | Hepatopancreas gluconeogenesis and glycogen content during fasting in crabs previously maintained on a high protein or carbohydrate –rich diet. | Comp. Biochem. Physiol. 137A, 383-390 | | Perry, S. F. and Walsh, P. J. (1989). | Metabolism of isolated fish gill cells: contribution o epithelial chloride cells. J. Exp. Boil. 144, 507-520. | | Racotta IS, Palacios E (1998). | Hemolymph metabolic variables in response to experimental manipulation stress and serotonin injection in Penaeus vannamei. | J. World Aquacult. Soc. 29: 351 -356. | | Roach P. J (1998); Cheng, C., Huang, D., Lin, A., Mu, J., Skurat, A.V., Wilson, W. And Zhai, L. (1998). | Novel aspect o the regulation of glycogen storage | J. Basic Clin. Physiol. Pharmacol. 9. 139-151. | | Santos, E. A and Nay, L.E.M. (1987): | Blood glucose regulation in an estuarine crab Chasmargnathus granulate (Dana, 1851) exposed to different salinities. | Comp. Biochem. Physiol., 4: 1033-1035. | | Telford M (1968). | Changes in blood sugar composition during the molt cycle of the lobster Homarus americanus. | Comp. Biochem. Physiol.26: 917-926. | |