African Journal of Biotechnology Vol. 6 (16), pp. 1944-1948, 20 August 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Biochemical studies on changes associated with enzymes of glucose metabolism in white spot syndrome virus (WSSV) infected with *Penaeus monodon* (Fabricius)

Suseela Mathew[1*], Ashok Kumar Kesavan Nair[2], Rangasamy Anandan[1], Pullanniyil Gopalan Nair Viswanathan Nair[1], and Koodenchery Devadasan[1]

[1]Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Matsyapuri (PO), Cochin 682 029, India.
[2]Quality Assurance and Management Division, Central Institute of Fisheries Technology, Matsyapuri (PO), Cochin 682 029, India.

Accepted 26 June, 2007

Tiger prawns (*Penaeus monodon*) were infected with white spot virus artificially by intramuscular injection of the virus inoculum. Haemolymph, hepatopancreas and muscle samples from the infected prawns were analyzed for glucose and enzymes viz aldolase, glucose-6-phosphatase, fructose-1,6-diphosphatase and glucose-6-phosphate dehydrogenase in the carbohydrate metabolism. Glucose content reduced to 33% of its original value in 24 h of infection and to 31% in 48 h. Almost 95% loss in activity was observed in the case of fructose 1,6-diphosphatase in hepatopancreas, whereas the reduction in activity in muscle was 67%, after 48 h of infection. Glucose-6-phosphatase showed a reduction of 16 and 13% in hepatopancreas and muscle respectively during 24 h of infection. The activity of glucose-6-phosphate dehydrogenase increased by 300% of original value in hepatopancreas, while in muscle the increase was only 30% of original value during 24 h of infection. No significant change in activity was noted in the case of aldolase. Even at moribund stage, the glycolytic pathway was not affected, as evident from the normal activity of aldolase observed in the present study.

Key words: WSSV, *P. monodon*, glucose, gluconeogenic enzymes, glycolytic enzymes.

INTRODUCTION

The diseases of cultured penaeid shrimp include infections (viral, rickettsial, bacterial, fungal, protistan and metazoan etiologies) as well as a number of non-infectious diseases caused by environmental hindrances, nutritional imbalances, toxicants and genetic factors (Lightner and Redman, 1998). White spot syndrome, one of the most serious viral diseases of cultured shrimp in the world (Wu et al., 2002) emerged in cultured kuruma shrimp (*Penaeus japonicus Bate)* in Japan (1993), and was named penaeid acute viremia (PAV). White spot syndrome virus (WSSV) infects a wide spectrum of hosts including shrimps (penaeid and nonpenaeid), crabs and aquatic insect larvae (Wu et al., 2002). It is currently the most important disease agent (van De Braak, 2002) in cultured prawns.

Diseases can be seen as the result of a complex interaction between host, pathogen and environment. Maintaining a healthy shrimp stock requires a multi disciplinary approach, which should include stress management and disease control (Sindermann and Lightner 1988). There is considerable evidence to support links between stress caused by environmental changes and diseases and a depression of the immune system (Dunier and Siwicki, 1993; Pipe and Coles, 1995). Once the immune system fails, it may lead to enormous changes in the metabolism of an organism. Though immunology has been studied in crustacea to a certain extent, not much attention has been paid to its relationship with metabolism, especially

*Corresponding author. E-mail: suseela@ciftmail.org. Tel: 00 91 484 2666845.Fax: 00 91 484 2668212

during disease conditions.

In general the metabolism of crustacea does not differ in broad outline from that of higher animal phyla and the principal pathways are more or less similar in crustacea as in vertebrates and the similarities overshadow the distinctions (Claybrook, 1983). There is evidence for the existence of glycolytic and pentose phosphate pathways in various crustacean species (Hohnke and Scheer, 1970). The production of NADPH by glucose-6-phosphate metabolism and the synthesis of ribose by the pentose phosphate pathway are critically important in the overall energy economy of the crustaceans. However carbohydrate metabolism has only been partially studied in shrimp (Rosas et al., 2001).

Glucose is the major component of sugars in circulating haemolymph (Hohnke and Scheer 1970). Glucose is of central metabolic importance in virtually all organisms from microbes to man. Metabolism of glucose through the pentose phosphate pathway generates NADPH and precursors required for a variety of anabolic pathways. Alternatively glucose may be converted to its polymeric form, glycogen, which is the storage form (Wilson, 2003). An efficient mechanism of glucose homeostasis seems to be unnecessary in crustaceans since they can tolerate large variations in blood glucose levels (Cuzon et al., 2000).

We have already reported the effect of WSSV infection on tissue defense system in *P. monodon* (Suseela et al. 2007). However the changes in the metabolism of glucose during white spot viral disease are not studied yet. The metabolic changes, if monitored regularly and effectively, can be used to predict the health status of an organism. Therefore an attempt is made to study the changes occurring to carbohydrate metabolism during WSSV infection of *P. monodon*.

MATERIALS AND METHODS

Collection of experimental animals

Healthy *P. monodon* (15.5 - 22.5 g body mass), were collected from grow-out ponds and maintained in 200 l fiberglass tanks with air-lift biological filters at room temperature (27 – 30°C) with salinity between 20 and 25 ppt. Natural filtered seawater was used in all experiments. The tanks were well aerated to maintain the oxygen level above 10 ppm. The animals were kept in the tanks for 10 days and fed with artificial pelleted feed for acclimatization before the experiments. From the experimental animals, 20 from a group of 100 were randomly selected and screened for the WSSV by polymerase chain reaction (PCR) using the primer designed by Takahashi et al. (1996) to ensure that animals are free from WSSV infection.

Experimental infection

Forty shrimp were kept separately as control and the remaining shrimps were used for experimental purpose. Animals were inoculated intramuscularly in the fourth abdominal segment with 100 µl of virus extract (9 mg protein/ml) prepared from infected shrimp for the induction of WSSV infection as per the method described by Van Hulten et al. (2000). The control group was injected with sterile normal physiological saline.

Ten shrimp from each group were randomly sacrificed at 0, 24 and 48 h (at the moribund stage) post injection. At 72 - 84 h, 100% mortality occurred in the virus-injected group, hence animals could not be collected after 48 h. 0.5 ml of haemolymph was drawn directly just before sacrifice with the help of a 26 gauge glass needle using saturated trisodium citrate as anticoagulant and preserved at 4°C. The shrimps were then dissected and, hepatopancreas and abdominal muscles were separated and stored at -20°C for biochemical analyses. The haemolymph of shrimp was subjected to PCR analysis using the primer designed by Takahashi et al. (1996) to confirm WSSV infection in the virus injected group.

Collection of haemolymph

Haemolymph was collected as per (Singh, 2001) using a specially designed sterile thin glass pipette with a tapered end. The pipette pre-rinsed with anticoagulant (10 mM Tris–HCl, 250 mM sucrose, 100 mM sodium citrate, pH 7.6) was introduced into the cephalothorax through the anterior portion of the head between the eyes. Adequate care was taken not to pierce the hepatopancreas. About 0.6 - 0·8 ml of haemolymph were withdrawn from cephalothorax using the pipette. The animal was held inclined slightly downwards to expedite the flow of heamolymph.

Haemolymph from 10 animals of each group was collected. The haemolymph was collected at 0, 24 and 48 h after the injection of viral extract and physiological saline. The collected haemolymph were stored in eppendorf tubes with 100 µl anticoagulant at 4°C. Enzyme assays were carried out without any delay after the collection of haemolymph.

Collection of hepatopancreas and muscle

The animals, after collection of haemolymph were dissected and hepatopancreas and muscles were removed and stored separately at -20°C for biochemical analysis. The muscle and hepatopancreas extracts were prepared in Tris HCl buffer pH 7.4.

Biochemical analyses

Glucose in haemolymph was estimated by the method of Sasaki et al**.** (1972). The enzymes aldolase (EC 4.1.2.13) and glucose-6-phosphatase (EC 3.1.3.9) were assayed by the method of King (1965a and b). Fructose-1,6-diphosphatase (EC 3.1.3.11) was assayed by the method of Gancedo and Gancedo (1971). Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity was determined by the method of Ellis and Kirkman (1961).

Statistical analysis

The data obtained from the experiments were subjected to appropriate statistical analysis. Student t test was done for obtaining probability of significance between infected and control organisms, assuming unequal variance (Snedecor and Cochran, 1967). All the determinations were carried out in triplicate and the average with ± standard deviation (SD) of these values were reported in results and discussion.

RESULTS

Upon injection, the prawns exhibited characteristic symptoms of WSSV infection, within 48 h. The symptoms in-

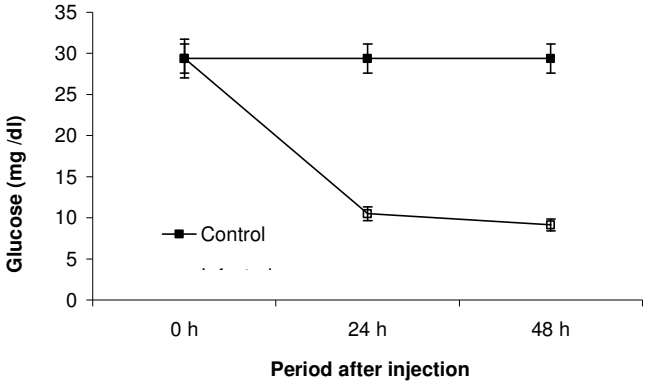

Figure 1. Vairations in glucose content in the haemoloymph of *P. monodon* during white spot syndrome virus (WSSV) infection.

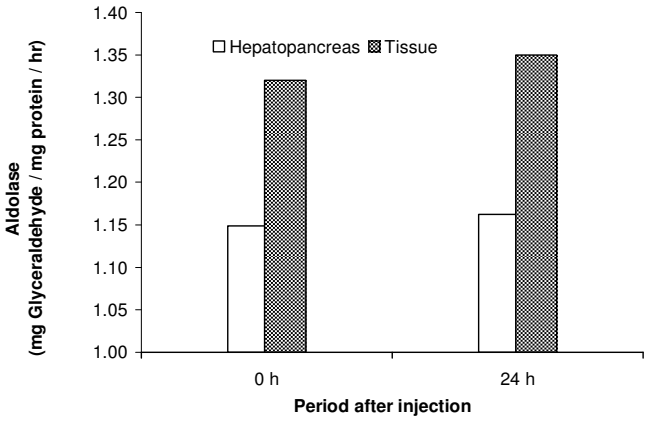

Figure 2. Vairations in aldolase content in hepatopancreas and muscle of *P.monodon* during white spot syndrome virus (WSSV) infection.

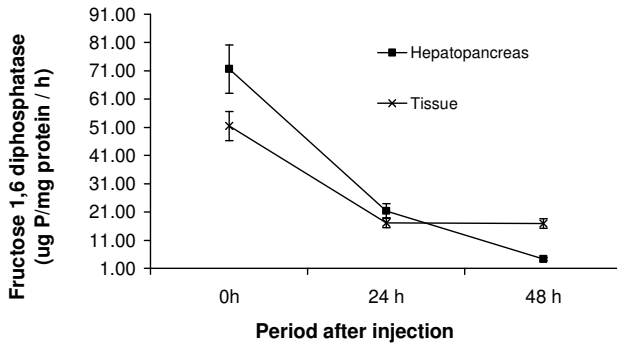

Figure 3. Vairations in fructose-1, 6-diphosphatase in hepatopan-creas and muscle of *P.monodon* during white spot syndrome virus (WSSV) infection.

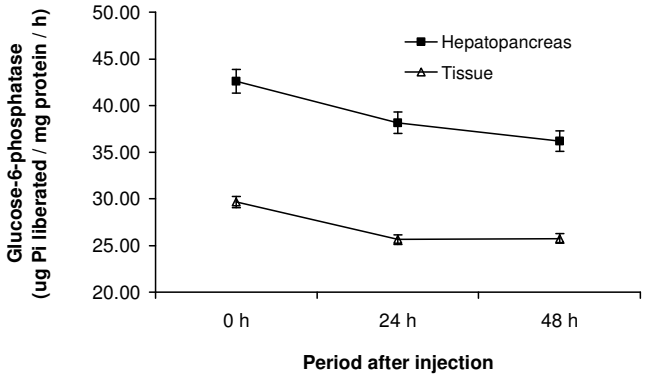

Figure 4. Variations in Glucose-6-phosphatase in hepatopancreas and muscle of *P. monodon* during white spot syndrome virus (WSSV) infection.

cluded white spots, reddening of the body, sluggish movement and stoppage of feed intake. The control shrimp, injected with PBS, did not show any of the symptoms and was active throughout. Further the WSSV injection was confirmed by PCR. Serum glucose exhibited a sudden decrease in the initial 24 h; afterwards, its rate of decrease was more or less slow (Figure 1). The glucose content reduced to 33% of its original value in 24 h of infection. Control shrimps, however, did not exhibit any significant variation at all.

To study the changes in glycolysis during white spot virus infection in *P. monodon,* the activity of the enzyme aldolase was measured both in hepatopancreas and in muscle. In both the cases, no significant change in activity was noted (Figure 2). The same trend was observed in the case of control shrimps also.

The enzymes in the anabolic pathway, viz. fructose-1,6-diphosphatase and glucose-6-phosphatase, were found to be affected adversely by WSSV infection, which is evident from their subdued activities in hepatopancreas and muscle (Figures 3 and 4). Almost 95% loss in activity was observed in the case of fructose 1, 6, di - phosphatase in hepatopancreas, whereas in muscle a decrease in activity by 67%, was seen after 48 h of infection. Glucose-6-phosphatase exhibited a higher activity in hepatopancreas than in muslce. The activity of this enzyme was reduced by 16 and 13% in hepatopancreas and muscle respectively during 24 h of infection. The activity of these enzymes, on the other hand, did not register any significant variation in the control shrimps.

Figure 5 shows the changes in the activity of glucose-6-phosphate dehydrogenase in hepatopancreas and muscle of *P. monodon* infected with white spot virus. The activity of this enzyme increased by 300% of original value in hepatopancreas, while in muscle increase was only 30% of original value during 24 h of infection. There was not much change in the activity of this enzyme in control shrimps.

DISCUSSION

The concentration of glucose in the haemolymph of unin-

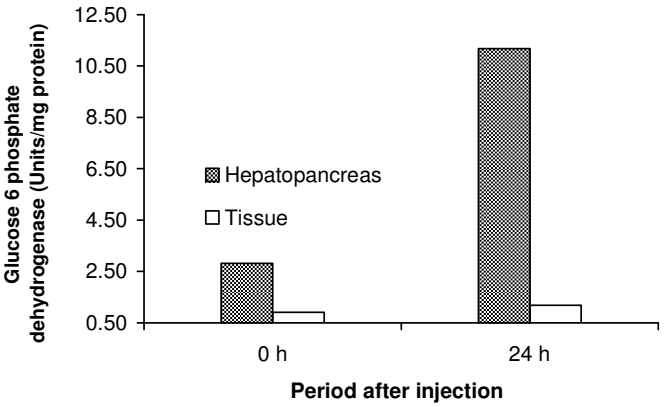

Figure 5. Variation in glucose 6 phosphate dehydrogenase content in hepatopancreas and muscle of *P. monodon* during white spot syndrome virus (WSSV) infection

fected *P. monodon* (0.29 mg/ml) obtained in the present study compares well with the values reported by Verri et al., 2001 (0.34 mg/ml). Hohnke and Scheer (1970) have reported glucose as the major sugar in the circulating haemolymph in crustaceans. Abdel Rahman et al. (1979) and Lynch and Webb (1973) reported that efficient mechanism of glucose homeostasis is absent in crustaceans and they tolerate large variations in blood glucose. The reduction in the glucose level during infection might be 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.

Stewart and Cornick (1972) also observed disappearrance of glucose and lactic acid from the haemolymph of the lobster infected with *Gaffkya homari*. Glycolysis is reported to be a major pathway for the generation of energy (ATP) in all living organisms. Glycolytic intermediates were also reported to serve as precursor for biosynthesis of other cellular constituents (Wilson, 2003). To study the changes in glycolysis during white spot virus infection in *P. monodon,* the activity of aldolase was measured both in hepatopancreas and in muscle. In both cases, no significant loss of activity was noted. This indicated that the enzyme aldolase was not affected adversely by the virus infection. Aldolase is a ubiquitous glycolytic enzyme that catalyzes the reversible change of fructose-1,6-diphosphate to glyeraldehyde-3-phosphate and dihydroxy acetone phosphate. This enzyme has a central position in the glycolytic pathway. The maintenance of aldolase activity indicated that the glycolysis continued and production of energy from glucose by catabolism also proceeded in the infected animal. It is interesting to note that even at moribund stage, the glycolytic pathway was not affected, as evident from the normal activity of aldolase observed in the present study.

The data from this investigation clearly show that the enzymes in the anabolic pathway i.e. production of glucose from pyruvate, the fructose-1,6-diphosphatase and glucose-6-phosphatase were adversely affected during viral infection. As can be seen from Figures 4 and 5, the loss of activity of glucose-6-phosphatase was not as high as that of fructose-1,6-diphosphatase.

Viral infection had resulted in significant reduction in feed intake. This, coupled with normal rate of glycolysis, as evidenced by the aldolase activity and near total inhibition of gluconeogenesis, because of loss of activity of fructose-1,6-diphosphatase must have contributed to the severe energy crisis in the infected animal. Another interesting point deserving special attention is that we have observed earlier that the activity of other enzymes viz ALT, AST, LDH etc involved in gluconeogenesis pathway increased significantly as a result of WSSV infection in *P. monodon* (Suseela, 2005). This can lead to condition of accumulation of excess pyruvate, which in anaerobic conditions is converted to lactate.

The activity of glucose-6-phosphate dehydrogenase in hepatopancreas and muscle of *P. monodon* infected with white spot virus was different from the activity in uninfected animals. This enzyme is involved in the metabolism of glucose through the pentose phosphate pathway to generate NADPH (Wilson, 2003). The increase in activity of this enzyme might therefore result in the production of more NADPH. The significance of this is that NADPH required for adequate levels of reduced GSH in turn would be helping to overcome oxidative stress. Pentose phosphate pathway is considered in fish as a minor pathway, but in decapods it is a major one during intermoult period (McWhinnie and Kuschenberg, 1962). Cuzon et al. (2000) also stated that the pentose phosphate pathway with the major enzyme glucose 6 - phosphate dehydrogenase provides the tissues with specific molecule, the reduced NADPH. The significant increase in the activity of the enzyme in the WSSV infected prawn may be part of the overall defense mechanism against the excessive oxidative stress during the infection.

It was observed during this study that some of the key enzymes involved in the glucose metabolism in *P. monodon* are affected during WSSV infection. It is possible that the levels of these enzymes can serve as indicators of the severity of infection and they can be used as markers in the diagnosis of the disease.

ACKNOWLEDGEMENT

The authors wish to acknowledge Director, CIFT, Cochin, for giving permission to publish this paper and for the facilities provided.

REFERENCES

Abdel Rahman SH, Kanazawa A, Teshima S (1979). Effect of dietary carbohydrates on the growth and levels of the hepatopancreas glycogen and serum glucose of shrimp. Bull. Jpn. Soc. Sci. Fish.12: 1491 – 1494.

Claybrook DL (1983). Nitrogen Metabolism *In.* Mantel, L.H (Ed) Internal Anatomy and Physiological regulation, The Biology of Crustacea Vol 5 Academic Press pp. 163-205.

Dunier M, Siwicki AK (1993). Effects of pesticides and other pollutants in the aquatic environment on immunity of fish: a review. Fish Shellfish Immunol. 3: 423-438.

Ellis HA, Kirkman HN (1961). A colorimetric method for assay of erythrocyte glucose 6 phosphate dehydrogenase. Proc. Natl. Exp. Biol.Med. 106: 607 -609.

Gancedo JM, Gancedo C (1971). Fructose 1,6 – diphosphatase, phosphofructokinase and glucose 6 – phosphate dehydrogenase from fermenting and nonfermenting yeast. Arch. Mikrobiol.76: 132–138.

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.

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.

King J (1965a). The dehydrogenases or oxidoreductases Lactate dehydrogenase In: Practical Clinical Enzymology, London. Van Nostrand, D. Company Ltd. pp 83–93.

King J (1965b). The hydrolases – acid and alkaline phosphatases. In: Van D (ed) Practical Clinical Enzymology, Nostrand Co., London, 1965 pp. 191–208.

Lightner DV, Redman RM (1998). Shrimp diseases and current diagnostic methods. Aquaculture 164: 201-220.

Lynch MP, Webb KL (1973). Variations in serum constituents of the blue crab Calinectus sapidus glucose. Comp. Biochem. Physiol. A. 45A: 127-139.

McWhinnie MA, Kurchenberg RJ (1962). Crayfish hepatopancreas metabolism and the intermolt cycle. Comp. Biochem. Physiol. 6: 117-128.

Pipe RK, Coles JA (1995). Environmental contaminants influencing immune function in marine bivalve molluscs. Fish Shellfish Immunol. 5: 581-595.

Cuzon G, Rosas C, Gaxiola G, Taboada G, Wormhoudt AV (2000). *in* Utilization of carbohydrates by shrimp *In.* Cruz-Suarez. L.E, Ricque-marie, D, Tapia-salazar, M, Olvera-novoa M.Ay C.Civera-cerecedo R (Eds) Avances en nutricon acuicola V. memorias del V. Simposium Internatcionjal de Nutricion Acuicola, Novembre, 2001. Nerida, Yucaton.

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.

Rosas C, Cuzon G, Gaxiola G, Priol VL, Pascual C, rossignyol J, wornboudt AV (2001). Metabolism and growth of juveniles of *Litopenaeus vannamei* – Effect of salinity and dietary carbohydrate levels. J. Exptl. Mar. Biol. Eco. 259: 1-22.

Sasaki T, Matsuv S, Sanne A (1972). Effect of acetic acid concentration of the colour reaction in the o-toluidine boric acid for blood glucose determination. Rinsho Kagaku. 1: 346-53.

Singh B (2001). A novel method of extracting haemolymph from shrimps. Personal Communication. pp. 1-5.

Snedecor GW, Cochran WG (1967). Statistical Method., Oxford and IBH Publication Co., Calcutta, India.

Stewart JE, Cornick JW (1972). Effect of Gaffkya homari on glucose, total carbohydrate and lactic acid of the haemolymph of the lobster (Homarus americanus. Can. J. Microbiol.18: 1511-1513.

Suseela M (2005). Haematological and enzymatic alterations during white spot syndrome virus infection in *Penaeus monodon* (fabricius) *Ph.D.* Thesis, Central Institute of Fisheries Education, Indian Council of Agricultural Research, Mumbai.

Suseela M, Ashok Kumar K, Anandan R, Viswanathan Nair PG, Devadasan K (2007) Changes in tissue defence system in white spot syndrome virus (WSSV) infected *Penaeus monodon*. Comp Biochem Physiol C Toxicol Pharmacol. 145: 315-320.

Telford M (1968). Changes in blood sugar composition during the molt cycle of the lobster Homarus americanus. Comp. Biochem. Physiol. 26: 917-926.

Takahashi Y, Itami T, Maeda M, Suzuki N, Kasornchandra J, Supamattaya K, Khongpradit R, Boonyaratpalin S, Kondo M, Kawai K, Kusda R, Hirona I, Aoki T (1996). Polymerase chain reaction (PCR) amplification of bacilliform virus (RV-PJ) DNA in Penaeus japonicus Bate and systemic ectodermal and mesodermal baculovirus (SEMBV) DNA in Penaeus monodon Fabricius. J. Fish Dis. 19: 399– 403.

Van De Braak K (2002). Haemocytic defence in black tiger shrimp (*Penaeus monodon*) Ph.D Thesis, Wageningen University, The Netherlands.Sindermann and Lightner 1988.

Van Hulten MCCW, Westenberg M, Goodall DS, Vlak JM (2000). Identification of two major virion protein genes of white spot syndrome virus of shrimp, Virology 266: 227-236.

Verri T, Mandal A, Zilli L, Bossa D, Mandal PK, Ingrosso L, Zonno V, Viella S, Aheam GA, Storelli (2001). D Glucose transport in decapod crustacean hepatopancreas. Comp. Biochem. Physiol. Part A 130: 585 -606.

Wilson JE (2003). Isozymes of mammalian hexokinase: structure, subcellular localization and metabolic function. J. Exp. Biol. 206: 2049-2057.

Wu JL, Nishioka T, Mori K, Nishizawa T, Muroga K (2002). a time course study on the resistance of Penaeus japonicus induced by artificial infection with white spot syndrome virus. Fish Shellfish Immunol. 13(5): 391–403.