Proc. 2nd Saudi Sci. Conf.,Fac. Sci., KAU, 15-17 March 2004, ***Part I,*** pp. 29-41 (2005 A.D./1425 A.H.

Na^+, K^+-ATPase Activity from Various Organs of Tilapia (*Oreochromis mossambicus*): A Bioindicator for Environment Monitoring

T.A. KUMOSANI
Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

ABSTRACT. In this report, Na^+, K^+-ATPase activity from various organs (gills, brain and RBC) of tilapia (*Orechromis mossambicus*) acclimated to sea water at standard conditions of temperature (22°C) and salinity (0 M) were assayed and used as indicators of environmental monitoring changes (*in vivo*) such as salinity and temperature. Gill's Na^+, K^+-ATPase activity was the highest activity (9.80±0.32 umol Pi/min. mg protein) followed by brain (2.79±0.82 umol Pi/min. mg protein) then RBC (1.42±0.22 umol Pi/min. mg protein), respectively. Tilapia during exposure to various concentrations of salt showed marked changes in enzyme activity, represented by an increase in the activity of brain enzyme and a decrease in gills enzyme at higher concentrations of salt. In addition, the euzyme activity during exposure to different temperatures was represented by an increase in the enzymes activity of gills and RBC and a decrease in brain enzymatic activity with increasing temperature. In conclusions, Na^+, K^+-ATPase from various organs of tilapia can be used to monitor some environmental changes, since a correlation was shown between Na^+, K^+-ATPase activity of gills, RBC and brain with the increase in ionic strengths or temperatures. Therefore, it is recommended to use this bioindicator in desalination plants using gills only, where some of fish gills can be removed and the fish return into the sea alive.

Keywords: Tilapia, *Orechromis mossambicus*, Na^+, K^+-ATPase, brain, gills, red blood cells (RBC), ionic strength, temperature.

Introduction

Na^+, K^+-ATPase, a member of the P-type ATPase, is composed of two subunits, α and β, and is responsible for translocation Na^+ out of the cell and K^+ into the cell using the energy of hydrolysis of one molecule of ATP. Na^+, K^+-ATPase, the ion translocation enzyme, is present in all animal cells. The electrochemical gradient it generates is necessary for many cellular functions, including establishment of the plasma membrane potential and transport of sugars and ions in and out of the cell. In addition to its fundamental importance to ion transport, Na^+, K^+-ATPase activity could be used as an indicator of physiological changes. Osmotic regulation in teleosts is intimately bound to control of ionic concentration as well as cell and body volume (Assem and Hankle, 1979; Abo-Hegab and Hankle, 1981, 1986).

Bronchial Na^+, K^+-ATPase plays an important role in facilitating the transfer of electrolytes across the epithelium (Jampol and Epstein, 1970; Zaugg and Mclain, 1970).

The activity of cation- and anion-stimulated ATPase was studied in a homogenate and in subcellular fractions of the osmoregulatory organs in representatives of marine fish (elasmobranches and teleost), freshwater teleost fish, amphibians, reptiles, birds and mammals. Close values of the Na^+, K^+-ATPase activity were detected in almost all the vertebrate osmoregulatory organs. In addition, to its importance to ion transport in fish, gill Na^+, K^+-ATPase activity is often used as an indicator of the parr-smolt transformation of juvenile salmonids in hatcheries and laboratories (Karnaky, 1986).

Several membrane ion transporters playing a role in gas transport and exchanges, cell volume regulation and intracellular acid-base regulation have been identified in fish red blood cells (RBCs). Na^+, K^+-ATPase plays an important role in establishing the ion gradients across the membrane, on the Cl^-/HCO_3^- exchanger and its key role in respiration and possibly in inducing a chloride conductance, on the Na^+/H^+ exchanger (Thomas and Egee, 1998).

Na^+, K^+-ATPase is used widely as an indicator for physiological changes and/or environmental pollution's monitoring. The oxidative injury to brain and kidney Na^+, K^+-ATPase using *in vitro* and *in vivo* approaches have been indicated by modification in Na^+, K^+-ATPase oligomeric structure formation and subsequent hydrolysis rate suppression (Dobrota *et al.*, 1999). Other studies showed that stress leads to inhibition on the erythrocyte Na^+, K^+-ATPase properties in rats (Kazennov *et al.*, 1999). Prolonged (6 hr) exposure of rats to emotional painful stress stimulated Na^+, K^+-ATPase activity by 40% from crude synaptosomal fractions of rat brain gray matter without affecting that of Mg-ATPase. The activation of Na^+, K^+-ATPase is a mechanism which is responsible for acceleration of reflex conditioning and for the maintenance of the conditioned reflexes in stress-exposed animals (Sazonotova *et al.*, 1984). Bronchial Na^+, K^+-ATPase activity has been shown to be affected by a variety of environmental contaminants although few studies have been carried out in the field along defined contaminant gradients. Enzyme activity was lower in gills from the more contaminated sites (Stagg *et al.*, 1992). The inhibition of the enzyme Na^+, K^+-ATPase was found to be a suitable indicator of toxicity for bleached pulp and paper mill waste waters (Araujo-Neto *et al.*, 1994).

Different cell components protect enzyme structure and function against different stress conditions, and this protection depends on the nature of components and enzyme (Sole *et al.*, 1997). Heat shock proteins may be involved in the *in vivo* modulation of Mg, K-ATPase enzyme activity during heat stress in rice (Mariamma *et al.*, 1997) and from *Escherichia coli* (Vickery *et al.*, 1997). Some of the heat shock-induced ionic changes are mediated by inhibition of the Na^+-H^+ exchanger, activation of Na^+, K^+-ATPase and changes of membrane conductance for ions (Skrandies *et al.*, 1997).

In the course of the past several decades, the industrialized nations of the world have been confronted with a variety of scientific and technological challenges. One of these problems is water availability. One way to solve this problem is to use desalination technology. Protection of the environment is among the most provocative of these challenges. The effect of increased water temperature of environment or salinity must be investigated before they become out of control. This work will describe one way to evaluate how well Red Sea fish tolerate the common problem of increased salinity and/or temperature. The aim is to evaluate the activity of Na^+, K^+-ATPase from different organs of fish in order to use it as an indicator of environmental water system. The biochemical

and physiological indices of stress may prove to be of special value in signaling the development of sublethal abnormalities which could cause an animal population to be less efficient or effective in coping with the normal stress and strain of survival.

Materials and Methods

This study was conducted at the end of 2002 at the Biochemistry laboratory of King Abdulaziz University, Jeddah-Saudi Arabia.

The Bovine serum albumin, Tris (Tris [hydroxymethyl] aminoethane), ATP (adenosine-5'-triphosphate, grade 1), phosphoenolpyruvate, rabbit muscle pyruvate kinase, rabbit muscle lactate dehydrogenase, NADH, EDTA (ethylenediamine tetraacetic acid, disodium salt, $2H_2O$) were obtained from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals were reagent grade.

Fish

Tilapia (*Oreochromis mossambicus*) chosen to be the experimental species, due to its importance as one of the most popular commercial fish. Salt-water acclimated fish were obtained from King Abdulaziz University hatchery at Abhor. They were juvenile, about 10 cm in total length. Glass tanks (30x30x20 cm) were used for keeping the experimental fish (12 fish in each), under the same experimental conditions with continuous aeration. A photoperiod of 16 hrs of light and 8 hrs of dark was maintained, oxygen was never below 8 mg/liter, acidity was monitored regularly (pH 7.3 ± 0.4) using hand pH meter. At the end of the experiment, fish were killed; the gills, brain and RBC samples were collected from each of the experimental group. Blood was collected from a severed portion posterior to the head on the dorsal side by syringe in heparanized tube. Blood was centrifuged at 3000 rpm for 10 min to remove plasma.

Temperature Studies

Each aquarium was connected with temperature regulator through water bath. Thermometers were placed in each aquarium to monitor the changes in water temperature. Experimental temperatures used were 15°C, 22°C and 30°C, respectively, for 96 hrs (Kumosani, 2002).

Ionic Strength Studies

Adding NaCl solution to the test Aquarius to have final concentrations of 0, 0.5 and 1.0 M and the experiments were run for 96 hrs (Kumosani, 2003).

Preparation of Tissue Homogenate

After scarifying the fish, brain and gills were rapidly excised and frozen at -20°C. The tissue were either worked upon immediately or stored at -20°C till the enzyme assay was started. The gill bars were cut off with scissors and the gill filaments were selected for assay. Part of the tissue was accurately weighed then homogenized in 0.25 M sucrose solution. The homogenate after centrifugation was diluted to give the proper enzyme activity which can be measured within a suitable absorbency range. Total protein concentration of each homogenate was determined according to (Lawry *et al.*, 1951).

Na^+, K^+-ATPase

The phosphomolybdic assay was adopted to measure the number of micromoles of inorganic phosphate released by the action of the ATPase as a measure of its activity according to the method of Serrano (1978). The method is based on selective inhibition of Na^-, K^+-ATPase by the glycoside ouabain (Kimelberg and Papahadjopoulos, 1972). Accordingly, the ATPase activity was measured in the presence of ouabain to give Mg^{2+}ATPase activity and in its absence to give total ATPase activity and by subtraction, the Na^+, K^+-ATPase activity could be calculated. The reaction mixture was buffered with 50 mM Tris pH 6.5 and contained different concentrations of $MgCl_2.6H_20$ (0.5-20 mM), 100 mM NaCl, 10 mM KCl, 0.1 mM EDTA and +/- 1.5 uM ouabain. After adding the brain or gills homogenate (50 ul) and incubation for 5 min at 30°C in a water bath shaker, the reaction was started by the addition of different volumes of 0.1 M ATP (0.25-10 mM). After 10 min incubation at 30°C, the reaction was stopped by the addition of 2 ml of a solution containing 2% (v/v) sulfuric acid, 0.5% (w/v) ammonium molybdate and 0.5% (w/v) sodium lauryl sulfate. The detergent was included here to avoid the development of any turbidity. The phosphomolybdate was reduced with 20 ul of 10% (w/v) ascorbic acid and the absorbency at 750 nm was read after 5 min according to the method of (Fiske and Subbarow, 1925).

Statistical Analysis

The data collected were logged into personal computer and analyses of data were performed using SPSS statistical package. T-test was used for comparing means. P value were considered to be statistically significant if < 0.005.

Results

Na^+, K^+-ATPase activity of fish gills showed an average value of 9.80 umoles Pi/min. mg protein (Fig. 1), whereas that of brain showed an average value of 2.79 umoles Pi/min. mg protein. On the other hand, Na^+, K^+-ATPase activity from RBC of *O. mossambicus* showed an average value of 1.42 umoles Pi/min. mg protein.

The total proteiu concentrations were expressed as mg of protein per 1 g of wet tissue. Total protein concentrations were 10.28, 384.91 and 66.15 mg/g for RBC, brain and gill tissues, respectively (Fig. 2).

Figure 3 shows the effects of salinity on the Na^+, K^+-ATPase activity in gills of *O. mossambicus* acclimated for 96 hrs to different NaCl concentration. Control fish showed Na^+, K^+-ATPase activity of an average value of 9.80 umoles Pi/min. mg protein. This value was dropped to 2.09 umoles Pi $min.^{-1}$ mg^{-1} protein when the fish were placed in saline water containing 0.5 M NaCl for 96 hrs. An increase was seen in the Na^+, K^+-ATPase activity from the fish gills (4.46 umoles Pi $min.^{-1}$ mg^{-1} protein) when the fish were placed in saline water containing 1.0 M NaCl.

Figure 4 shows the total protein concentration of the control fish gills (66.15 mg per 1 gm of homogenized gill tissue). The total protein concentrations was increased when ionic strength increased (0.5 M), it becomes 99.80 mg/g, and decreased (6.67 mg/g) when ionic strength of NaCl becomes 1 M.

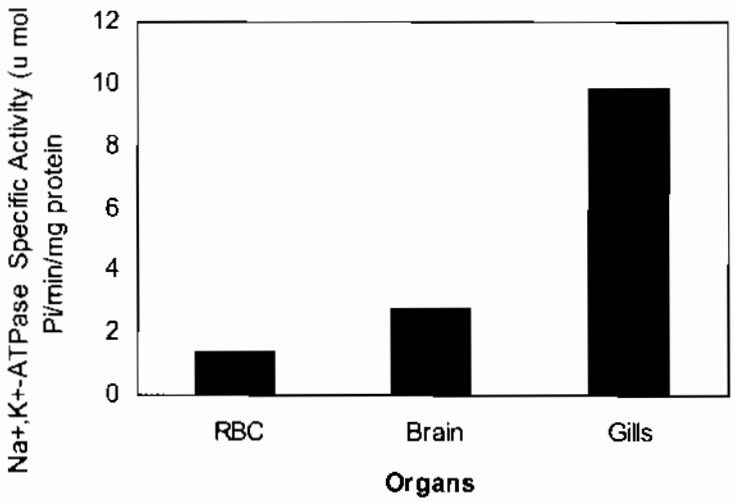

Figure (1). Na^+, K^+-ATPase from RBC, brain and gills of *O. mossambicus*.

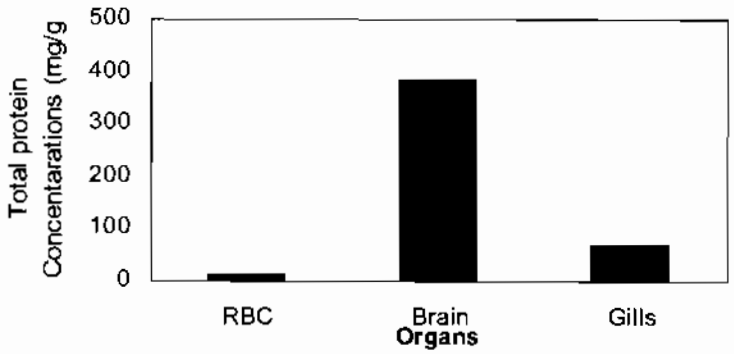

Fig. (2). Total protein conceutrations from RBC, brain and gills of *O. mossambicus* .

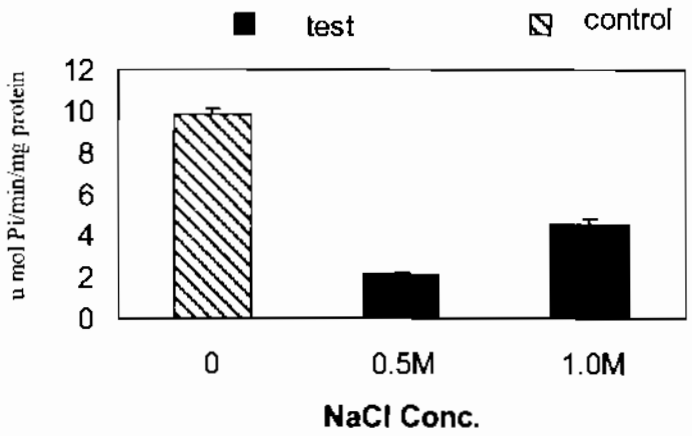

Fig. (3). Na^+, K^+-ATPase activity from the gills of *O. mossambicus* a function of ionic strengtb.

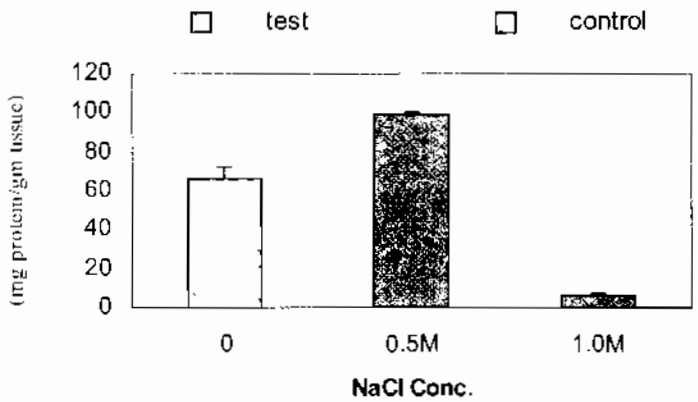

Fig. (4). Total rotein concentrations from the gills of *O. mossambicus* as a function of ionic strength.

Figure 5 shows the effects of salinity on the Na^+, K^+-ATPase activity in brain of *O. mossambicus* acclimated for 96 hrs to different NaCl concentration. Control fish showed Na^+, K^+-ATPase activity of an average value of 2.79 umoles Pi $min.^{-1}$ mg^{-1} protein. This value was increased to 3.26 umoles Pi $min.^{-1}$ mg^{-1} protein when the fish were plaeed in 0.5 M NaCl medium and to 9.68 umoles Pi $min.^{-1}$ mg^{-1} protein when the fish were placed in water containing 1.0 M NaCl, respectively.

Figure 6 shows the total protein concentration of the control fish brain (384.91 mg per 1 gm of homogenized brain tissue). The total protein concentrations was increased when ionic strength of NaCl was increased to 0.5 M, it becomes 546.19 mg/g, and decreased to 88.2 mg/g when ionic strength of NaCl increased to 1 M.

Figure 7 shows the effects of salinity on the Na^+, K^+-ATPase activity in RBC of *O. mossambicus* acclimated for 96 hrs to different NaCl concentration. Control fish showed Na^+, K^+-ATPase activity of an average value of 1.42 umoles Pi $min.^{-1}$ mg^{-1} protein. This value was dropped to 0.31 umoles Pi $min.^{-1}$ mg^{-1} protein when the fish were placed in saline water containing 0.5 M NaCl. An increase was seen in the Na^-, K^+- ATPase activity from the fish RBC (0.73 umoles Pi $min.^{-1}$ mg^{-1} protein) when the fish were placed in saline water containing 1.0 M NaCl.

Figure 8 shows the total protein concentration of the control fish RBC (10.28 mg per 1 gm of homogenized RBC tissue). The total protein concentrations increased when ionic strength of NaCl changed to 0.5 M, it becomes 38.03 mg/g, and increased to 12.85 mg/g (compared to control group) when ionic strength of NaCl becomes 1 M.

Figure 9 show the effect of different temperatures on the Na^+, K^+-ATPase activity in gills, brain and red blood cell of *O. mossambicus* acclimated to different temperature. Control fish showed Na^+, K^+-ATPase activity of average values of 9.80, 2.79 and 1.42 umoles Pi/min. mg protein, respectively. These values were changed to 4.76, 32.21 and 0.58 umoles Pi/min. mg protein, respectively when the fish were placed in water environment that was maintained at 15°C. An increase was seen in the Na^+, K^+- ATPase activity 15.07, 32.21 and 4.37 umoles Pi/min. mg protein, respectively when fish were placed in a water environment that was maintained at 30°C.

Figure 10 shows the total protein concentration of the control fish gills, brain and red blood cell (66.15, 384.91 and 10.28 mg per 1 gm of homogenized gill tissue, respectively). The total protein concentration dropped when temperatures was maintained at 15°C, it was

34.09, 105.5 and 10.61 mg/g, respectively, and changed to 3.35, 584.86 and 4.45 mg/g, respectively at 30°C.

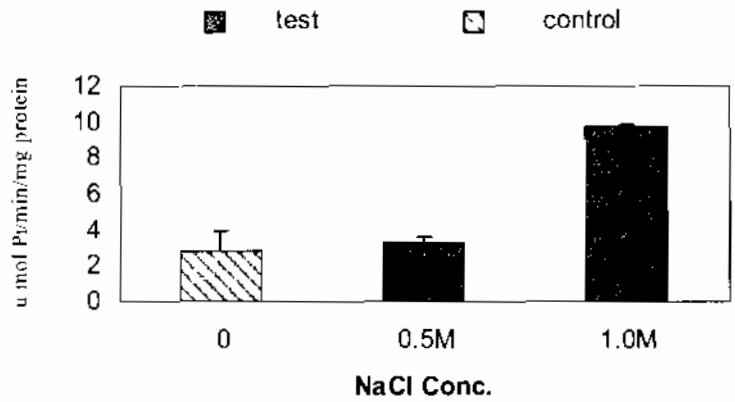

Fig. (5). Na^+, K^+-ATPase activity from the brain of *O. mossambicus* as a function of ionic strength.

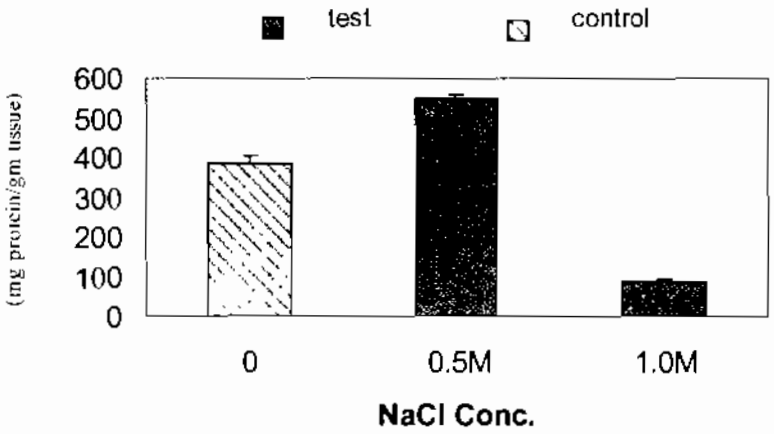

Fig. (6). Total protein concentrations from the brain of *O. mossambicus* as a function of ionic strength.

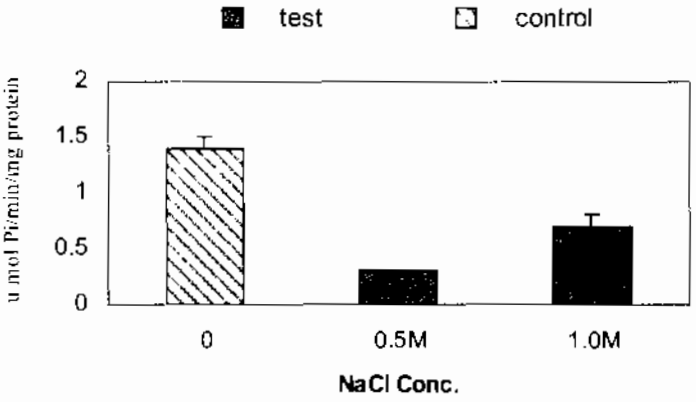

Fig. (7). Na^+, K^+-ATPase activity from the RBC of *O. mossambicus* as a function of ionic strength.

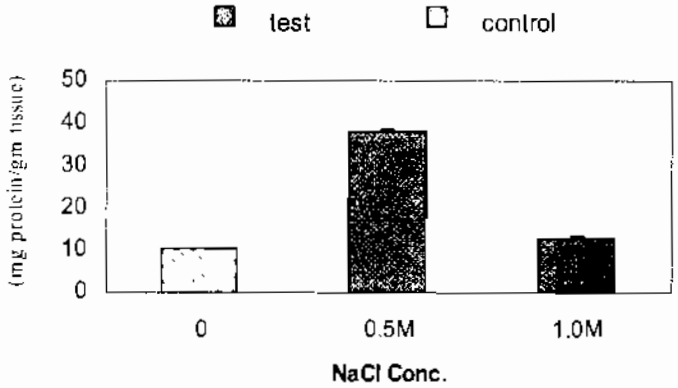

Fig. (8). Total protein concentrations from RBC of *O. mossambicus* as a function of ionic strength.

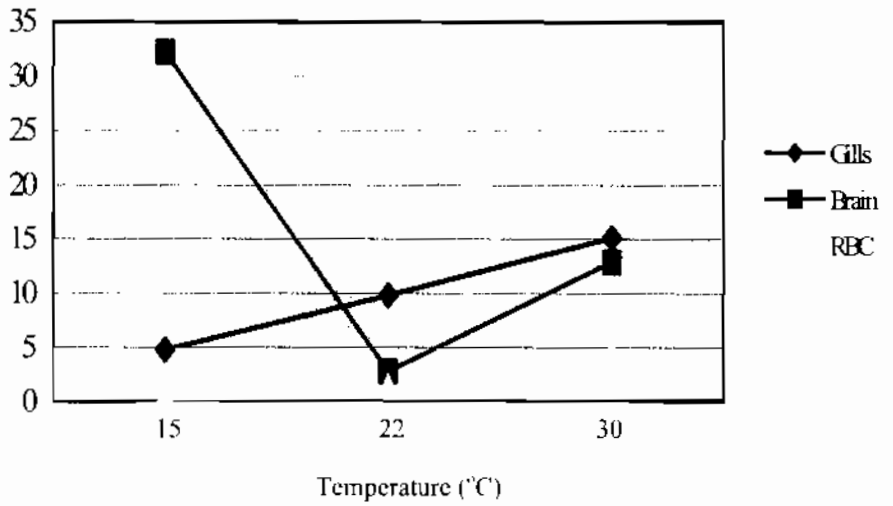

Fig. (9). Na^+, K^+-ATPase activity from the gills, brain and RBC of *O. mossambicus* as a function of temperature.

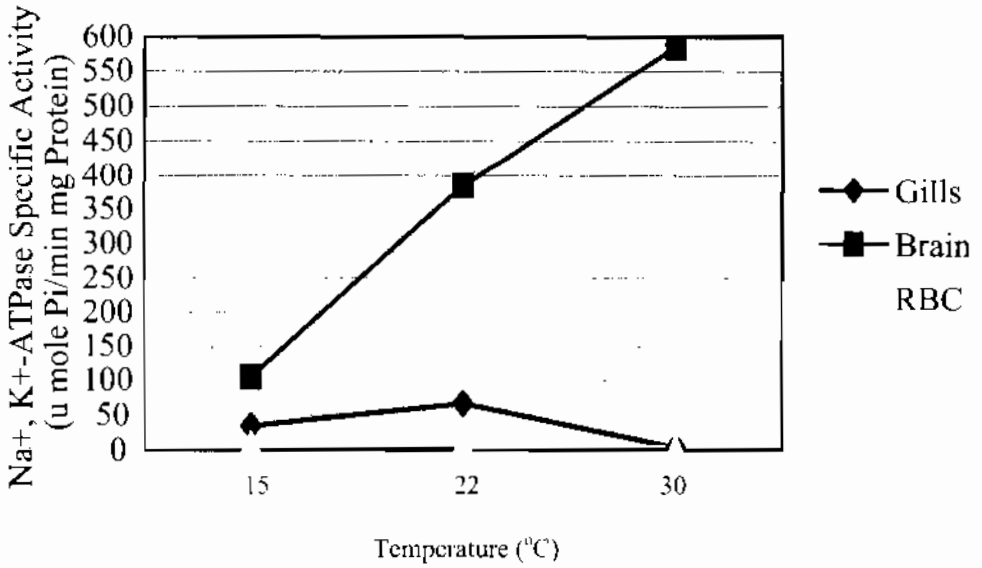

Fig. (10). Total protein concentration from the gills, brain and RBC of *O. mossambicus* as a function of temperature.

Discussion

The finding results obtained in this study for the specific activity of Na^+, K^+-ATPase at standard conditions of temperature (22°C) and salinity (0 M) are similar to a certain extent to published results (Dobrota *et al.*, 1999). The variation in those values may allow for using them as an indicative and confirmatory indices for monitoring water pollution and physiological changes of the animals.

This study showed a direct correlation between Na^+, K^+-ATPase activity of gills and RBC and inversely with brain ATPase with the increase in temperature. Therefore, it can be used as index to monitor temperature increase in the fish environment.

The results obtained from brain and RBC'c concerning Na^+, K^+-ATPase activity as a response of environmental salinity was considered new, since no body, to the best of my knowledge, was working with them. These findings may suggest strongly to use the Na^+, K^+-ATPase activity as an indicator to monitor fish environmental regarding salinity and temperature.

The function of the gills made it necessary for this organ to be in direct contact with the water. As a result, this tissue is vulnerable to changes in the external environment. For example any toxicants, abrasive materials, or parasites can affect the gill epithelium and reduce the respiratory efficiency.

There is extensive information on the concentrations of many pollutants or toxicants that kill fish and other organisms. There is much less information about the mechanisms by which these toxicants act. There are several reasons for this. Most government regulatory agencies do not need to know how a substance acts before they can set limits on its discharge into a body of water.

The changes in enzyme activity as a function of ionic changes and/or salinity can be explained as follows. In some cases the lowest concentration enhanced the enzyme activity to some extent (Verma *et al.*, 1979). Since Na^+, K^+-ATPase is an integral component of the membrane, the active site of the enzyme would be altered, and the energy needed to pump out ions would be reduced. Ionoregulatory failure occurs by chemical-biological interactions with at least two discrete sites. At low external concentrations, ion influx is inhibited and at higher concentrations, ion efflux is stimulated, as seen here with gills and RBC ATPases of tilapia. The inhibition of influx is due in large part to the binding of metal ions to sulphydryl groups on transport proteins (ATPases), while the stimulation of efflux appears to be governed by the displacement of calcium from the intercellular tight junctions of the epithelial cells. Chloride cell proliferation and ATPase induction are frequently reported as results of metal exposure. Ultrastructural changes in tight junctions have also been reported recently. The role of metallothionein or other metal-binding proteins in acclimation is equivocal. Inorganic anions also, appear to affect ionoregulation (Lauren, 1991).

The European sea bass, *Dicentrarchus labrax*, tolerates salinity's ranging from freshwater (FW) to hypersaline conditions (HSSW). Gill Na^+, K^+-ATPase activity was unchanged in 15 ppt SW but doubled in FW and HSSW-groups after transfer. In both groups, this was preceded by a 2- to 5-fold elevation of the gill α-subunit Na^+, K^+-ATPase m-RNA level. Thus increased expression of α-subunit Na^+, K^+-ATPase m-RNA is part of the molecular mechanism of both FW and SW acclimation in sea bass (Jensen *et al.*, 1998). The salinity-dependent stimulation of mRNA of gill Na^+, K^+- ATPase α-subunit is associated with corresponding stimulation at the protein level. This provides direct

evidence of enhanced transcription and translation of Na^+, K^+-ATPase α-subunit gene upon salinity challenge (Hwang *et al.*, 1998).

Also, it was seen an increase of the Na^+, K^+-ATPase activity in the gills of *Carcinus maenas* adapted to diluted seawater (Welcomme and Devos, 1988).

Growth hormone (GH) receptors from fish have been characterized in ovary, testis, fat, skin, cartilage, gill, blood platelet, brain, spleen, kidney and muscles. GH has the property of hypo- osmoregulatory through improving the action of gill Na^+, K^+-ATPase (Xu *et al.*, 1997).

It has been found that the low temperatures induced on the non-chilled organs gave similar changes to organs exposed to a reduced temperature. The changes consisted of a parallel depolarization of leaf and root membranes and similarly a decrease in the ATPase activity (Filek and Koscienlniak, 1996). The same was seen with gills and RBC ATPases.

The result obtained in this paper agrees to a certain extent with the previous findings, and ATPase activity can be used to monitor the fish health in the area of desalination stations in order to evaluate how well Red Sea fish tolerate the common problem of inereased salinity.

References

Abo-Hegab, S. and **W. Hankle, W.** (1981) Electrolyte changes and volume regulatory processes in Carp, *Cyprinus carpio*, during osmotic stress. *Comp. Biochem. Physiol.*, **71A:** 157-164.

Abo-Hegab, S. and **W. Hankle,** (1986) Electrolyte changes, ecll volume regulation and hormonal influences during acclimation of rainbow trout, *Salmo gairdneri*, to salt water. *Comp. Biochem. Physiol.*, **83**A: 47-52.

Assem, H. and **W. Hankle,** (1979) Potential use of physiological changes as indices of stress in the teleost,Cyprinus carpio. *Bull. Inst. Oceangor. Fish. A.R.E.*, **10:** 111-121.

Araujo-Neto, J.S., A.J. Jr. Martins and **G.L. Sant'-Anna,** (1994) Use of rat brain Na+, K+-ATPase assay to determine effectiveness of biological treatments to reduce toxicity of paper mill effluents. *Water Res.*, **28**(12): 2583-2584.

Dobrota, D., M. Matejovcova, E.G. Kurella and **A.A. Boldrev.** (1999) Na^+, K^+-ATPase under oxidative stress: molecular mechanisms of injury. *Cell. Mol. Neurobiol.*, **19**(1): 141-9.

Filek, M. and **Koscienlniak, J.** (1996) The effect of chilling maize shoots and roots on the electric potential, composition of fatty acids and the ATPase activity in the non-chilled organs of seedlings. *J. Agro. Crop. Sci.*, 177(4): 261-267.

Fiske, C. H. and **Y. Subbarow,** (1925) The colorimetric determination of phosphorus. *J. Biol. Chem.*, **66:** 375-400.

Hwang, P.P., M.J. Fang, J.C. Tsai, C.J. Huang and **S.T. Chcu,** (1998) Expression of mRNA and protein of Na+, K+-ATPase alpha subunit in gills of ilapia (*Oreochromis mossambicus*). *Fish Physiol. Biochem.*, **18**(4): 363-373.

Jampol, L.M. and **F.H. Epstein,** (1970) Sodium-potassium activated adenosine riphosphatase and osmotic regulation by fishes. *Am. J. Physiol.*, **218:** 607-611.

Jensen, M.K., Madsen, S.S. and **Kristiansen, K.,** (1998) Osmoregulation and salinity effects on the expression and activity of Na^+, K^+-ATPase in the gills of European sea bass, *Dicentratchus labrax* (L.). *J. Exp. Zool.*, **282**(3): 290-300.

Karnaky, K.J. (1986) Structure and Function of the chloride cells of *Fundulus heteroclitus* and other teleosts. *Am. Zool.*, **26:** 209-224.

Kazennov, A.M., M.N. Maslova, V.A. Dubrovskii, E.A. Skverchinskaia, F.A. ustamov and **T.V. Tavrovskaia,** (1999) Effect of stress and ecetylcholinesterase inhibition in vivo on the erythrocyte Na+, K+-ATPase roperties in rats. *Zh. Evol. Biokhim. Fiziol.*, **35:** 29-32.

Kimelberg, H.K. and **Papahadjopoulos, D.** (1972) Phospholipid requirement for Na^+, K^+ ATPase activity: Head group specificity and fatty acid fluidity. *Biochem. Biophys. Acta.*, **282:** 272.

Kumosani, T.A. (2002) *In Vivo* Changes in the Activity of (Gill, Brain and RBC) ATPases from *Oreochromis mossambicus* as a Response of Environmental Temperatures, Pak. *J. Sci. Ind. Res.*, **6:** 413-414.

Kumosani, T.A. (2003) *In Vivo* Changes in the Activity of (Gill, Brain and RBC) ATPases from *Oreochromis mossambicus* as a Response of Environmental Ionic Changes, (In Press).

Lawry, O.H., N.J. Rosebrough, A.I. Farr and **R.J. Randall,** (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193:** 262-275.

Lauren, D.J., (1991) The fish gill: A sensitive target for waterborne pollutants. *Aquatic Toxicology and Risk assessment,* **Mayes, MA** and **Barron, MG,** (eds.) Philadelphia, PS-USA. American Society for Testing and Materials, pp. 223-244.

Mariamma, M., Muthukumar, B. and **Gnanam, A.** (1997) Thermotolerance and effect of heat shock on the stability of the ATPase enzyme in rice. *J. Plant Physiol.*, **150**(6): 739-742.

Reznik, L.V., M.A. Belen'-kii, E.M. Myazina, O.A. Nikitina and **S.A. Nikitina,** (1986) Activity of adenosine triphosphatases in the osmoregulatory organs of ertebrates. *J. Evol. Biochem. Physiol.*, **22:** 99-103.

Sazontova, T.G., I.U.V. Arkhipenko and **V.S. Lakushev,** (1984) Na K-dependent TPase activity of synaptosomes of rat cerebral hemispheres with ischemic ecrosis of the myocardium, occurring after emotional-painful stress. *Vopr. ed. Khim.*, **30:** 61-3.

Serrano, R. (1978) Purification and properties of proton translocation ATPase complex of Bovine heart mitochondria. *Mol. Cell. Biochem.*, **72:** 248.

Skrandies, S., Bremer, B., Pilatus, U., Mayer, A., Neuhaus, S.U. and **Rensing, L.** (1997) Heat shock- and ethanol-induced ionic changes in C6 rat glioma determined by NMR and Fluorescence spectroscopy. *Brain Research,* **746**(1-2): 220-230.

Sole, P.M., Ferreira, P.A., Dos-Passos, L.A. and **Meyer, F.J.R.** (1997) Carbohydrate protection of enzyme structure and function against guanidinium ehloride treatment depends on the nature of carbohydrate and enzyme. *Eur. J. Biochem.*, **248**(1): 24-29.

Stagg, R.M., J. Rusin and **F. Brown,** (1992) Na+, K+-ATPase activity in the gills of the flounder (Platichthys flesus) in relation to mercury contamination in the irth of Forth. *Mar. Environ. Res.*, **33:** 255-266.

Thomas, S. and **S. Egee,** (1998) Fish red blood cells: Characteristics and physiological role of the membrane ion transporters. *Comp. Biochem. hysiol.*, **-A, 119A:** 79-86.

Verma, S.R., Mohan, D., Tyagi, A.K., and **Dalela, R.C.** (1979) *In vivo* response of ATPase in few tissues of the fish *Mystus vittatus*-(Ham.) to the synthetic detergent Swascofix-Reg TM- E45. *Bull. Environ. Contam. Toxicol.*, **22**(3): 327-331.

Vickery, L.E., Silberg, J.J. and **Ta, D.T.** (1997) Hs66 and Hs20, a new heat shock cognate molecular chaperone system from *Escherichia coli. Protein Science,* **6**(5): 1047-1056.

Welcomme, L. and **Devos, P.,** (1988) Cytochrome c oxidase and Na^+, K^+-ATPase activities in the anterior and posterior gills of the shore crab *Carcinus maenas*, L. after adaptation to variousm ,salinities. Comp. *Biochem. Physiol.*, **-B. 89B**(2): 339-341.

Xu, B., Zhang, P., and **Li, D.,** (1997) Advances in the studies on the plasma metabolism, regulation and action mechanism(s) of fish growth hormone. *Oceanol. Limnol. Sin.*, **28**(3): 328-333.

Zaugg, W.S. and **L.R. Mclain,** (1970) Adenosinetriphosphatase activity in gills of Salmonids: Seasonal variations and salt water influence in coho salmon (Oncorhynchus kisntch). *Comp. Biochem. Physiol.*, **35:** 587-596.

استخدام نشاط إنزيم الصوديوم ، بوتاسيوم – اتيبيز من الأعضاء المختلفة لسمكة البلطي كمؤشر للتغيرات البيئية

طه عبدالله قمصاني

قسم الكيمياء الحيوية ، كلية العلوم، جامعة الملك عبدالعزيز

جـــــــدة – المملكة العربية السعودية

المستخلص. في هذا التقرير ، تم قياس نشاط إنزيم الصوديوم ، البوتاسيوم – اتيبيز من الأعضاء المختلفة لسمكة البلطي (الخياشيم والمخ وكريات الدم الحمراء) التي تم تكييفها لماء البحر عند الظروف القياسية في درجة حرارة (٢٢مْ) والملوحة (٠ مولار) لاستخدامها كمؤشر لمراقبة التغيرات البيئية كالتغير في درجات الملوحة والحرارة . أظهرت النتائج أن أعلى نشاط للإنزيم كانت من الخياشيم (٠،٣٢ + ٩،٨ ميكرمول فوسفات/دقيقة. مليجرام بروتيني) يتبعه نشاط الإنزيم من المخ (٠،٨٢ + ٢،٧٩ ميكرمول فوسفات/دقيقة. مليجرام بروتيني) ثم يتبعه نشاط الإنزيم من كرات الدم الحمراء (٠،٢٢ + ١،٤٢ ميكرمول فوسفات/دقيقة. مليجرام بروتيني)، على التوالي. أظهرت سمكة البلطي عند تعرضها لتركيزات مختلفة من الملح تغيرات واضحة في نشاط الإنزيم التي كانت ممثلة بزيادة في نشاط إنزيمات المخ ونقصان في نشاط الإنزيم من الخياشيم عند التركيزات العالية من الملح. بالإضافة إلى أن نشاط الإنزيم خلال التعرض لدرجات الحرارة المختلفة مثلت بزيادة نشاط الإنزيم من الخياشيم وكريات الدم الحمراء ونقصان النشاط من إنزيم المخ بزيادة درجة الحرارة . ويستنتج هذا البحث أن إنزيم الصوديوم ، بوتاسيوم – اتيبيز في الأعضاء المختلفة للبلطي يمكن استخدامه كمؤشر لمراقبة بعض التغيرات البيئية ، حيث تم إيجاد علاقة بين نشاط الإنزيم في الخياشيم وكريات الدم الحمراء والمخ مع زيادة درجات الملوحة والحرارة . لذلك يوصي باستخراج هذا المؤشر الحيوي في مراكز تحلية المياه باستخدام الخياشيم فقط حيث يمكن نزع جزء من الخياشيم من السمكة وإرجاعها إلى الماء بدون الاضطرار إلى قتلها.