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

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

ABSTRACT. The study concerns with the effects of different ionic strength concentrations of NaCl in fish environment on the *in vivo* activity of Na^+, K^+-ATPase from the gills, brain and red blood cells of tilipia, *Oreochromis mossambicus.* The most important changes in Na^+, K^+-ATPase activity during exposure to the NaCl concentrations were represented by an increase in the brain and a decrease in gills and RBC Na^+, K^+-ATPases activity at higher concentrations. Total protein concentrations of gills and brain homogenates were decreased but increased in RBC's homogenates at higher ionic strengths.

Introduction

The physiological legends for Na^+, K^+-ATPase (the Na, K-pump) are ions, and electrostatic forces that could be revealed by their ionic strength dependence are therefore expected to be important for their reaction with the enzyme[1].

Previous studies have demonstrated that osmoregulating capacity (OC: osmotic gradient between hemolymph and external medium at a given salinity) is an indication of physiological state in crustaceans. This parameter was used to study respectively the effects of ambient ammonia and turbidity of suspended sediments on *Penaeus japonicus* late juveniles. In both experiments, depressed osmoregulation was the result of reduced Na^+ and Cl^- regulation. Increased gill Na^+, K^+-ATPase activity may reflect a compensating reaction to the impairment of ionic regulation. The fact that sublethal levels of ambient ammonia or turbidity could be reflected by the decrease of OC confirmed that it is possible to use OC to detect sublethal effects of environmental stresses in crustaceans, in particulate panaeid shrimps[2].

Osmotic regulation in teleosts is intimately bound to control of ionic concentration as well as cell and body volume[3-6]. Bronchial Na^+, K^+-ATPase play an important role in facilitating the transfer of electrolytes across the epithelium[6, 7].

The present investigation aims to evaluate physiological changes in fish under the influence of different ionic strength for toxicity test in environmental water system. The biochemical and physiological indices of stress may prove to be of special value in signalling 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. In the present study the tilapia (*Oreochromis mossambicus*) was exposed to different concentrations of NaCl for a period of 96 hr and the effect of the *in vivo* activity of Na^+, K^+-ATPase from the gills, brain and red blood cells were studied.

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 with the common problem of increased salinity.

Materials and Methods

Chemicals

The Bovine serum albumin, Tris (Tris [hydroxymethyl] aminoethane), ATP (adenosine-5'-triphosphate, disodium salt, grade I), ascorbic acid, ouabain, EDTA (ethylenediamine tetraacetic acid, disodium salt, 2 H_2O) were obtained from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals were reagent grade.

Fish

Tilapia (*Oreochromis mossambicus*) was chosen to be the experimental species, due to its importance as one of the most popular commercial fish. Saltwater acclimated fish were obtained from King Abdulaziz University hatchery. They were juvenile, weighted 10 gm, about l0 cm in total length. Glass tanks ($30 \times 30 \times 20$ cm) were used for keeping the experimental fish, under the same experimental conditions with continuous aeration. A photo period of 16 hr of light and 8 hr 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 fish of the experimental group (25 each). 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 l0 min to remove plasma.

Experimental Design

Adding the NaCl solution to the test Aquarius to have final concentrations of 0, 0.5 and 1.0 M before starting the experiments.

Preparation of Tissues Homogenate

In all test groups, after killing, brain or gills or RBC were rapidly excised or collected and frozen at –20ºC. The tissues were either worked upon immediately or stored at –20ºC till the enzyme assay was started.

Part of the tissue was accurately weighed, then homogenized in 0.25 M sucrose solution. The homogenate was diluted to give the proper enzyme activity that can be measured within a suitable absorbency range. Total protein concentration of each homogenate was determined according to Lawry *et al*.[8].

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[9]. The method is based on selective inhibition of Na^+, K^+-ATPase by the glycoside ouabain[10]. 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 $MgCl_2.6H_2O$ (2 mM), 100 mM NaCl, 10 mM KCl and +/– 1.5 uM ouabain. After adding the brain homogenate (50 ul) and incubation for 5 min at 30ºC in a water bath shaker, the reaction was started by the addition of ATP (2 mM). After 10 min incubation at 30ºC, 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[11]. The data presented here are the mean of triple experiments each.

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[12].

Results

Figure 1 shows the effects of different concentrations of NaCl on the Na^+, K^+-ATPase activity in gills of *Oreochromis mossambicus.* Control fish showed Na^+, K^+-ATPase activity of a 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 water environment that has 0.5 M ionic strength. 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 water environment that has 1.0 M ionic strength. This might due to removing some of the lipids that might hinder the accessing of the substrate into the active site.

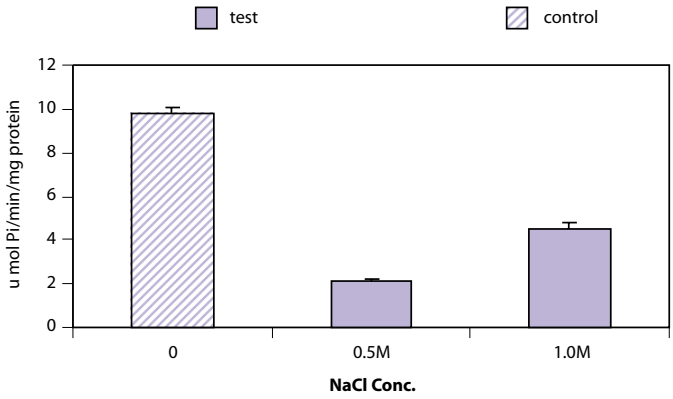

FIG. 1. Na^+, K^+-ATPase activity from the gills of *Oreochromis mossambicus* a function of ionic strengths.

Figure 2 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 becomes 1 M.

Figure 3 shows the effects of different concentrations of NaCl on the Na^+, K^+-ATPase activity in brain of *Oreochromis mossambicus*. Control fish showed Na^+, K^+-ATPase activity of a 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 placed in water environment that has 0.5 M ionic strength and 9.68 when the fish were placed in water environment that has 1.0 M ionic strength, respectively.

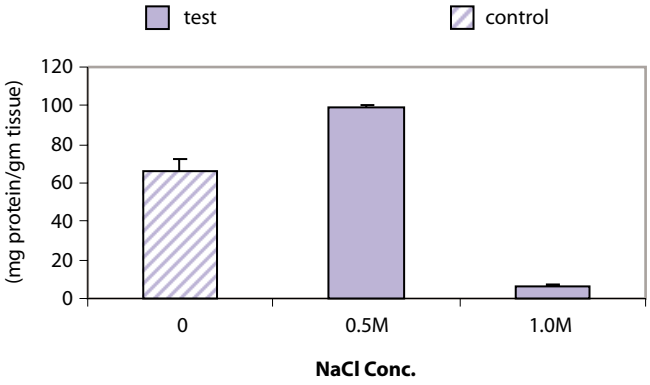

FIG. 2. Total protein concentrations from the gills of *Oreochromis mossambicus* a function of ionic strengths.

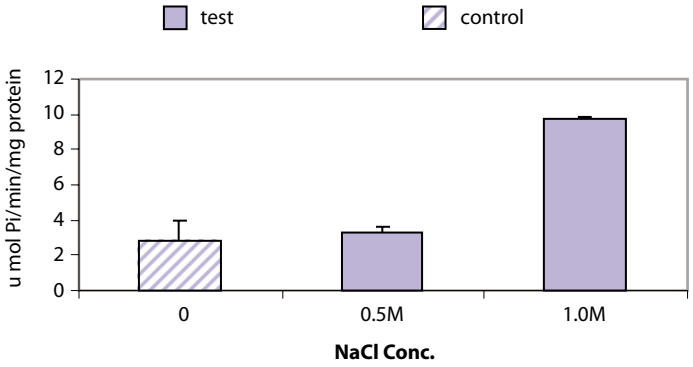

FIG. 3. Na^+, K^+-ATPase activity from the brain of *Oreochromis mossambicus* as a function of ionic strengths.

Figure 4 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 was increased to 0.5 M, it becomes 546.19 mg/g, and decreased to 88.2 mg/g when ionic strength increased to 1 M.

Figure 5 shows the effects of different concentrations of NaCl on the Na^+, K^+-ATPase activity in RBC of *Oreochromis mossambicus*. Control fish showed Na^+, K^+-ATPase activity of a 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 water environment that has 0.5 M ionic strength. 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 water environment that has 1.0 M ionic strength.

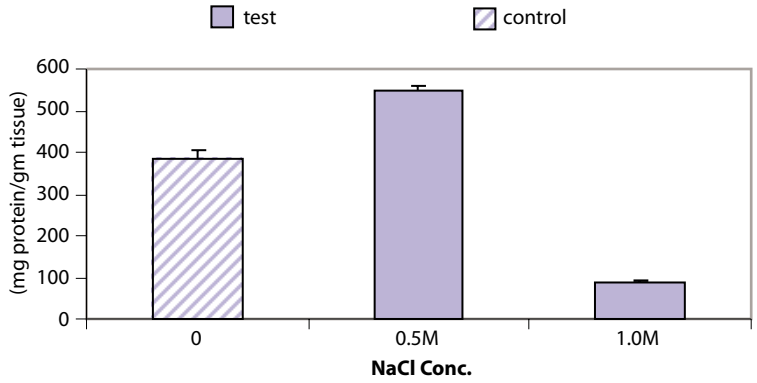

FIG. 4. Total protein concentrations from the brain of *Oreochromis mossambicus* as a function of ionic strengths.

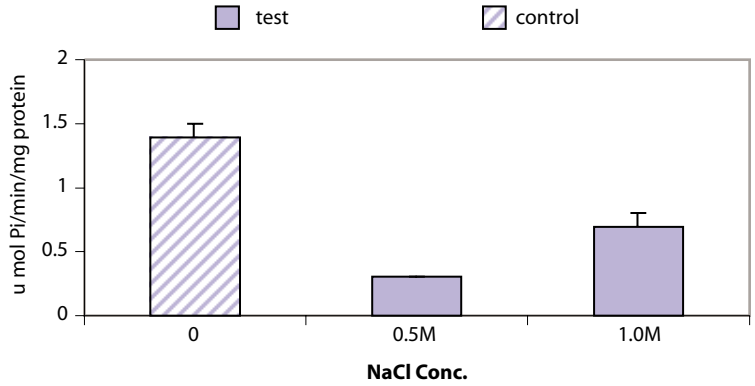

FIG. 5. Na^+, K^+-ATPase activity from the RBC of *Oreochromis mossambicus* as a function of ionic strengths.

Figure 6 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 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 becomes 1 M.

Discussion

The ions of salts and water can be exchanged through the gill membrane that is called osmoregulation. Such functions for the gills gave it an important role in the life of fish. 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

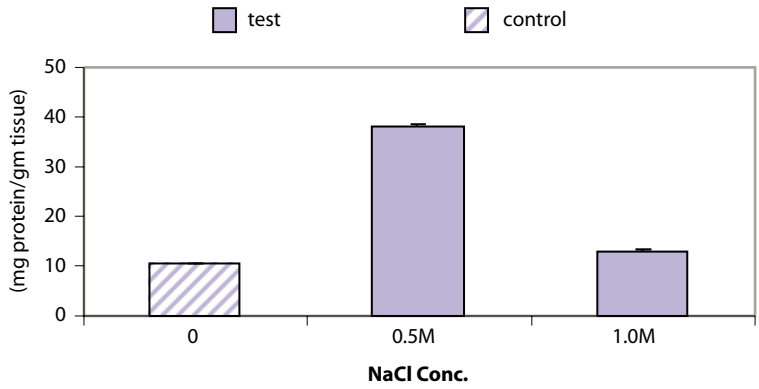

FIG. 6. Total protein concentrations from RBC of *Oreochromis mossambicus* as a function of ionic strengths.

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.

In some cases the lowest concentration enhanced the enzyme activity to some extent[13]. Since ATPase is an integral component of the membrane, the active site of the enzyme would be altered, and the energy needed to pump out 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[14].

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[15].

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[16].

There is an increase of the Na^+, K^+-ATPase activity in the gills of *Carcinus maenas* adapted to diluted seawater[17].

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[18].

Using RT-PCR, partial cDNA of Na^+, K^+-ATPase alpha subunit of tilapia (*Oreochromis mossambicus*) was cloned and sequenced. Clone TG3, with 1685 bp encoding a protein of 565 amino acids, showed higher than 85% identity in deduced amino acid sequence with previously published animal Na^+, K^+-ATPase alpha-1 subunit genes. Northern blot showed that TG3 expressed in gills, kidneys and other organs in tilapia. The amount of mRNA in gill tissue increased with the level of environmental salinity[l6].

The result obtained in this paper agrees to certain extent on 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 increased salinity.

The results obtained from brain and RBC'c concerning Na^+, K^+-ATPase activity as a response of environmental ionic changes was considered new, since nobody to my knowledge was working with them. It is hoped to use the Na^+, K^+-ATPase activity as an indicator to monitor fish environmental regarding ionic changes.

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التغيرات الفسيولوجية لنشاطية أتيبيزات الخياشيم والمخ وكريات الدم الحمراء في سمكة أوروكروميس موسامبيكس كاستجابة للتغير في تركيز الملوحة البيئية

طه عبدالله قمصاني

قسم الكيمياء الحيوية ، كلية العلوم ، جامعة الملك عبدا لعزيز

جـــدة – المملكة العربية السعودية

المستخلص. تتضمن هذه الدراسة تأثير التركيز الملحي المختلف لكلوريد الصوديوم في بيئة الأسماك على النشاطية الفسيولوجية لإنزيم الصوديوم ، وبوتاسيوم – أتيبيز من خياشيم ، ومخ ، وكريات الدم الحمراء لسمكة البلطي، أوروكروميس موسامبيكس. أكثر تغير ملحوظ لنشاطية إنزيم الصوديوم ، وبوتاسيوم – أتيبيز خلال التعرض لتركيزات ملح كلوريد الصوديوم كان ممثلاً في زيادة نشاطية الإنزيم في المخ وانخفاض نشاطيتة في الخياشيم وكريات الدم الحمراء عند التركيزات المرتفعة. البروتينات الكلية لمتجانس الخياشيم والمخ انخفضت عند تركيزات الملح المرتفعة بينما ارتفعت في حالة كريات الدم الحمراء.