Short Communication

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IN VIVO CHANGES IN THE ACTIVITY OF (GILL, BRAIN AND RBC) ATPASES FROM *OREOCHROMIS MOSSAMBICUS* AS A RESPONSE TO ENVIROMENTAL TEMPERATURES

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The effect of increased water temperature due to desalination plant on marine life must be investigated before it becomes a major problem. Different cell components protect enzyme structure and function against different stress conditions, (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 in *Escherichia coli* (Vickery *et al* 997). 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). This study was perfrmed to investigate the effects of temperature in fish environment on the *in vivo* activity of Na+,K+- ATPase from the gills, brain and red blood cells of Tilapia, *Oreochromis mossambicus.*

Salt-water acclimated Tilapia *(Oreochromis mossambicus)* were obtained from King Abdulaziz University hatchery. They were juvenile, about 10 cm in total length. Glass tanks (30x30x20 cm) were used for keeping the experimental fish (12fish in each), under the same experimental conditions with continuous aeration. A photoperiod of 16 h of light and 8 h of dark was maintained, oxygen was never below 8 mg l^{-1}, acidity was monitored regularly (pH 7.3 ± 0.4) using hand pH meter.

Each aquarium was connected with temperature regulators through water bath. Thermometers were placed in each aquarium to monitor the changes in water temperature. Experimental temperatures were 15°C, 22°C and 30°C, respectively

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

At the end of the experiment, fish were killed; the gills, brain and RBC samples were collected from each of the experiment 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 fro 10 min to remove plasma.

In all groups, brain or gills or RBC were rapidly collected and were either worked upon immediately or stored at -20°C until 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, which can be measured within a suitable absorbency range. Total protein concentration of each homogenate was determined according to Lowry *et al* (1951).

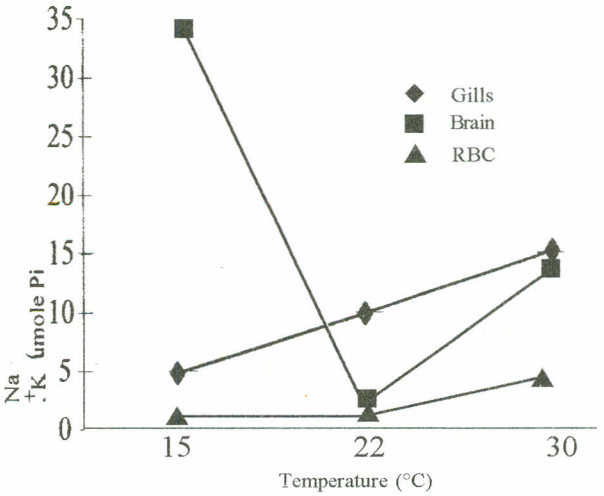

Fig 1. Na^+,K^+-ATPase activity from the gills, brain and RBC of *Oreochromis mossambicus* as a function of temperature.

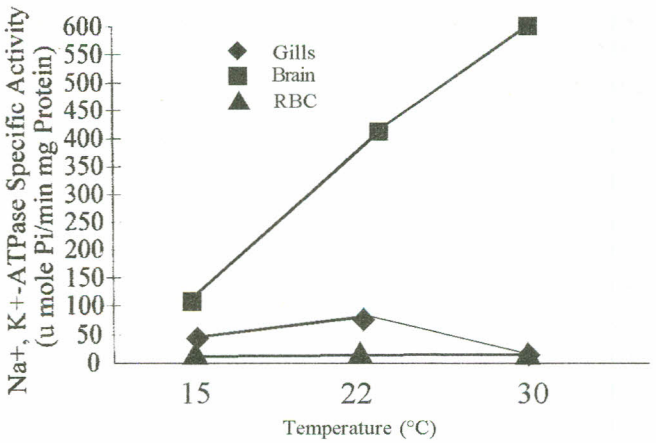

Fig 2. Total protein concentration from the gills, brain and RBC of *Oreochromis mossambicus* as a function of temperature.

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 activiy according to the method of Serrano (1978). The method is based on selective inhibition of Na^+, K^+-ATPase by the glycoside ouabain (Kimelberg *et al* 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^+-APase activity could be calculated.

The reaction mixture was buffered with 50 mM Tris pH 6.5 and contained different concentrations of $MgCl_2.6H_2O$ (0.5-20 mM), 100 mM NaCl, 10 mM KCl, 0.1 mM EDTA and ± 1.5 uM ouabain. After adding the brain 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 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). The data presented here are the result of triple experiments each.

In the experimental results the control fish *O. mossambicus* showed Na^+, K^+-ATPase activity of a value of 9.80, 2.79 and 1.42 umoles Pi/min mg protein, respectively (Fig 1). This value was 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 maitained 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.

The total protein concentration of the control fish in gills, brain and red blood cell was 66.15, 384.91 and 10.28 mg per 1 gm of homogenized gill tissue, respectivity (Fig 2). The total protein concentration dropped when temperatures was maintained at 15°C; it was 34.09, 105.5 and 10.61 mg g^{-1}, respectively, and changed to 3.35, 584.86 and 4.45 mg g^{-1} respectively at 30°C. The gills remain in direct contact with the water and so this tissue is vulnerable to changes in the external environment (Brum *et al* 1994).

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.

In some cases it was seen that low temperature enhanced the enzyme activity to some extent (Verma *et al* 1979). Since ATPase is an integral component of the membrane, the active site of the enzyme would have been altered and the energy needed to pump out ions would be reduced. This was seen with the brain Na^+, K^+-ATPase.

It has been seen 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.

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

Key words: *Oreochromis mossambicus*, Na^+, K^+-ATPase, Tilapia, Temperature.

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