Effect of Electromagnetic Field on Na\(^+\), K\(^+\)-ATPase from Gills of *Oreochromis mossambicus*

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**Abstract.** The Na\(^+\), K\(^+\)-ATPase (adenosine triphosphatase), the ion pump enzyme located in cell membrane, is a well-defined protein whose properties can be used to study the mechanism of electromagnetic field (EMF) interaction with biological system. Electromagnetic field of 60 Hz, and field strength of 10 kV/m was applied for 2 weeks on *O. mossambicus* to measure its effects on the Na\(^+\), K\(^+\)-ATPase activity. The enzyme from gills showed a 30% decrease in the specific activity after exposure to EMF compared to the control ones. The ATPase specific activity of gills homogenate of post-exposed fish showed an increase (180%) over the directly affected ones after 2 weeks outside the field (late 1). No further increase in the specific activity of the enzyme for the fish kept 4 weeks outside the field (late 2). A change seen in enzyme activity was non-reversible (no return to normal value), when the pre-exposed fish were left outside the field for a period up to 4 weeks. It is concluded from this result that the applied field strength has an effect on Na\(^+\), K\(^+\)-ATPase activity and aquatic environment has no shielding effect. Further research needed before establishing limits of exposure on human.

**Introduction**

Interest in the biological effects of electromagnetic fields (EMFs) has been driven mainly by epidemiological studies which relate cancer incidence with exposure to such fields. Until now, the scientific communities could not answer how weak EMF interacts with molecules or how molecular reactions initiate complex cellular processes. To approach the problem of transduction of EMF exposure into molecular changes, the effects of EMF have been studied on the enzyme activity of a well defined membrane protein, the Na\(^+\), K\(^+\)-ATPase. The Na\(^+\), K\(^+\)-
ATPase is a ubiquitous transport enzyme, the molecular structure and properties of the enzyme have been reviewed in monographs by Lauger (1991) and Tonomura (1986). The splitting of ATP by the ATPase provides enough energy for the transport of Na\(^+\) and K\(^+\) ions against their concentration gradient, but how the energy from breaking a chemical bond is transduced into a directed ion flux is not known. Perhaps the most puzzling aspect of this problem is how the widely separated sites for catalysis and ion binding are coordinated to achieve the fluxes. Two molecular processes are generally believed to be involved in the active transport mechanism: conformational changes and charge shifts within the protein during enzyme activity. These processes are linked through the surface charge. The effects of charges in protein surface charge on molecular conformation can be illustrated in terms of channels, which function as molecular switches in biological membranes (Blank, 1987). The effect of different electromagnetic fields on the production, activity and/or association of different enzymes of different membranes or organs has been reported (Byus et al., 1984; Moses and Martin, 1992; Robertson and Astuian, 1992 & Uckum et al., 1995).

Electromagnetic field of 60 Hz and field strength of 10 kV/m was applied for 2 weeks on whole Swiss Albino mice to measure its effects on the hemoglobin spectra, total proteins, albumin, creatinine, urea, uric acid, glucose contents, triglyceride, cholesterol, HDL, LDL, GOT, GPT and level of Na, K-ATPase from different organs (Kumosani and Mashak, 1995, 1997a; Kumosani et al., 1996, 1997b, 1997c & Kumosani, 2001). However, this is the first report on studying the effect of EMF on aquatic species, to monitor if the aquatic environment has any shielding effect.

**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\(_2\)O) 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 g, about 10 cm in total length. Glass tanks (70 × 30 × 80 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/l, pH was monitored regularly (pH 7.3 ± 0.4) using hand pH meter.

**Experimental Design**

A 6000 V stabilized power supply was used. The fish exposure technique is discussed in details in previous works with other animal (Kumosani and Mashak, 1995; 1997a; Kumosani et al., 1996, 1997b, 1997c & Kumosani, 2001). It is briefly as follows: the fish were placed in glass aquarium (70 × 30 × 80 cm), throughout the length and from outside of the aquarium have two aluminum plates for the electromagnetic field application. The applied frequency was 60 Hz while the field strength was 10kV/m.

The experimental fish were divided into two groups, control and the groups exposed to EMF. Control fish were kept in the same environmental conditions and given the same food, on the sides of the aquarium were two dummy wooden plates fixed. The exposed fish to EMF were divided into three groups, each contains 15 fish, namely direct (D), first late (L1) and second late (L2). Fish of group D were decapitated after direct exposure to the field for two weeks. The gills were used to test the Na⁺, K⁺-ATPase. The first late L1 and second late L2 groups are the pre-exposed fish left outside the field for 2 and 4 weeks before decapitation, respectively.

At the end of the experiment, fish were killed; the gills samples were collected from each fish of the experimental group (15 each).

**Preparation of Tissues Homogenate**

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

Samples of gill tissues of exposed groups and control were 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 (Lowry 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
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$^+$-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$_2$O (0.5-20 mM), 100 mM NaCl, 10 mM KCl, 0.1 mM EDTA and +/- 1.5 µM ouabain. After adding the gills homogenate (50 µl) and incubation for 5 min at 30ºC in a water bath shaker, the reaction was started by the addition of 20 µl of 0.1 M ATP (0.25-10 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 µl 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 mean of triple experiments each.

**Statistical Analysis**

The data collected as means + SE and logged into personal computer and analyses of data were performed using SPSS statistical package. Post Hoc test was used for comparing means (Norusis, 1989).

**Results**

**Direct Effects**

Figure 1 shows the ATPase specific activity of gills homogenate after the direct exposure of the fish to EMF at frequency of 60 Hz with field strength 10kV/m. The enzyme showed a 30% reduction in the specific activity compared to the control ones with highly significant value (P = 0.001).

**Late Effects**

From Fig. 1, it is clear that the ATPase specific activity of gills homogenate of pre-exposed fish showed an increase (180%) over the directly affected one after 2 weeks outside the field (late 1) with very highly significant value (P = 0.000). No further increase in the specific activity for the fish kept 4 weeks outside the field (late 2) (P = 0.07).

**Discussion**

The result obtained in this study is similar to large extent to the study done on Na$^+$, K$^+$-ATPase from the brain tissues of Swiss Albino mice exposed to similar
EMF field strength (Kumosani and El-Mashak, 1995). It seems that aquatic environment has no protective effect. The effects of electric fields can be accounted for through ion activation at the surface of the enzyme. Theoretically an alternating square wave leads to an increase in ion binding (Blank and Soo, 1992), but the exposed changes are far too small. Perhaps the field accomplishes the same changes in charge distribution inside the ATPase as ion binding causes on the surface. In any case, the proposed mechanics, based on increases in ion activation at surface of the enzyme explains qualitatively the observed dependencies on electrical field and on basal level of Na⁺, K⁺-ATPase activity through increases in cation binding at activation sites. The ion activation hypothesis can also explain both inhibition and stimulation, and the difference in optimal frequency for cation influx and efflux in red blood cells as seen in red blood cells (Blank and Soo, 1992).

Magnetic fields affect the enzyme in the same way as an increase in ion binding at the enzyme surface, and magnetic fields appear to increase charge flow within the protein, thereby coordinating binding sites at the two surfaces of the enzyme. Magnetic fields can not act through the induced electric fields because the magnitudes of the induced currents are much too small and the effects are in the opposite direction (Britten and Blank, 1973).
From studies of the effects of electric and magnetic fields on Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity, we have been able to learn about changes in molecular properties that may be characteristic of the ways in which membrane proteins are affected by the EMS. Magnetic fields and electric fields appear to act at different sites on the ATPase, but both change the surface charge density. Changes in charge density affect the electric gradient across a channel, the number of ions bound or released, and channel opening and closing, all of which affect ion transport across membranes, which consists with the enzyme inhibition seen in the direct effect experiment. However, enzyme activity was restored to higher level and not to the normal level after removing the electromagnetic fields which might be due to changes in charge density. Further studies are needed at molecular level to verify this hypothesis.

**Acknowledgement**

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**References**


تأثير المجال الكهرومغناطسي على إنزيم الصوديوم، بوتاسيوم -
إتبيز من خياشيم أسماك البلطي

طه عبدالله قماني و عبدالرحمن لبد المالكي
قسم الكيمياء الحيوية، كلية العلوم، جامعة الملك عبدالعزيز،
جدة - المملكة العربية السعودية

المستخلص. إنزيم الصوديوم، بوتاسيوم - إتبيز، عبارة عن مضخة
أيونية موجودة في الغشاء البلازمي، وهو بروتين يمكن استخدام خواصه
لدراسة آلية تداخل المجال الكهرومغناطسي مع النظام الحيوي. طبق
المجال الكهرومغناطسي ذو قوى 60 هرتز وشدة 10 كيلو فولت/ متر لمدة
أسبوعين على أسماك البلطي لقياس تأثيره على نشاطية إنزيم الصوديوم،
بوتاسيوم - إتبيز. تناقصت نشاطية الإنزيم المختصة في الخياشيم بقدر
30% بعد التعرض للمجال الكهرومغناطسي مقارنة بنشاطية الإنزيم
المختصة في الأسماك غير المتعرضة. ارتفعت نشاطية الإنزيم المختصة
من الخياشيم بمقدار 180% للتي تركت بعد التعرض لمدة أسبوعين مقارنة
بنشاطية الإنزيم المختصة من الأسماك المتعرضة. ولم يلاحظ أي زيادة
في نشاطية الإنزيم المختصة من الخياشيم التي تركت بعد التعرض لمدة
أربعة أسابيع. يمكن الاستنتاج من النتائج المحصلة أن المجال
الكهرباً/مغناطسي المطبق قد أثر على نشاطية إنزيم الصوديوم، بوتاسيوم -
إتبيز وأن البيئية المائية لم تلعب أي دور في الخصائص. والأمر يحتاج
لأبحاث أكثر قبل البدء في وضع حدود للتعرض للإنسان.