

Full Length Research Paper

# Effect of 50 Hz electromagnetic fields on acid phosphatase activity

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**The effect of extremely low frequency (ELF) electromagnetic field (EMF) (50 Hz 0.5 mT) on the activity of acid phosphatase (EC 3.1.3.2) was studied. In addition the factors affecting the enzyme activity such as the temperature, pH and substrate concentration were also investigated. The results show that ELF EMF have significant influence on enzyme activity. Upon EMF exposure  $K_m$  increased from  $0.014 \pm 0.005$  to  $0.040 \pm 0.008$  mM whereas  $V_{max}$  increased from  $0.991 \pm 0.254$  to  $1.638 \pm 0.345$   $\mu\text{mol}/\text{min}$ . Further studies can probably help in finding suitable applications for ELF based modulation of enzyme activity.**

**Key words:** ELF EMF, acid phosphatase, enzyme activity.

## INTRODUCTION

Daily exposure to electromagnetic field is unavoidable as a consequence of living in a society that depends heavily on the use of the electricity. Over the past several years there has been a growing concern in the general public of a perceived health risk associated with exposure to EMF. The EMF generated by the 50 Hz alternating current traveling through electrical power lines has been of special concern. The first indication of a possible health risk developed from epidemiological studies (Wertheimer and Leeper, 1979). Further investigation in the laboratory has shown that a variety of biological processes can be influenced by 50 Hz EMF (Shang et al., 2004; Lupke et al., 2006; Mehri et al., 2008). Although EMFs are usually associated with high voltage power lines and power stations, they are also provided by any electrically powered device, typical of those found in households or the workplace. Appliances such as video display terminals, TV's, hair dryers and cellular phones emit EMF's (Goodman et al., 1993).

Human exposures are normally to extremely low frequency ELF EMF's (defined as less than 300 Hz). Heightened public awareness has led to the inclusion of exposure to ELF EMF's as a part of a growing series of environmental conditions related to the "quality of life" in

the industrial world. In recent years studies investigating the interaction of extremely low frequency EMF with human subjects, laboratory animals, organ cultures and individual cells have become substantial (Zmyslony et al., 2000; Claudio et al., 2004; Thomas et al., 2006).

There have been reports in the literature that this ELF EMF affects the various biochemical processes. Various surveys (Brix et al., 2001; Kelsh et al., 2003; Henderson et al., 2006) and epidemiological studies (Kuane et al., 2000; Wartenberg, 2001; Szabo et al., 2006) have been carried out to find the effects of these low frequency electromagnetic fields. Several studies have been carried out to investigate the effects on DNA (Ivancsits et al., 2002; Nikolai et al., 2004), enzyme activity (Blank et al., 1995, 1998a, b; Farrell et al., 1997; Morelli et al., 2005; Blank, 2005; Manoliu et al., 2006) and cells (Jin et al., 1997; Chang et al., 2005).

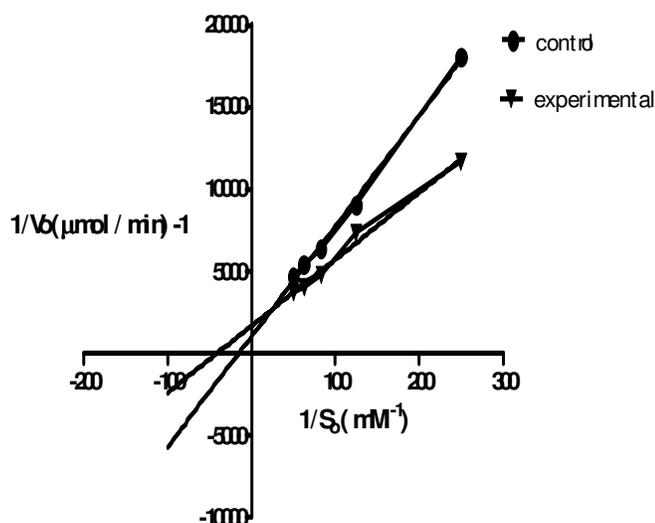
Enzymes play a vital role in the biological processes; also cell communication is facilitated by these biocatalysts. Any alteration in the activity of the enzyme may affect these biological processes. Elevated prostrate acid phosphatase may indicate the presence of prostrate cancer (Bull et al., 2002). Acid phosphatase is a phosphatase, a type of enzyme, used to freely attached phosphate groups from other molecules during digestion. Different forms of acid phosphatase are found in different organs, and their serum levels are used as a diagnostic for disease in the corresponding organs (Dattoli et al.,

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**Table 1.** Effect of EMF on the activity of the acid phosphatase from *Ipomoea batatas*.

Trial	Enzyme activity in the presence of EMF ( $\mu\text{moles/min}$ )	Enzyme activity without EMF (Control) ( $\mu\text{moles/min}$ )
1	0.042	0.022
2	0.050	0.023
3	0.053	0.029
4	0.045	0.026
5	0.042	0.025
Mean	$0.0458 \pm 0.005^a$	$0.0232 \pm 0.002^a$

Acid phosphatase activity measured after 60 min exposure to a field of 0.53 mT, p-value = 0.0054 (paired t-test). <sup>a</sup> mean value with standard deviation of 5 measurements.



**Figure 1.** Lineweaver-Burk plot for acid phosphatase in the absence and the presence of EMF. Each value represents the mean  $\pm$  SD of five measurements.

1999; Giraldo et al., 2000; Tsu et al., 2004). In this paper we have chosen to study the effect of ELF EMF on the activity of acid phosphatase enzyme.

## MATERIALS AND METHODS

### Exposure system and EMF characteristics

The exposure system consisted of a Helmholtz coil pair 17 cm in diameter, mounted on a wooden frame. Each coil had 500 turns of 0.25 mm diameter copper wire. The inner radius of each Helmholtz coil was 7 cm, while the outer radius was 10 cm. The system arrangement generated a uniform magnetic field of 0.53 mT ac rms. The distance between the two coils was 7 cm. At the centre of the arrangement a shelf was placed to hold the samples to be exposed. The signal was provided using step down ac transformer 6 V, 50 Hz duty cycle and the field intensity 0.53 mT rms measured by a gauss meter GM O5 (Hirst magnetic instruments UK, range 0 mT -3 T,

frequency 15 Hz to 10 KHz and with an accuracy of  $\pm 1\%$ ) the gauss meter was connected to a laptop with a RS 232 interface and using Microsoft Visual Basic software programming tool the real time data was captured and stored in the system. The magnetic field of 0.53 mT was provided within Helmholtz coils supplied by electric generator able to deliver 50 Hz electric current. A PVC test tube stand to hold the samples was positioned in the middle of the Helmholtz coils. This arrangement allowed a uniform magnetic field of ac rms 0.53 mT.

### Assays for enzyme activity from *Ipomoea batatas*

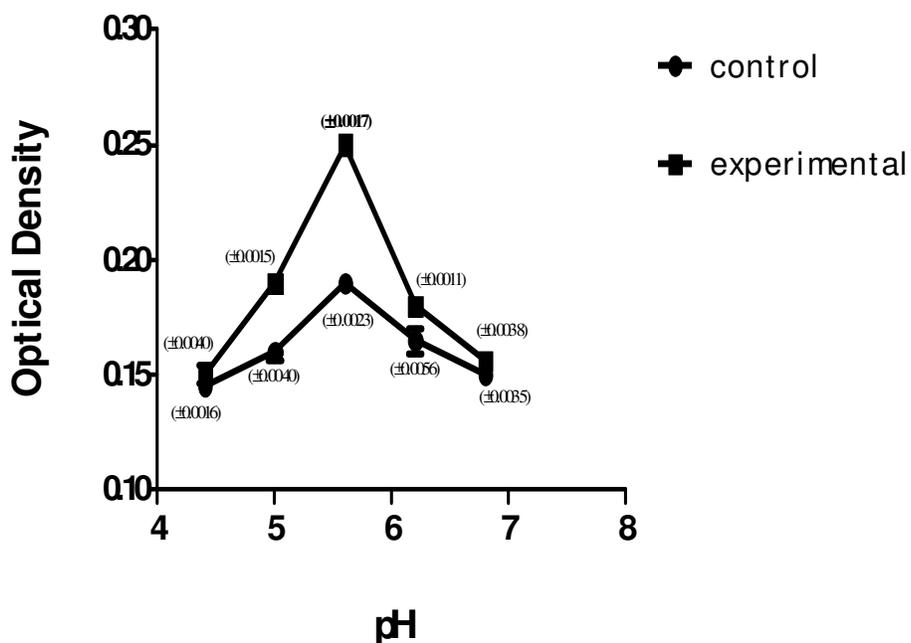
Acid phosphatase activity was determined by the method of Peter Bernfield (1955). Sodium  $\beta$ -glycerophosphate was used as the substrate. Acid phosphatase reactions were performed at 37°C in 0.1 M citrate buffer containing 0.1 M magnesium acetate, pH 5.6. Kinetic measurements were determined by activity assays at pH 5.6 with various substrate concentrations. Acid phosphatase activity was measured before and after 60 min exposure to a field of 0.53 mT based on the absorbance at 660 nm (Systronics 117 type spectrophotometer). The kinetic parameters  $V_{max}$  and  $K_m$  were evaluated by Lineweaver Burk method. Acid phosphatase was assayed for its pH optimum and temperature optimum with the same substrate to check the effect of EMF over a range of pH and temperature.

### Statistical analysis

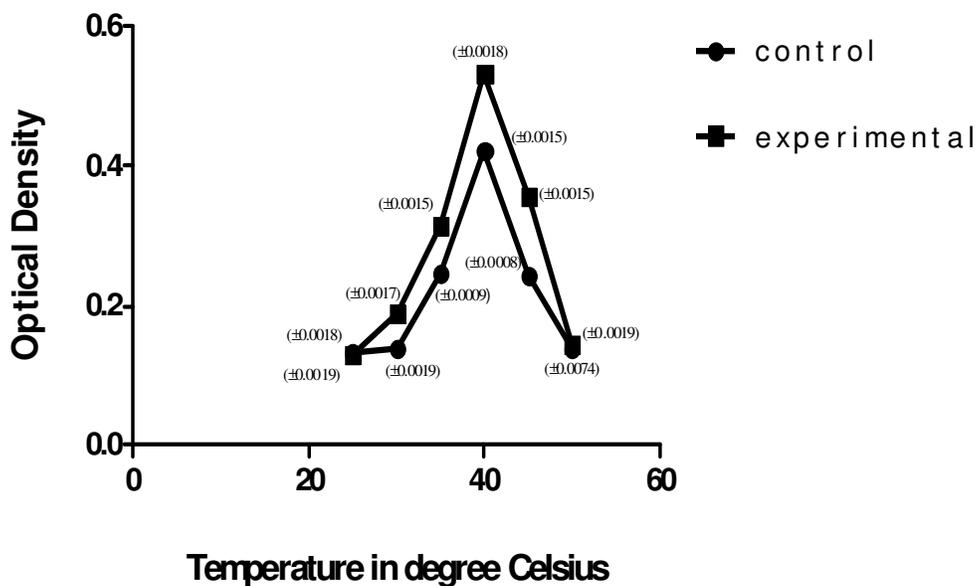
Statistical analysis was done using Graph pad prism version 5.0. Two tailed paired t-tests were applied to compare the enzyme activities which were exposed to electromagnetic fields and the control samples which were not exposed to electromagnetic fields and results were considered to be statistically significant with  $P < 0.05$ .

## RESULTS AND DISCUSSION

Table 1 shows that the acid phosphatase activity increased significantly ( $P < 0.05$ ) when it was exposed to EMF. The exposure to electromagnetic field induced about 50% increase in the enzyme activity ( $0.0468 \pm 0.005$ ) when compared to control group ( $0.0232 \pm 0.002$ ). Figure 1 represents a Lineweaver Burk plot with control and exposed samples. Upon EMF exposure  $K_m$  increased from  $0.014 \pm 0.005$  to  $0.040 \pm 0.008$  mM whereas  $V_{max}$  in



**Figure 2.** Effect of pH in the absence and the presence of EMF. Each value represents the mean  $\pm$  SD of five measurements.



**Figure 3.** Effect of temperature in the absence and the presence of EMF. Each value represents the mean  $\pm$  SD of five measurements.

increased from  $0.991 \pm 0.254$  to  $1.638 \pm 0.345$   $\mu\text{mol}/\text{min}$ . Figure 2 represents the effect of pH on enzyme activity in the absence and the presence of EMF. No significant differences were detected for the optimum pH between control and exposed samples when acid phosphatase was assayed over a range of pH. Figure 3 represents the

effect of temperature on enzyme activity in the absence and the presence of EMF. No significant differences were detected for the optimum temperature between control and exposed samples when acid phosphatase was assayed over a range of temperature. However the curves in Figures 2 and 3 for the exposed samples show a

different trend with an optimal pH and temperature where maximal activity is seen in the presence of ELF EMF.

Dick et al. (1983) showed that the optimum pH for corn roots' acid phosphatase was 4, while Ullah and Gibson (1988) showed that the optimum pH for acid phosphatase was pH 5. In soil, the pH optimum for acid phosphatase ranges from pH 4 to 6.5 (Eivazi and Tabatabai, 1976). Figure 3 represents the effect of temperature on enzyme activity which is similar to that reported by Panaral et al. (1990) for acid phosphatase from barley and by Basha (1984) for peanut.

Based on the experimental work, and theoretical calculations (Laurence et al., 2000; Pickard and Moros, 2001; Laurence et al., 2003; Stuerger and Gaillard, 1996; Saxena et al., 2003) several mechanisms have been proposed of how ELF EMF is likely to interact with tissue, cells and proteins in solutions. The Moving Charge Interaction (MCI) model proposes that moving electrons affect enzyme activity. The activation of gene expressions (Olivares et al., 2004) and synthesis of stress proteins can be initiated by an EM field (both electric and magnetic fields) by moving electrons. The optimal frequency dependence is related to the turnover numbers (Blank and Soo, 1998b; 2001; Blank and Goodman, 1997, 1999, 2000, 2002) of the enzyme reaction. It is interesting to notice this effect of ELF EMF on enzyme functions in spite of small amount of energy carried by applied field; which is well below relevant ionizing or binding energy.

Balcavage et al. (1996) have shown that EMFs indeed affect metabolic processes by regulating flow of cellular cations. Wolf et al. (2005) have reported that EMF, in the presence of a transition metal, generates reactive oxygen species to cause DNA damage. Low frequency EMF, and pulsed EMF affect biological systems via information transfer; this information transfer can trigger biochemical processes such as ion binding and signal transduction (Rosch and Markov, 2004). Theoretical models have depicted existence of low frequency (ELF) magnetic field interactions with biosystems at ion cyclotron resonance frequencies, that is, at frequencies corresponding to charge to mass ratios of ions such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$  (Bruce et al., 1992). Experimental investigations reveal that through calcium signaling pathways and cytosolic calcium oscillator low frequency electromagnetic fields effects living systems (Galvanovskis and Sandblom, 1998). The models on the effects of ELF-EMFs on enzyme activities refer to changes of motion of ions at the active site (Edmonds, 1993).

Structural investigations indicate that the catalytically active form of sweet potato purple acid phosphatase contains binuclear Fe-Mn clusters (Gerhard et al., 2001).

Susan and Bruce, (1987) predicted iron in sweet potato purple acid phosphatase is expected to be most likely in the Fe (III) state. The structural study by Gerhard et al. (1999) revealed the presence of a strongly antiferromagnetically coupled binuclear Fe(III) - Mn (II) center in sweet

potato purple acid phosphatase which is responsible for the enzyme's biological activity. The presence of thioether bond would put more structural constraints at the active site and henceforth increases the electron density (Atila et al., 1999). Therefore one may speculate EMF would affect the electron density which may in turn cause deprotonation which may lead to the enhancement of the enzyme activity.

More important results have been reported for enzymes exposed to ELF EMF. Venkatachalam et al. (2005) have shown a reduction in the activities of lysosomal enzymes; cathepsin D, acid phosphatase and myeloperoxidase in the liver and serum of arthritic rats subjected to a field of 4  $\mu\text{T}$ . Zhang and Liu (1992) have shown that magnetic field effect on acid phosphatase is physically reversible by studying the effects of a strong constant magnetic field on the activity and localization pattern of acid phosphatase in *Blepharisma*. The magnetic fields in the range 0 - 70 Hz and 0 - 2 G increased Na, K-ATPase activity by 5 - 10% (Blank et al., 1995).

It was reported that 60 Hz magnetic fields accelerate the oxidation of cytochrome C, a reaction catalyzed by cytochrome oxidase, an electron transport enzyme of the mitochondrial redox chain; the acceleration varied with the magnetic field strength, the increase in the oxidation rate constant was 20 - 30% at field strengths below 3 mT (Blank et al., 1998). Farrell et al. (1997) have demonstrated that 4 pT, 60 Hz magnetic field enhances ornithine decarboxylase activity during gastrulation while studying the biochemistry of developing chicken embryos. Morelli et al. (2005) have shown that ELF EMF of 75 Hz, 2.5 mT above a threshold produces a decrease of about 54 - 61% of the enzymatic activities of three membrane-bound enzymes: alkaline phosphatase, phosphoglycerate kinase and acetylcholinesterase from blood cell or from synaptosomes. A more recent study by Manoliu et al. (2006) shows that the exposure to the field strength of 10 mT induced a 55% increase in the catalase activity and a 50% increase in the peroxidase activity in a fungi culture medium. The data reported in this article show that ELF EMF of 50 Hz, 0.53 mT induced about 50% increase in the enzyme acid phosphatase activity.

Blank and Soo (2001) while explaining the EMF interaction mechanism with  $\text{Na}^+\text{-K}^+$  ATPase suggests the electrons move regularly and the threshold force producing the effect on the enzyme is due to acceleration of the electron regardless of direction. Therefore, it is very likely that the effects of the field on the acid phosphatase could involve electron density at the active site and the antiferromagnetically coupled binuclear Fe (III) - Mn (II) center. The results of the study clearly indicate that ELF EMF influence enzyme activity irrespective of temperature, pH and substrate concentration.

Further studies are required, however it is possible that a whole new process of controlling enzyme activity using ELF EMF with commercial implications in industrial enzymology.

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