30th Annual International IEEE EMBS Conference
Vancouver, British Columbia, Canada, August 20-24, 2008

Non-thermal Effects of 500MHz – 900MHz Microwave Radiation on Enzyme Kinetics

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Abstract— **Enzymes are essential for the catalysis of biochemical reactions and in the regulation of metabolic pathways. They function by greatly accelerating the rate of specific chemical reactions that would otherwise be slow. It has been shown that extremely low-power microwaves can influence enzyme activity [1-5]. This study is focused at investigating the effects of low level microwave exposures ranging from 500MHz to 900MHz on L-Lactate Dehydrogenase (LDH) enzyme activity. The results obtained revealed the increased bioactivity of the LDH upon microwave radiation at two particular frequencies 500MHz and 900MHz.**

INTRODUCTION

The number of devices using microwave irradiation has been increasing the previous years rapidly, and the concern of military, industrial and government organizations with the possible health hazards associated with exposure to microwave irradiation was grown. The heating effect of the microwaves was already well-known but a doubt remained on the existence of a non-thermal effect. Isolated enzymes in aqueous medium were taken as model to determine if there was an effect of the radiation on the biological processes. These first studies have been carried out with various microwave apparatuses, with frequencies and powers of irradiation used varied significantly from one study to another [1].

Thirty years ago, Frohlich postulated that extremely weak microwave signals may act as a trigger for certain bioeffects. During the last few decades, the use of microwave radiation has greatly increased in radar and communication systems as well as in food-processing technology and in other industrial applications. The development of consumer and medical microwave devices for clinical diagnosis and therapy also has prompted widespread interest and has stimulated much research on the mechanisms of interaction of microwave radiation with living organisms [2-5].

Manuscript received April 7, 2008. This work was proudly supported by the Australian Centre of Radiofrequency Bioeffects Research (ACRBR).

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According to the literature, two types of effects can be ascribed to microwaves, i.e. thermal and non-thermal [2-5]. The thermal effects are related to the heat generated by the absorption of microwave energy by the water medium or by organic complex systems, both characterized by a permanent or induced polarization. At present, very little is known about the molecular mechanisms involved in the putative non-thermal effects which could involve direct energy transfer from the electromagnetic field to the vibrational modes of macromolecules altering their conformation [6-7].

Biological molecules, once perceived as rigid structures, are now known to show rapid, continuous changes in shape that may well be important in their biological functions. It was reported that microwaves radiation may be used to selectively manipulate biochemical processes remotely and non-thermally [8]. Therefore, proteins, and more specifically enzymes, present themselves as target systems for microwave electromagnetic radiation. Experiments have shown that extremely low-power microwaves can affect enzymes activity. For example, Bose reports a tripling in the rate at which the enzyme cellulose breaks down cellulose compounds with the enhancing effect of microwave radiation [9]. Also reported is the accelerated hydrolysis of albumin [10], and the rapid disruption of a peptide bond cleavage [11]; neither of which can be attributed to thermal heating effects. It was suggested that two particular parameters seem to influence the enzymatic activity under microwave irradiation: the hydration state and the polarity of the reaction medium.

However, the results obtained on enzyme systems are so far controversial, probably due to the experimental difficulties in the proper control and monitoring of the temperature. No measurable non-thermal effect on catalytic activity has been observed in a number of isolated enzymes irradiated *in vitro* [9-12]. Conversely, other enzyme systems such as lymphocyte protein kinases [13], hepatoma cell ornithine decarboxylase [14] and acid phosphatase [15] respond to low or high intensity and amplitude-modulated microwave fields. Moreover, a significant inhibition of red cell Na/K. ATPase, presumably related to conformational changes of the protein, has been reported [16].

In our previous studies [17,18] we have tested the influence of visible light in a range of 550-850 nm and infrared radiation ranging from 1140 up to 1200 nm on enzyme kinetics of the LDH enzyme. This study extends our investigation of non-thermal biological effects of low power electromagnetic radiation (EMR) exposures on the LDH biological activity in the microwave range. In particular, this paper reports on results of our experimental

study of the LDH enzyme aqueous solutions exposed to 500MHz – 900MHz microwave radiation with the power of -50dBm, -40dBm and -30dBm (0.01μW, 0.1μW and 1μW respectively).

I. Materials and Methods

A. Microwave exposure system

As a source of microwave radiation we used TC-5062A UHF TEM Cell (100kHz – 3GHz), TESCOM Ltd (Pic. 1). This TEM Cell generates the electromagnetic field for irradiating the object of investigation. Through the input port, an external signal is applied to generate predictable field inside the TEM Cell.

Picture1. Sample exposure system (TC-5062A UHF TEM Cell)

A GTEM (model TC-5062A) was used to expose the enzyme solution samples. The GTEM was calibrated using a broadband electric field probe to determine the electric field produced at the sample position inside the GTEM for a given input power. For the input powers used in this experiment the test fields at each frequency are shown in Table 1.

We scale the field values we provide for 10 dBm (10 mW) input to the power levels we used, using:

$E_1 = E_0 * \mathrm{sqrt}(P_1/10\mathrm{mW})$

Where E_1 is the exposure field of the sample (that we would like to know), E_0 is the calibration field we found using a test power of 10 mW, and P1 is the test power we used in our exposure. The results have shown that the estimated uncertainty in the test field is ± 30%.

B. Experimental study

Dehydrogenases are the enzymes that catalyze a variety of oxidation-reduction reactions within the cells. As the protein example we have chosen the L-Lactate Dehydrogenase (rabbit muscle, EC1.1.1.27). This enzyme has been selected fort this experimental investigation on the basis of its commercial availability, simplicity of the assay, and the possibility of measuring its bioactivity using the standard, well accepted procedure, i.e. Continuous Spectrophotometric Rate Determination. To measure the absorption coefficients of the analyzed samples we have used an Ocean Optics USB2000 spectrometer coupled with a CCD array, which can detect in the 190-870 nm range (Pic. 2). Software control automatically monitors and saves the optical density values (absorption coefficients) at the required wavelength every 30 sec.

Picture 2. Spectrophotometer USB2000

L-lactate dehydrogenase (LDH) catalyses the inter-conversion of the l-lactate into pyruvate with the Nicotinamide Adenine Dinucleotide Oxidised form (NAD+) acting as a coenzyme. The suitability of the LDH enzyme for this reaction is attributed to the absorption characteristics of the NADH (Nicotinamide Adenine Dinucleotide Reduced form). NADH is able to absorb light at 340 nm in contradiction to the NAD, which is inactive at this frequency. Due to the different optical characteristics of the NADH and NAD we are able to optically asses if the reaction of conversion Pyruvate → Lactate in the presence of the LDH as an accelerator has occurred and then determine the amount of the reactants.

Assay preparation:

2.5 ml cuvettes are filled with the following components:

- 0.1 ml of 0.0027M Sodium Pyruvate (BioWhittaker);
- 0.1 ml NADH; disodium salt (C21H27N7O14P2Na2 Roche);
- 0.005M Phosphate buffered saline (SIGMA);
- 1.5 ml of deionized water;
- 0.3ml LDH diluted in 2mg/ml of Phosphate buffered saline with BSA (SIGMA).

The experimental procedure:

1. The cuvettes were filled with 0.3ml of the LDH samples. The samples were irradiated for 5 min using the GTEM exposure system set at the activation microwave

frequencies ranging from 500 MHz to 900 MHz and radiation power of -30dBm, -40dBm and -50dBm (1μW, 0.1μW and 0.01μW respectively).

2. The irradiated samples were then added to the already prepared solution of NADH and pyruvate. We have diluted the stock coenzyme solution with the 0.003 M potassium phosphate assay buffer.

3. The spectrophotometer was set to 100% transmittance (zero absorbance) at each wavelength using the 0.003 M K_2HPO_4 assay buffer blank.

4. Using the spectrophotometer, the absorption coefficients of NADH (rate of change in absorbance) were measured at 340 nm. The measurements were repeated three times for each analyzed radiating frequency and power values. The obtained data were collected and presented in Table II and Fig. 1. The experiments were performed at room temperature 27°C (Temperature controller, Quantum Northwest).

II. Results and Discussion

The results obtained have shown the change in NADH absorbance of analyzed samples under the influence of the irradiated LDH enzyme. From Fig.1 we can observe that after being irradiated by microwaves at the specific frequencies 500MHz and 900MHz, the LDH bioactivity has increased resulting in accelerating the reaction:

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+ + \text{H}^+.$$

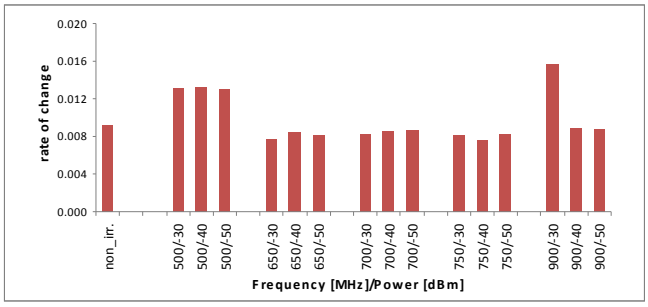

Figure 1. Rate of change in NADH absorbance upon microwave irradiation of the LDH enzyme

The results obtained revealed (Fig.1, Table II) that the maximum value of NADH optical density/rate of change in NADH absorbance is **0.0157 (**achieved at the frequency 900MHz and power of -30dBm), which corresponds to 73% increase in comparison with the rate of change value **0.0091** of the non-irradiated enzyme. Moreover, from Table II and Fig.1 we can also observe that microwaves at 500MHz/-30dBm, 500MHz/-40dBm and 500MHz/-50dBm have also affected the LDH bioactivity that resulted in the increased rate of change in NDAH absorbance. These values (0.0132, 0.0133, and 0.0131 respectively) are higher (45%, 47%, and 43%) than the rate of change 0.0091 in the presence of the non-irradiated LDH (Table II).

Interesting to note, when the LDH enzyme was irradiated by all other selected microwave frequencies 650MHz, 700MHz and 750MHz (Table II, Fig.1), the measured NADH absorption coefficients were lower than in the case of non-irradiated enzyme. Thus, we can conclude that the microwave exposures at these specific frequencies 650MHz, 700MHz and 750MHz have also affected the LDH kinetics leading to the decreased rate of change in NADH absorbance (2%-15% depending on the irradiating frequency), i.e. have slow down the overall chemical reaction (Table II).

III. Conclusion

In our previous work it was shown that enzyme activity can be modulated by electromagnetic radiation at the specific frequency of visible and infrared light. Here we have shown that the biological activity of the studied LDH enzyme can be increased by 40%-70% using low power microwaves at 500MHz/-30dBm, 500MHz/-40dBm, 500MHz/-50dBm and with the maximum increase achieved at 900MHz/-30dBm.

On the other hand, we have observed the opposite effect with the microwave frequencies of 650MHz, 700MHz and 750MHz (radiation power of -30dBm, -40dBm and -50dBm), when the LDH activity was decreased by 2%-15%.

Hence, the results suggest that this specific biological process can be modulated by the defined frequencies of microwave radiation. The possibility of influencing on protein biological activity using microwave radiation would benefit the development of new biomaterials, non-invasive treatments and advanced technologies. This could have major implications in drug design, medicine, agriculture, pharmacology and biotechnology.

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TABLE I. GTEM exposure system calibration results

Freq (MHz)	P (dBm)	E (means)	P (mW)	E (10dBm)	d (m)
500	9.53	2.01	8.974288	2.121759	0.333264
600	9.48	2.24	8.871560	2.378198	0.297329
700	9.45	2.53	8.810489	2.695383	0.262340
750	9.46	2.73	8.830799	2.905110	0.243401
800	9.48	3.29	8.871560	3.492978	0.202437
9.46	9.46	4.21	8.830799	4.480042	0.157835

TABLE II. Frequency and power values of microwave radiation, and rate of change in absorbance

Frequency, MHz	Power, dBm	Rate of change in NADH absorbance Averaged values and (max-min)	Relative difference irrad. vs non-irrad. (%)
non-irradiated	0	**0.00911**	0%
500	-30	**0.0132 (0.0007)**	**45**
500	-40	**0.0133 (0.0010)**	**47**
500	-50	**0.0131 (0.0002)**	**43**
650	-30	0.0077 (0.0012)	-15
650	-40	0.0085 (0.0003)	-6
650	-50	0.0081 (0.0002)	-10
700	-30	0.0083 (0.0020)	-9
700	-40	0.0086 (0.0011)	-6
700	-50	0.0087 (0.0004)	-4
750	-30	0.0081 (0.0002)	-11
750	-40	0.0076 (0.0016)	-16
750	-50	0.0082 (0.0003)	-10
900	-30	**0.0157 (0.0027)**	**73**
900	-40	0.0089 (0.0011)	-2
900	-50	0.0089 (0.0002)	-3