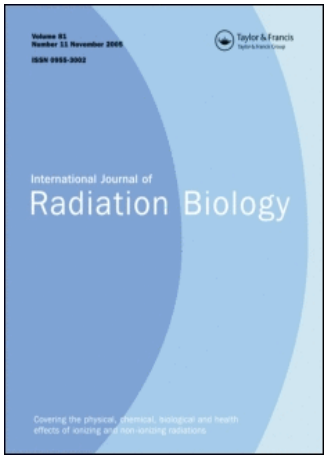

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The effect of electromagnetic radiation (550 - 850 nm) on l-Lactate dehydrogenase kinetics

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The effect of electromagnetic radiation (550 – 850 nm) on l-Lactate dehydrogenase kinetics

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Abstract

Purpose: This work is based on our earlier research of the Resonant Recognition Model (RRM), where we have proposed that protein activation is electromagnetic in its nature. In this study we investigated experimentally the possibility of modulating the protein activity by the electromagnetic radiation of the specific frequency. The concept is studied here by applying a visible light radiation to example of l-Lactate Dehydrogenase enzyme (LDH).

Materials and methods: The selected LDH example is radiated by monochromatic visible light in a frequency range predicted computationally by the RRM. The kinetics of the irradiated LDH is measured by continuous monitoring of the NADH absorption at 340 nm.

Results: A comparative analysis of the LDH enzyme activity before and after the electromagnetic field (EMF) exposures is performed. It was found that the LDH activity is selectively increased only by the radiation at the particular wavelengths of 595 nm and 828 nm. These experimentally determined wavelengths of the applied EMF are within the range predicted by the RRM.

Conclusions: Results reveal the LDH activity was modulated by the EMF exposures at the computationally predicted frequencies. The RRM concept presented provides new insights into proteins susceptibility to perturbation by electromagnetic radiation and possibility to program, predict, design and modify proteins and their bioactivity.

Keywords: *Electromagnetic radiation, RRM, enzyme activity*

Introduction

Experimental investigations of the interaction of electromagnetic field (EMF) and living systems remain intensively controversial. Some bioeffects observed are attributed to the extremely low frequency field (ELF) and others to the radiofrequency (RF) exposures (Lerner ed. 1984). It was reported that Low Frequency (LF) magnetic fields increase the activity of the membrane enzymes, Na,K-ATPase and cytochrome oxidase, and the increased activity varies with frequency. Optimal frequencies for increases in the reaction rate constant of cytochrome oxidase and in the rate of splitting of ATP by Na,K-ATPase differ by an order of magnitude, and are in the ranges of the turnover numbers of the respective enzyme reactions. The researchers summarized that the two frequency dependence curves are similar in that the slope of the low frequency portion is about 10 times greater than the slope of the high frequency portion. The greater slope indicates greater ability to adjust quickly in the low frequency range, which may be significant for optimal biological control of activity (Blank & Soo 2001).

In addition, the influence of an extremely low frequency (ELF) magnetic field (50 Hz and 1 mT, EMF) on the activity of a soluble and insoluble horseradish peroxidase (E.C. 1.11.17) has been studied as a function of time. Results have shown that the field affects the inactivation rate of the soluble enzyme, while no effects are observed with insoluble derivatives (Portaccio et al. 2003).

Several studies published have suggested the mechanisms underlying the process of interaction between the EMF and living systems. It was reported that EMF stimulated transcription appears to require specific DNA sequences, and these bases may be sites where EM fields generate large repulsive forces between chains by accelerating electrons that move within DNA (Blank & Goodman 2001). Diverse biophysical and biochemical studies have sought to

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understand electron transfer (ET) in DNA in part because of its importance to DNA damage and its repair. However, the dynamics and mechanisms of the elementary processes of ET in this medium are not fully understood and have been heavily debated. The DNA polymer appears to provide an efficient intervening medium to couple donor and acceptor metal complexes for electron transfer (Wan et al. 1999).

The ideas of the resonant absorption and resonant interactions have been proposed as an explanation for the marked sensitivity of living systems to EMF (Frohlich 1986). Each biological process involves a number of interactions between proteins and their targets. These interactions are based on the energy transfer between the interacting molecules. Protein interactions are highly selective, and this selectivity is defined within the protein structure. However, the physical nature of these interactions is not yet well understood.

There is much evidence that biological processes can be induced or modulated by induction of light of the particular characteristic frequencies (Blum 1959, Karu 1987, 1999, Ahmad & Cashmore 1993). This is caused directly by the light-induced changes of energy states of proteins. For instance, a visible light provides the energy required for the carbon fixation process in photosynthesis reactions. Light also excites the rhodopsin/bacteriorhodopsin molecules involved in the hyperpolarisation process of the cell membrane that can be used either in generating of the nerve impulse, ATP synthesis or light regulated embryogenesis (i.e., seed germination in plants). A number of different investigations (Fedoseyeva et al. 1988, Karu et al. 2004) focused on study of the effects of the visible light on cell proliferation and their metabolisms have been conducted up-to-date. There are various existing methodologies that incorporate a low-intensity light in their therapeutical procedures which are proven to be efficient and already widely used in modern medicine.

In recent years the studies of the effects of low-intensity non-thermal light irradiation (impulse and continuous regime) on eukaryotic and prokaryotic cells have been undertaken and results are published. The accelerated proliferation rate was observed in yeast and mammalian cells after irradiating them by the He-Ne laser (Fedoseyeva et al. 1988, Karu 1999) and in *E. coli* cultures affected by the Argon laser light. The increased proliferation rate was also shown in cultures of different bacteria irradiated by the laser light of 0.015 W/cm^2 and radiant exposures of 1 – 50 J/cm^2 (Nussbaum et al. 2002) at 630 nm and 810 nm wavelengths.

The stimulating effect of various light-emitting diode (LED) diodes and monochromator lights on eukaryotic cells was documented (Whelan et al. 2001, 2002). These effects were observed at the pulsed and continuous light exposures. Several studies reported the change in acetylcholinesterase activity of human erythrocytes after the low-intensity light radiation at 810 nm (Kujawa et al. 2003). There is also evidence that the light exposures caused the effects on the endocrine system by influencing on the function of the submandibular glands (Luis et al. 2003). It was suggested that Cytochrome c oxidase and certain dehydrogenases may play a key role in the photoreception process, particularly in the near infra-red (NIR) frequency range (Karu 1999).

All these frequency selective effects of light on biological processes of protein activation imply that protein activation involves energies of the same order and nature as the electromagnetic irradiation of light. Thus, the ability of light to stimulate or inhibit the proliferation rate of some bacteria and tissues lead to enormous possibilities of its further applications in the fields of bio- and nanotechnology.

To elucidate the possible mechanism of interaction between light radiation and proteins we have investigated *in vitro* the activity of the LDH enzyme, which plays a central role in metabolic pathways of almost every cell. Up-to-date the measurement of the protein bioactivity versus the light radiation frequency has not been well understood. Therefore, our study is directed on measurement of the possible changes in the LDH activity upon radiation by light in the wavelengths ranging from 560 nm up to 850 nm with the resolution of 1 nm, the light irradiance of 1.5 mW m^{-2} nm^{-1} at 560 nm and 5 mW m^{-2} nm^{-1} at 850 nm.

In our previous work (Cosic et al. 1989, Cosic 1994, 1995) a relationship between the RRM spectra of some protein groups and their interaction with visible light has been established. Within the RRM it was conceptualised that external EMF at the particular activation frequency would produce the resonant effects on protein biological activity (Cosic 1997).

Materials and methods

Resonant recognition model

It has been shown in our previous research that all protein sequences with the common biological function have common frequency component in the distribution of free energy of electrons along the protein backbone. This characteristic frequency is related to the protein biological function as it was found in our previous investigations (Cosic 1994, 1997). Furthermore, it was also shown that proteins and their targets have the same characteristic frequency in common. Thus, it can be postulated that RRM frequencies characterize not only a general

function but also a recognition/interaction between the particular protein and its target at the distance. Thus, protein interactions can be considered as the resonant energy transfer between the interacting molecules. This energy can be transferred through oscillations of a physical field, possibly electromagnetic in nature (Cosic et al. 1989, Cosic & Birch 1994, Cosic 1994, 1995, 1997).

Enzymes are proteins crucial in accelerating metabolic reactions in the living organism. Dehydrogenases are the enzymes that catalyse a variety of oxidation-reduction reactions within the cells. In this study we have performed the structure-function analysis of dehydrogenases using the Resonant Recognition Model (RRM), which is based on Digital Signal Processing (DSP) methods, Fourier and Wavelet Transform, applied to the proteins sequence (Cosic 1994, 1997, Pirogova et al. 2002). The application of the RRM in structure-function analysis of protein molecules involves several stages of calculation.

The application of the RRM involves two stages of calculation. The first is the transformation of the amino acid sequence into a numerical sequence. Each amino acid is represented by the value of the Electron-Ion Interaction Potential (EIIP) describing the average energy states of all valence electrons in a given amino acid. The EIIP values for each amino acid were calculated using the following general model of pseudo-potentials (Veljkovic & Slavic 1972, Veljkovic 1980):

$$\langle k+q|w|k\rangle = 0.25\frac{Z\sin(1.04^{*}\pi Z)}{2\pi}$$

where q is a change of momentum of the delocalized electron in the interaction with potential w, while:

$$Z = \frac{\sum_i Z}{N}$$

where Z_i is the number of valence electrons of the i-th component of each amino acid and N is the total number of atoms in the amino acid. A unique number can thus represent each amino acid or nucleotide, irrespective of its position in a sequence. Numerical series obtained this way are then analysed by digital signal analysis methods in order to extract information relevant to the biological function.

As the average distance between amino acid residues in a polypeptide chain is about 3.8 Å, it can be assumed that the points in the numerical sequence derived are equidistant. For further numerical analysis the distance between points in these numerical sequences is set at an arbitrary value $d = 1$. Then the maximum frequency in the spectrum is $f_{max} = 1/2d = 0.5$. The total number of points in the sequence influences the resolution of the spectrum only. Thus for N-point sequence the resolution in the spectrum is equal to 1/N. The n-th point in the spectral function corresponds to the frequency $f = n/N$. In order to extract common spectral characteristics of sequences having the same or similar biological function, the following cross-spectral function was used:

$$S_n = X_n Y_n^{*} \quad n = 1, 2, \ldots, N/2$$

where X_n are the DFT coefficients of the series x(m) and $Y_n^{\star}$ are complex conjugate discrete Fourier transform coefficients of the series y(m). Peak frequencies in the amplitude cross-spectral function define common frequency components of the two sequences analysed. To determine the common frequency components for a group of protein sequences, the absolute values of multiple cross-spectral function coefficients M have been calculated as follows:

$$|M_n| = |X1_n| \,.\, |X2_n| \ldots |XM_n| \quad n = 1, 2, \ldots, N/2$$

Peak frequencies in such a multiple cross-spectral function denote common frequency components for all sequences analysed. Signal-to-noise ratio (S/N) for each peak is defined as a measure of similarity between sequences analysed. S/N is calculated as the ratio between signal intensity at the particular peak frequency and the mean value over the whole spectrum.

As was mentioned above in the RRM protein interactions are considered as resonant energy transfer between the interacting molecules. This energy can be transferred through oscillations of a physical field possibly electromagnetic in nature. Since there is evidence that proteins have certain conducting or semi-conducting properties, a charge, moving through the protein backbone and passing different energy stages caused by different amino acid side groups, can produce sufficient conditions for a specific electromagnetic radiation or absorption. In our previous research we have shown that such charge transfer through protein backbone is possible through exciton process (Cosic 1997, Ciblis & Cosic 1997). The frequency range of this field depends on a charge velocity estimated to be 7.87×10^5m/s and on the distance between amino acids in a protein molecule, which is 3.8Å. Therefore, the maximum frequency due to the exciton transfer is estimated to be: $Fmax < V/(2d) < 1 \times 10^{15}$ Hz, ($Lmin > 330$ nm). The minimum frequency depends on the total length of the protein and is estimated for protein of about 200 amino acids to be about 10^{13} Hz. (30000 nm) (Cosic 1994, 1997). The range from 30000 – 300 nm is very wide, starting from the far infrared through the visible to the ultraviolet regions. For larger structures (e.g.,

longer proteins, DNA, protein clusters, membrane proteins and membrane channels) relevant bioactivity frequency range can be estimated to start in high microwave area in the range of 10^{10} Hz to 10^{11} Hz which is in accordance with the study published by other researchers (Frohlich 1986). The frequency range predicted for protein interactions is from 10^{13} Hz to 10^{15} Hz. This estimated range includes infra red (IR), visible and ultra violet (UV) light. The characteristic frequency identified for a particular protein group characterises one particular biological function/interaction. It was postulated that the protein function is directly related to the absorption of light of the defined wavelength.

Within the RRM it was found that a strong linear correlation exists between the predicted and experimentally determined frequencies corresponding to the absorption of electromagnetic radiation of such proteins (Cosic 1994, 1997). It is inferred that approximate wavelengths in real frequency space can be calculated from the RRM characteristic frequencies for each biologically related group of sequences. These calculations can be used to predict the wavelength of the light irradiation, which might affect the biological activity of proteins exposed (Cosic et al. 1989, Cosic & Birch 1994, Cosic 1994, 1997).

l-Lactate Dehydrogenase

Our experimental study consists of the series of experiments that can confirm a possibility that protein activity can be influenced by external light radiation as predicted within the RRM. L-lactate dehydrogenase (LDH) EC1.1.1.27 catalyses the inter-conversion of the l-lactate into pyruvate with the nicotin amide adenine dinucleotide (NAD+) acting as a coenzyme. There are several different forms of LDH found in the body having different kinetic properties. These enzyme forms are referred to as the isozymes. The 'M' form, of the LDH was found in the anaerobic tissue, while the 'H' form is found in the highly aerobic tissues, such as the heart. H form has an effective lower turnover rate for pyruvate, which assists to shunt the pyruvate into the mitochondria and hence into the Krebs cycle. 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 contradict to the NAD (Nicotinamide Adenine Dinucleotide Nicotinamide Adenine Dinucleotide, Oxidized form), which is inactive at this frequency. Due to the different

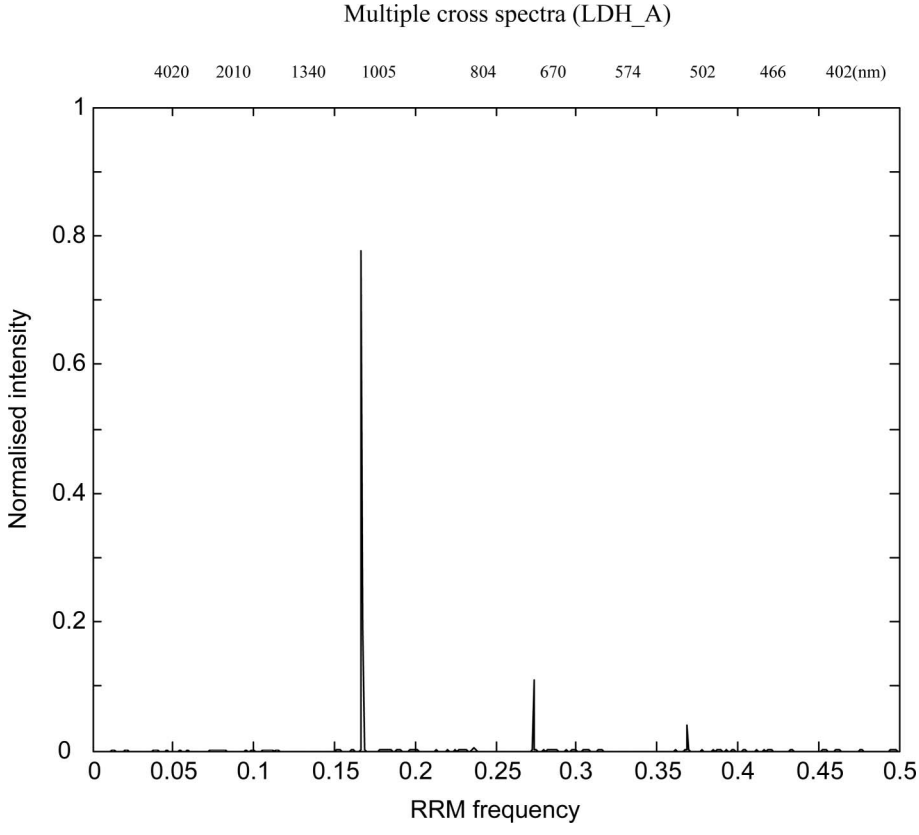

Figure 1. Multiple cross spectral function of LDH_A sequences. The x axis represents the relative RRM frequency and corresponding wavelength of the electromagnetic radiation. The y axis represents the normalized intensity of the multiple cross spectral function in the RRM spectra.

optical characteristics of the NADH and NAD we are able to optically asses if the reaction Pyruvate → Lactate in the presence of the LDH as an accelerator has occurred and then determine the amount of the reactants. The reaction rate depends on the concentration of the enzyme and substrate.

Equipment

(1) As a source of VIS/near IR we use monochromator SPEX 270M: (Princeton Instruments, Trenton, NJ, USA) 1200 g/mm grating, focal length 270 mm, resolution 0.1 nm at 500 nm, dispersion 3.1 nm/mm, lamp Olympus 68v5A TP1 (35W), range 400 – 890 nm, RS232 connection with HP 34001A, controlled by LabView 6.1 (National Instruments).

(2) Spectrometer USB2000 coupled with USB-ISS-UV/VIS, (Ocean Optics, Inc. Dunedin, FL, USA) range 190 – 870 nm, CCD detector with 2048 pixels, USB-2 connection with Pentium IV (Windows XP), controlled with OOIBase32 software. Software automatically monitors and saves the absorption coefficient at 340 nm every 30 sec.

Enzyme activity measurement

The activity of the LDH example is measured by determining the rate of substrate utilization during the enzyme-catalysed reaction. There are a relatively small number of reasons that can distract the observation of this reaction. The revers reaction rate is very slow in comparison to the direct reaction rate. The temperature has been controlled during the LDH irradiation as well as during the activity measurement procedures.

The assay contains the following components: 3.00 ml reaction mix includes 10 mM sodium phosphate, (Sigma, St Louis, MO, USA) 0.12 mM –NADH, disodium salt (ROCHE, Roche Diagnostics GmbH Nonnenwald 2 DE-82377 Penzberg, Germany) reduced form, 2.3 mM pyruvate, (Bio-Whittaker™ Cambrex Bio Science Walkersville, MD, USA) 0.033% (w/v) of bovine serum albumin (Sigma, St Louis, MO, USA) and 0.05 units of L-lactic dehydrogenase (Roche, Roche Diagnostics GmbH Indianapolis In, USA).

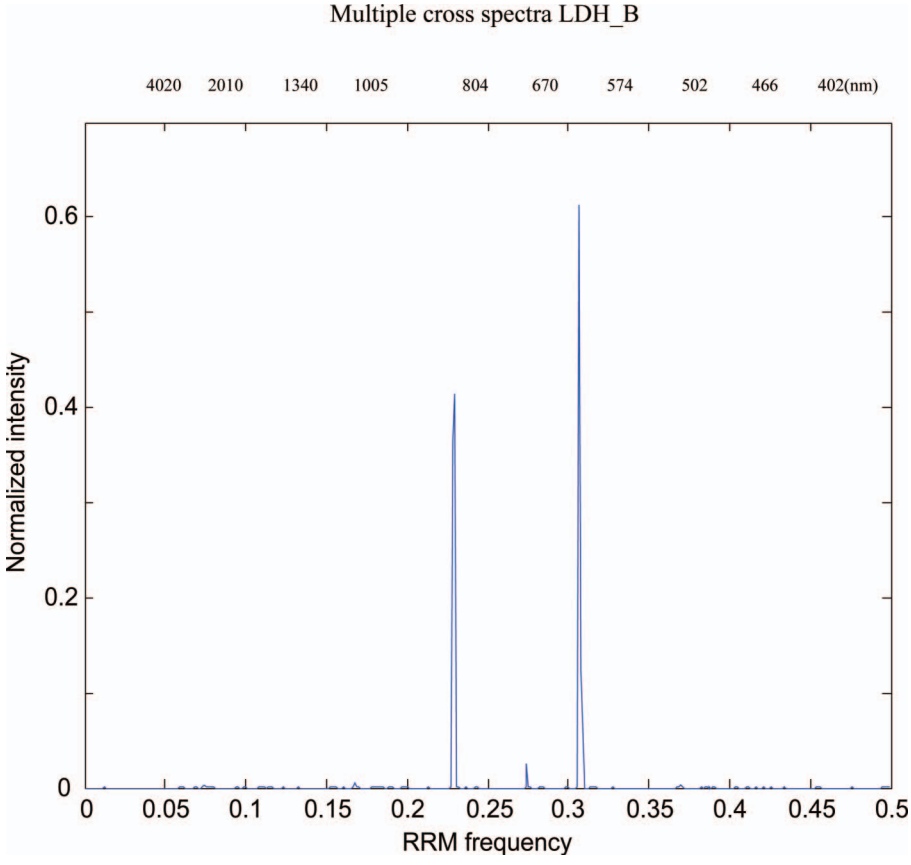

Figure 2. Multiple cross spectral function of LDH_B sequences. The x-axis represents the relative RRM frequency and corresponding wavelength of the electromagnetic radiation. The y axis represents the normalized intensity of the multiple cross spectral function in the RRM spectra.

The LDH kinetics is measured by the continuous monitoring of the NADH absorption at 340 nm. The protein concentration is determined by the extinction coefficient.

For each irradiated sample we have measured the absorption spectra for a 10 min by recording the solution's absorption values every 30 sec as follows:

$$A_\lambda = -\log_{10}\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right)$$

where S_λ is the sample intensity at wavelength λ defined as an intensity of incident light that passes the cuvette with the sample, D_λ is the dark intensity at wavelength λ, or intensity measured on the surface of the CCD detector (spectrometer) when the light source is switched off. R is the reference intensity at the wavelength λ.

The activity rate is determined by calculating the gradient of A_λ (t). For the time period of 0–10 min the increase of the absorbance is a linear process and can be calculated as a value of the gradient of the line representing the relation between the absorbance and time.

For each wavelength of the light radiation we have undertaken 3–5 control experiments to measure the activity of non-radiated protein solutions. In result, a non-significant increase in enzyme activity was detected. This effect can be attributed to the pH or heating artefacts, as well as the variations in the enzyme concentrations rather than to the light irradiation itself. With the aim at eliminating the effect of all possible artefacts we have repeated our measurements with the irradiated LDH example at the particular wavelengths of light that correspond to the resonant effects. Also we have randomized an order of measurements (596, 844, 724 etc.) to eliminate a possible modification of the enzyme or substrate during the experimental procedure.

Results and discussion

In our previous work (Pirogova et al. 2003) we analysed 72-dehydrogenase protein sequences using the RRM approach. A multiple cross-spectral analysis was performed resulting in one prominent frequency identified at f = 0.1680 with S/N = 511.1. The presence of only one prominent peak with the significant S/N ratio in a consensus spectrum implies that all of the analysed sequences within the group have this frequency component in common. This frequency is related to the biological activity as it was found in our previous investigations (Cosic 1997).

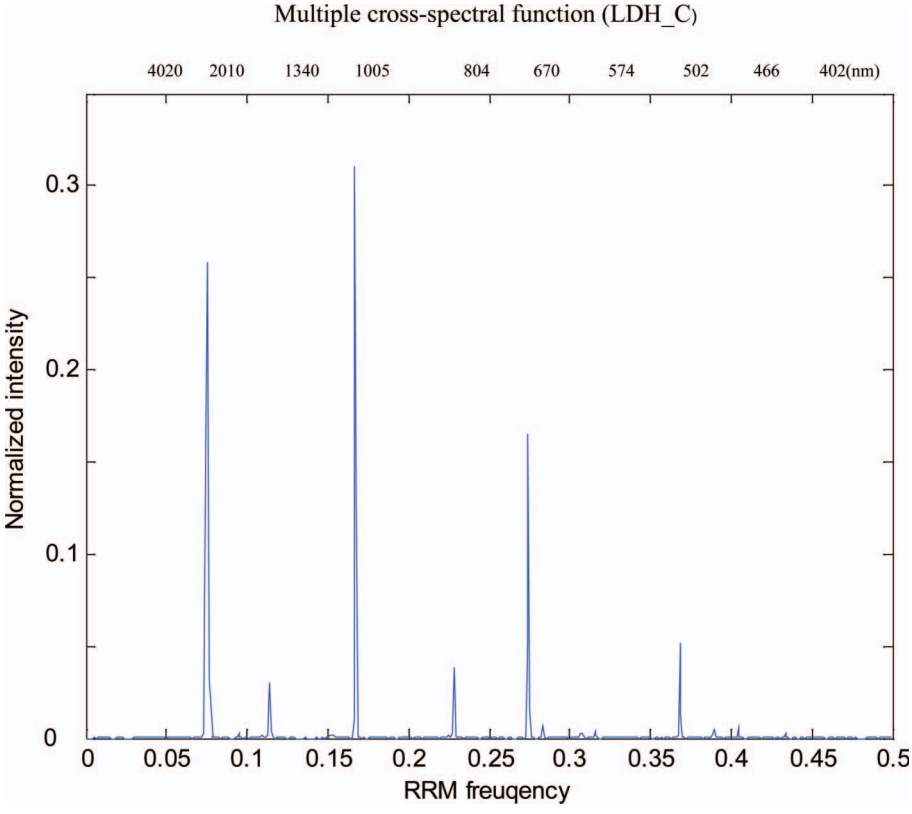

Figure 3. Multiple cross spectral function of LDH_C sequences. The x-axis represents the relative RRM frequency and corresponding wavelength of the electromagnetic radiation. The y axis represents the normalized intensity of the multiple cross spectral function in the RRM spectra.

As was mentioned above a linear correlation between the absorption spectra of proteins and their RRM spectra with a regression coefficient of $K = 201$ and predetermined frequency range was established (Cosic 1994, 1997). Thus, based on the characteristic frequency determined for the whole dehydrogenase functional group, we can calculate the wavelength of irradiation, which assumingly would activate protein sequences and modify their bioactivity:

$$K = 201/f_{RRM}$$

The dehydrogenases' characteristic frequency is at $f = 0.1680$, thus the wavelength of the electromagnetic exposure required for dehydrogenase enzymes activation will be at 1196 nm.

In this study, however, we cannot irradiate the selected enzyme sample by the EMF at the required wavelength due to the limitations of Monochromator Spex 270 (Instruments CA, Inc) with a range of 400 – 900 nm. To solve this problem we decided to look at the single spectrum of the studied LDH enzyme (1.1.1.27 rabbit muscle) that is shown in Figure 2. The RRM characteristic frequency of this enzyme is identified at $f_{RRM} = 0.3066$ that corresponds to $\lambda = 656$ nm. Therefore, to test the concept of the possible affect of the EMF on enzyme activity we have used the external radiation in a range of 550 – 900 nm.

In order to computationally predict the activation frequency/wavelength of light irradiation that could produce significant effects on LDH function, a database of 176-LDH protein sequences was established and analysed using the RRM approach. All protein sequences have been taken from SWISSPROT database. Accordingly to their different structural origins and functions the selected LDH sequences were divided onto 4 sub-groups: LDH_A, LDH_B, LDH_C and bacterial LDH. The cross spectral functions were calculated for each of these groups and are shown in Figures 1, 2, 3, 4. The characteristic frequencies of the LDH enzymes were identified at the following prominent frequencies: 0.1688, 0.332 ± 0.08 and 0.242 ± 0.08, which correspond to the light radiation with a wavelength of 1190 nm, 605 nm $\pm$ 10 nm and 830 nm $\pm$ 12 nm respectively. The RRM frequencies 0.332 ± 0.08 and 0.242 ± 0.08 are particularly prominent in the LDHB cross-spectral function. While for the LDH_A, LDH_C and bacterial LDH amplitude ratio of the peaks at the frequencies 0.332 ± 0.08 and 0.242 ± 0.08 are significantly smaller.

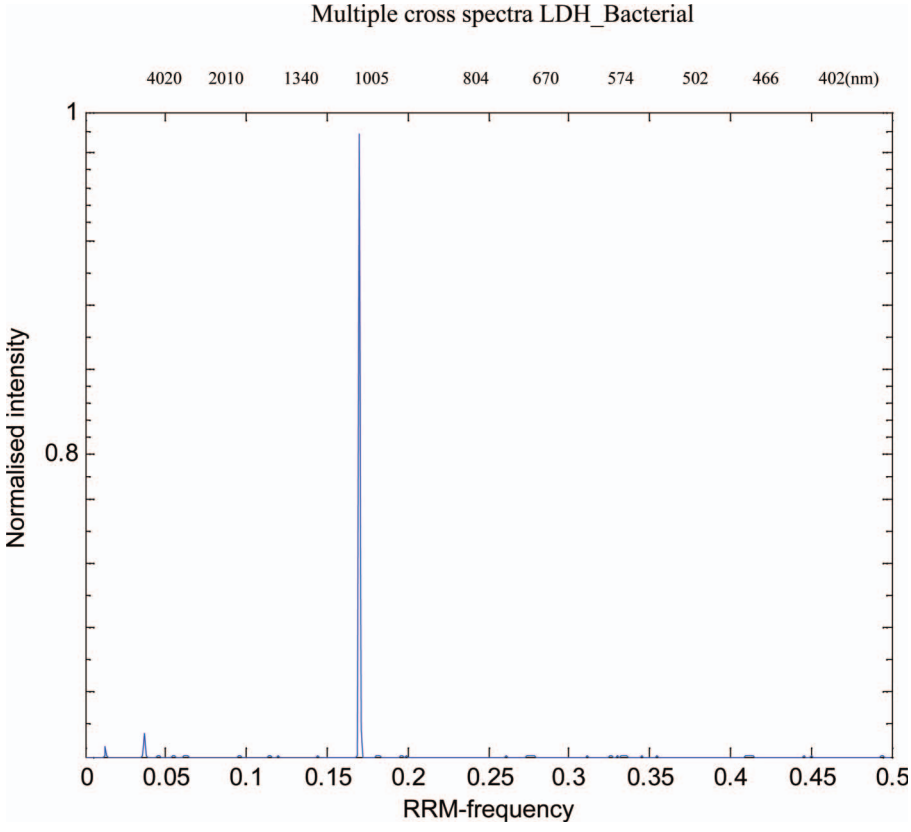

Figure 4. Multiple cross spectral function of LDH_BACTERIAL enzymes. The x-axis represents the RRM frequency and corresponding wavelength of the electromagnetic radiation. The y axis represents the normalized intensity of the multiple cross spectral function in the RRM spectra.

In the experimental part of our study the effect of light radiation of various wavelengths in a range of 550–900 nm on the LDH enzyme activity was examined. The data were collected and presented in Figure 5. The effects of light exposures on the l-LDH activity are measured as the rate of change of the NADH concentration per second. There is the evident increase in the l-LDH activity after irradiation by visible light at the particular wavelengths: 829 nm and 595 nm (Figure 5). The enzyme solutions were irradiated for 15 min. The enzyme activity measured immediately after irradiation at 829 nm is 0.025 ± 0.001 and at 595 nm is 0.025 ± 0.001 respectively. However, there is no significant difference in activity observed between LDH example, which was radiated by light of other wavelengths, and activity of the control non-radiated solutions. In comparison to the non-radiated LDH solutions that have average rate of 0.022 with a standard deviation of $\pm$ 0.0015, the results obtained demonstrate the increase of LDH activity in order of 11.9% ($p < 0.001$) at 596 nm and 12.67% ($p < 0.001$) at 829 nm respectively. To evaluate how significant is the difference between the mean values of the activity of irradiated and non-irradiated samples, we have used an independent two-sided *t*-test. This value is much higher then the value of the variability σ caused by the variations in concentrations of the LDH, NADH and pyruvate that were determined within the control experiment with the non-irradiated solutions. It is important to note, the increase of biological activity achieved upon radiation by light of the particular wavelengths at 596 nm and 829 nm has been already reported in literature. Study of the effect of burn healing in non-diabetic rats produced by LED light at 596 nm was published (Al-Watban & Andres 2003). Also light radiation at 829 nm is close to the 810 nm that was proved to be effective for bacteria's growth rate. In particular, the low-level laser at 830 nm has produced the effects in control of the painful stomatitis in patients with hand-foot (Toida et al. 2003).

Summarizing the results of our study, we have to point out that the computationally predicted activation frequencies of the LDH enzymes using the RRM approach closely correspond to our experimental data and findings of other researchers showing the maximum change in the LDH activity after irradiation at 596 nm and 829 nm. Hence, the results reveal that this specific biological process can be modulated by irradiation with the defined frequencies strongly supporting the main concept

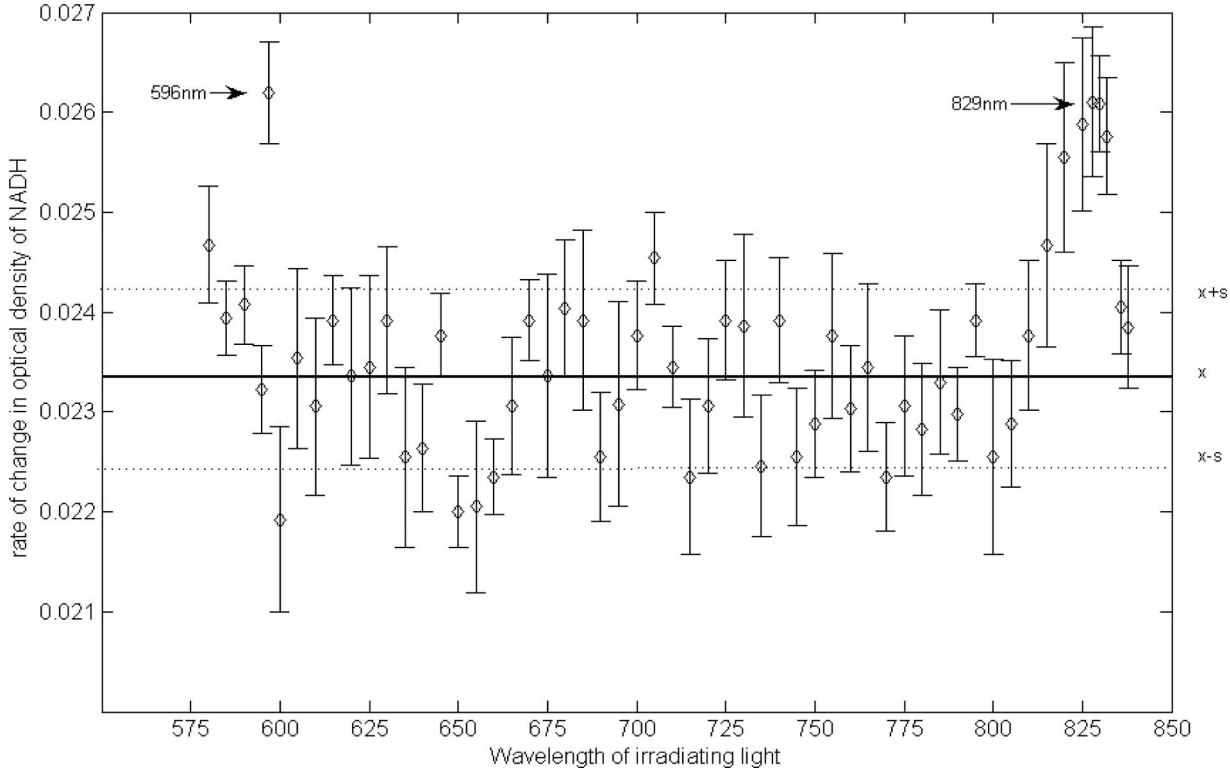

Figure 5. The activity of the LDH is measured after irradiation of the LDH with different wavelengths of light. The ordinate represents the rate of change in optical density of NADH per minute. By Beer-Lambert low the optical density is proportional to the concentration of absorbing molecule or NADH. Thus, the rate of change represents practically the rate of change of the NADH concentration per unit of time, or activity of the LDH. Horizontal bold line represents an average (x) for the non-iradiated samples. Horizontal dashed lines ($x+s$; $x-s$) values distant for one standard deviation(s) up and below from average value.

of the RRM methodology. Moreover, if we observe the increase of the LDH activity at 550–900 nm wavelength range, it is expected that much stronger effect in protein activation would be obtained if we could perform the experiment with the predicted by the RRM characteristic frequency $f = 0.1680$ ($\lambda =$ 1156 nm) that correspond to the common dehydrogenase activity. Such study would be the next step of our research of protein interactions with the EMF.

Conclusion

This study is an attempt to shed a new light on possible deeper physical grounds underlying the process of protein interactions. The results obtained reveal that the frequencies obtained for the LDH enzymes using the RRM approach can be directly related to the resonances in electron differential scattering cross section of these macromolecules.

To elucidate the possible mechanism of influence of the EMF on proteins we have conducted a series of *in vitro* experiments where l-Lactate dehydrogenase was irradiated by visible light of different wavelengths ranging from 550–900 nm. The results obtained have shown the sensitivity of the reaction rate depends on the frequency of the visible light, albeit a low-intensity visible light has energy incomparable to the characteristics energies of electron excitations. Our experimental results are in a close agreement with the characteristic wavelengths corresponding to the activation of LDH enzyme predicted computationally within the RRM. Based on similarity between the RRM spectra calculated and the LDH activity spectra obtained experimentally we conclude that the distribution of the electron-ion potentials along the whole peptide chain could have a crucial impact on the interactive process between visible light and enzymes. Our further experimental studies with other enzyme examples are necessary to valuate this phenomenon. The presented methodology may allow the generalization of the main advantage of the RRM in the case when the space structure of macromolecules is taken into account in a more realistic way. Based on the RRM spectral characteristic we can calculate the wavelength of the electromagnetic energy that can be used to modulate protein activity, hence giving rise to an innovative efficient methodology to program, predict, design and modify proteins and their bioactivity.

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