medicinska revija
medical review

UDK : 543.645.4

Pirogova E. et al. Med Data Rev 2010;2(4): 317-324 *MEDICAL DATA / Vol. 2. No 4 / December 2010.*

Opšti pregled/
Opšti pregled

REVIEW STUDY: INFLUENCE OF ELECTROMAGNETIC RADIATION ON ENZYME ACTIVITY AND EFFECTS OF SYNTHETIC PEPTIDES ON CELL TRANSFORMATION

REVIJALNA STUDIJA: UTICAJ ELEKTROMAGNETNOG ZRAČENJA NA AKTIVNOST ENZIMA A EFEKTI SINTETIČKIH PEPTIDA NA ĆELIJSKU TRANSFORMACIJU

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Ključne reči
Elektromagnetna radijacija; Proteini; Karakteristične Frekvencije; Peptidi; Kancerogene ćelije

Key words
Electromagnetic radiation; Proteins; Characteristic Frequency; Peptide; Cancer cells.

Abstract

With a large number of DNA and protein sequences already known, the crucial question is to find out how the biological function of these bio-molecules is "written" in the sequence of nucleotides or amino acids. Biological processes in any living organism are based on selective interactions between particular bio-molecules, mostly proteins. However, the rules governing the coding of the protein's biological function, i.e. its ability to selectively interact with other molecules, are still not elucidated.

With the rapid accumulation of databases for protein primary structures, there is an urgent need for theoretical approaches that are capable of analysing protein structure-function relationships. The Resonant Recognition Model (RRM) (1) is one attempt to identify the selectivity of protein interactions within an amino acid sequence. The RRM approach (2,3) proposes that protein interactions are electromagnetic in nature and their selectivity is based on resonant energy transfer between the interacting molecules that occurs at the same frequency. Once this frequency is identified, it is possible to predict the functionally important "key" amino acids in the protein sequence as well as to design de novo proteins/peptides with desired biological functions.

In this paper we present the RRM basic concepts and discuss its biological applications both theoretically and experimentally. A number of test examples are presented: protein (enzyme) activation using external electromagnetic radiation (EMR) of computationally defined wavelengths, and experimental evaluation of biological activity of de novo peptides designed using the RRM approach.

INTRODUCTION

It is known that biological processes in living organisms are based on selective interactions between bio-molecules. These interactions are very specific. However, it is still an unsolved puzzle as to where and how this specificity is encrypted within a protein's primary structure. The currently accepted explanation behind protein interactions' specificity is that this information is written in the protein's 3-D structure and is based on the "key-and-lock" fit between the 3-D conformation of a given protein and its interactive target. Interestingly, this fit in most cases is very "loose", and it is difficult to believe that this is the sole important parameter required for extremely selective and specific recognition between different interacting bio-molecules.

The design of new proteins, protein synthesis and DNA cloning are very well developed methodologies. Nowadays, practically any macromolecule can be synthesised and then implemented within the studied biological system. However,

our knowledge and understanding of the biological activity of these newly produced macromolecules is very limited. The current state of protein design involves the use of existing proteins, analysis of proteins' homology and design of a protein with a high degree of similarity in its amino acid sequence to a parent protein, with only a few changes (substitutions) in its primary structure. These changes are made randomly and thus, a large number of mutated proteins/peptides are then required to be extensively tested for validating their biological activity. This is a long, laborious and expensive process. As such a process usually does not involve the fundamental knowledge of how a protein's activity is "written" in the amino acid sequence; there is a danger of producing new macromolecules with unknown activities.

Here we propose an alternative physico-mathematical approach to understanding a protein's biological function from analysis of its primary structure. The approach is based on the assumption that protein interactions involve resonant recognition between interacting molecules and thus, the model is named the Resonant Recognition Model (RRM). The main foundation of the RRM is that there is a significant correlation between spectra of the free electron energy distribution along an amino acid sequence and protein biological activity. It was found that proteins with the same biological function or interactive activity have the same periodic components (same frequency) in the distribution of delocalised electron energies along the protein molecule. This frequency was found then to be a characteristic feature for a particular protein biological function or interaction [1-3].

To prove this idea we undertook research in two different directions:

- Elucidate theoretically a possible physical basis behind protein selective interactions.
- Use the RRM approach for biochemical applications by predicting protein functions and functional mutations as well as designing new proteins/peptides with desired biological activities.

Physical Basis

As it was found that proteins with the same biological function have a common frequency in the spectrum of free electron energies distribution, it suggests that their function is characterised by this frequency which can be resonant in nature. There is evidence that biological processes can be induced or modulated by light of particular frequencies. For instance, light-activated excitation of the rhodopsin/bacteriorhodopsin molecules involved in the hyperpolarisation process of the cell membrane can either generate nerve impulses or ATP synthesis [4-6]. It has also been 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. A number of different investigations [7-11] have explored the effects of visible light on cell proliferation and metabolism. Studies of effects of low-intensity non-thermal light irradiation on eukaryotic and prokaryotic cells have reported positive results of increased proliferation rate in yeast and mammalian cells after irradiating with a He-Ne laser and increased E. coli proliferation during Ar laser light exposures [4,10]. An increased proliferation rate has also been observed in various bacterial cultures irradiated by laser light with exposures at wavelengths of 630 nm and 810 nm [7-11]. The stimulating effect of various light-emitting diodes (LEDs) and monochromatic light on eukaryotic cells have been documented with pulsed and continuous light exposures. Several studies reported a change in the acetylcholinesterase activity of human erythrocytes after low-intensity light radiation at 810 nm. All these frequency selective effects of light on biological processes imply that protein activation involves energies of the same order and nature as the electromagnetic irradiation of light [7-11].

According to the RRM concepts, protein interactions present resonant energy transfer between interacting bio-molecules, and this energy is in the frequency range of 10 13 to 10 15 Hz, which incorporates infra-red (IR), visible and a small portion of the ultra-violet (UV) radiation (1-3). The mathematical and physical basis of this assumption is discussed here in the Methodology section. To test this postulate we have conducted a number of experiments where we studied the changes in protein activity using EMR within the range of certain frequencies. The results revealed that it is possible to modulate protein activity by irradiating the studied protein example with the EMR of particular frequency predicted by the RRM. These experiments are presented and discussed in details below [12,13].

Biochemical applications – prediction of functionally relevant amino acids

Once the characteristic frequency for a particular protein function/interaction is identified, it is possible then to predict the amino acids and segments in the protein sequences, which predominantly contribute to this specific frequency. We have examined a number of proteins and the findings showed that the computationally predicted amino acids, mostly contributed to the RRM characteristic frequency, are indeed relevant (critical) for the protein biological function. For example, in globular proteins with heme pocket (Hemoglobins, Myoglobins, Cytochrome C etc.) the predicted "key" amino acids are found to be not linearly linked, however in its 3-D protein conformation these amino acids are close together and are positioned around the heme pocket (14). In the case of proto-oncogene p21, using the RRM we determined that the amino acid that mostly contributed to the RRM characteristic frequency is Cyt 17. This is exactly the amino acid, experimentally found to be critical for the transformation of proto-oncogene p21 into the oncogene [15]. The RRM approach can also be used in analysis of protein-DNA interaction. For example, we investigated interactions of trp- repressors and found that amino acids, predicted by the RRM and mostly contributing to the RRM characteristic frequency, are actually positioned in the 3-D structure exactly around the DNA binding surface [16].

Biochemical applications – de novo protein/peptide design with desired function

If the RRM characteristic frequency is really one of the main parameters characterising the protein biological function, than the most exciting consequence is a possibility to use this frequency for design of *de novo* proteins with the desired biological functions. In our previous studies we have designed *de novo* FGF peptidic antagonists [2,17]. The aim of the study was to design the peptides which can bind to the FGF receptor without inducing cell growth. This apparently became possible as we identified two different frequencies characterising the receptor recognition process and growth activity. The designed peptide was tested and it indeed revealed the predicted activity [17].

In other experiment, the task was to design the HIV envelope agonists (2,18), which will have potential use in vaccine development. The challenge of that study was that HIV envelope proteins are increasingly variable and thus, it is difficult to design any general target for vaccine. However, using the RRM approach we identified a common characteristic frequency for all viral strains. Using this finding, a number of peptides for positive and negative controls were designed and tested in the

rabbit models aiming to produce anti-sera. Interestingly, only anti-sera for peptides with the exact RRM characteristics reacted with the virus. Additionally, it was found that the reaction of anti-sera of other proteins was directly related to the frequency they were designed for [18].

Here, we present and discuss another example of the *de novo* peptides designed for IL-12 (based on the murine IL-12 protein), TNF-2 (based on the murine tumour necrosis factor-2 protein) and MV-T5 (based on the NM-T5 protein). These three peptides were designed to express the anti-tumor activity, which has been tested experimentally and presented below [19].

MATERIALS AND METHODS

Computational analysis

Resonant Recognition Model (RRM)

Protein primary structures are linear sequences of their constitutive elements, i.e. amino acids. The RRM is designed for analysis of protein and DNA interactions and their interaction with EMR [1-3]. The RRM model interprets this linear information using digital signal analysis methods that include a spectral and space-frequency analyses. It has been found that the spectrum of the distribution of the energies of free electrons along the protein molecule is critical for protein function (interaction) [1-3].

The ability to predict the functions and 3-D shapes of biological molecules would certainly be useful in designing therapeutic drugs. The structure of the drug molecule that can specifically interact with a particular bio-molecule could be modelled using computational tools. These tools allow drug molecules to be constructed using knowledge of its structure and the nature of its active site.

It was postulated that the RRM characteristic frequency is a relevant parameter for mutual recognition between bio-molecules and is significant in describing the interaction between proteins and their substrates or targets. Therefore, it has been concluded that the RRM characteristic frequency may dictate the specificity of the protein interactions [1-3].

In order to design biologically active peptides it is of primary importance to determine which amino acids are responsible for the biological activity of the native protein. In the RRM a protein primary structure is presented as a numerical series by assigning to each amino acid a physical parameter value relevant to the protein's biological activity. Once the characteristic frequency for a particular protein function/interaction is identified, it is possible then to utilize the RRM approach to predict the amino acids in the protein sequence, which predominantly contribute to this frequency and thus, to the observed function, as well as to designed de novo peptides having the desired periodicities [1-3,20-22].

The application of the RRM approach 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 its Electron-Ion Interaction Potential (EIIP) value which describes the average energy states of all valence electrons in a given amino acid [23,24]. A unique number can thus represent each amino acid or nucleotide, irrespective of its position in a sequence [25]. Then the numerical series obtained are analysed by digital signal analysis methods, Fourier and Wavelet transform, in order to extract information pertinent to the biological function. A multiple cross-spectral function is defined and calculated to obtain the common frequency components from the spectra of a group of proteins. Peak frequencies in such a multiple cross-spectral function denote common frequency (feature) components for all sequences analysed.

In our previous work [1-3,26] a relationship between the RRM spectra of some protein groups and their interaction with visible light was established. It has been shown that all protein sequences with a common biological function have a common frequency component in the free energy distribution of electrons along the protein backbone. This characteristic frequency was shown to be related to protein biological function [1-3]. Furthermore, it was also shown that proteins and their targets share a characteristic frequency. Thus, it can be further postulated that RRM frequencies characterise not only a general function but also a recognition/interaction between the particular proteins and their target at a distance. 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 the protein backbone is possible through an exciton process [2,27].

It has been found that the strong linear correlation exists between the predicted and experimentally determined frequencies corresponding to the absorption of electromagnetic radiation of such proteins [1,2]. It is inferred that approximate wavelengths in electromagnetic frequencies 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 exposed proteins (Table 1). The frequency range predicted for protein interactions is from 1013 Hz to 1015 Hz. This estimated range includes IR, visible and UV light. These computational predictions were confirmed by comparison of:

a) Absorption characteristics of light absorbing proteins and their characteristic RRM frequencies [1,2]

b) Frequency selective light effects on cell growth and characteristic RRM frequencies of growth factors [1-3,26].

c) Activation of enzymes by laser radiation [4,10,28].

protein	nm	rrm	cm-1	K
cyt c	415	0.473	24096.39	196
blue	430	0.475	23255.81	204
green	540	0.355	18518.52	191
red	570	0.346	17543.86	197
hem.	14770	0.02	677.0481	295
purple	860	0.281	11627.91	241
flavodoxin	470	0.379	21276.6	178
igf	400	0.492	25000	196
fgf	441.6	0.453	22644.93	200
insulin	552	0.344	18115.94	189
growth f.	633	0.293	15797.79	185
	650	0.293	15384.62	190
pdgf	830	0.242	12048.19	200
chymotr.	851	0.236	11750.88	200
calculative	400	0.5	25000	200

Table 1*. The RRM frequencies and characteristic absorption frequencies of different visible light-absorbing protein groups and their scaling factor, K(1,2)*

All these results indicate that the specificity of protein interaction is based on a resonant electromagnetic energy transfer at the frequency specific for each interaction observed. A linear correlation between the absorption characteristics of proteins and their RRM spectra with a regression coefficient of K=201 has been established (Figure 1). Hence, the calculated RRM characteristic frequencies can be converted into the wavelengths of external EMR using the relationship (2):

$$\lambda = 201/f_{RRM}$$

Here we have utilized this relationship to calculate the frequencies/wavelengths that might modulate the bioactivity of the selected enzymes and evaluated experimentally their biological activity.

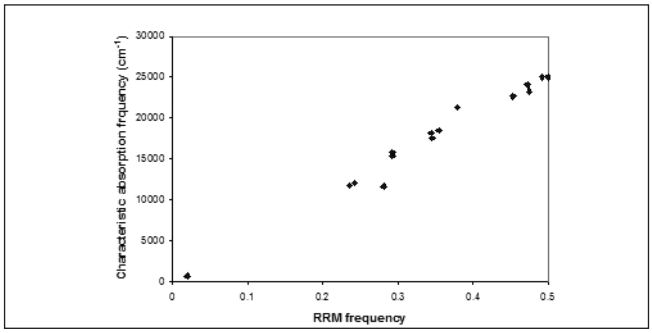

Figure 1. *Linear correlation between RRM frequencies and corresponding absorption frequencies of different visible light-absorbing protein groups*

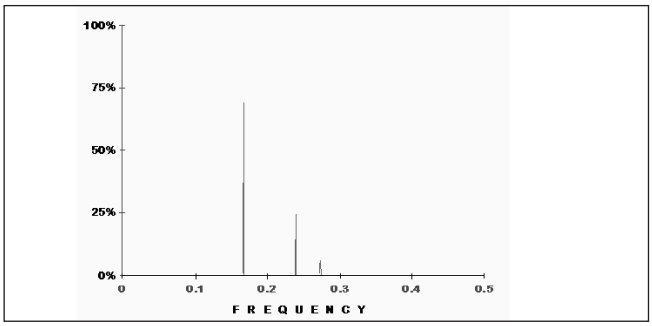

Figure 2*. Multiple cross-spectral function of the Dehydrogenase proteins (32 sequences) (1). The prominent peak(s) denote common frequency components. The abscissa represents the RRM frequencies, and the ordinate is the normalized intensity.*

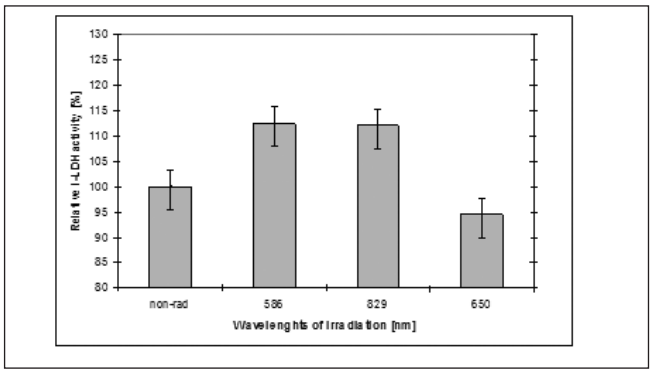

Figure 3. *Summary of the effects of EMR on LDH activity Š1Ć. A bar graph +/- SD for no irradiation, irradiation at 586 nm, 829 nm and 650 nm shows that EMR of visible and IR light does modulate LDH activity. No effect was observed for irradiation between 575 and 580 nm; 600 and 645 nm; 665 and 810 nm. Y axis corresponds to žRelative LDH activity' with 100% corresponding to the activity in the absence of irradiation.*

Experimental part

Irradiation of L-Lactate Dehydrogenase with the EMR of computationally defined wavelength

Various existing methodologies that incorporate low-intensity light into therapeutical procedures have now been integrated into modern medicine. Enzymes are proteins crucial in accelerating metabolic reactions in the living organism. Dehydrogenase enzymes catalyse a variety of oxidation-reduction reactions within cells. As a protein example we studied L-Lactate Dehydrogenase (rabbit muscle). This enzyme was selected on the basis of simplicity of its assay and possibility of measuring its bioactivity using the standard well accepted procedure, i.e. Continuous Spectrophotometric Rate Determination. LDH rabbit muscle EC1.1.1.27 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 optically active and 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 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.

As a source of IR and visible light we used SpectraPro 2300i monochromator (Acton Research Corporation) with a wavelength range of 400-1200 nm, grating 600 g/mm and a resolution of 0.1 nm. For measurement of absorbance of the analyzed enzyme solutions we used Ocean Optics USB2000 spectrometer which can detect in the 190-870 nm range.

To elucidate the possible mechanism of interaction between light radiation and proteins, we investigated the effects of EMR (400nm-900nm) on the enzymatic activity of L-Lactate Dehydrogenase (LDH). Our experimental studies were focused on measuring changes in absorbance and enzymatic activities due to EMR exposures. In this study we used the RRM (1,2) to predict theoretically the EMR frequency for activation of the LDH enzyme (Figure 2). A direct relationship between the RRM spectra of some protein groups and their interaction with visible light has been established in our previous work by showing that external EMR, at particular frequencies, can produce resonant effects on the biological activity of specific proteins (12,13).

The experimental procedure is presented below:

1. The experiments are performed at room temperature 27°C (Temperature controller Quantum Northwest).

2. The cuvettes were filled with 0.3ml of the LDH samples. The samples were irradiated using Monochromator SpectraPro 2150i (ACTON RESEARCH CORPORATION) set at the activation wavelength identified computationally using the RRM approach, at 1140-1200 nm and 550-850 nm respectively for 10 min.

3. The irradiated LDH samples were added to the already prepared solution of NADH and Pyruvate (standard enzyme assay, Sigma Alrdich).

4. The optical density of NADH is measured at 340 nm for each wavelength of irradiating light.

5. The values of rate of change in absorbance of NADH and changes of absorption coefficient values (at 340 nm) in time are collected and presented graphically (Figure 3).

Design of de novo peptides and their experimental testing

Computational peptide design

There are enormous scientific efforts and funding directed to understanding the cause of cell oncogenic transformation and thus, tumour development. However, so far there is no explicit answer, despite all the advances in basic and applied cancer research. The approach proposed here is intended to investigate cell transformation using the computationally designed bioactive peptides. The synthetic short peptide sequences were designed using the RRM (1,2). These three peptides are as follows:

(a) IL-12 (based on the murine IL-12 protein Original sequence interleukin 12 Mus musculus NP_032377, 215 amino acids). The designed analogue is 18 amino acids in length, and MW 2.2 kDa (19)

(b) TNF-2 (based on the murine tumour necrosis factor-2 protein TNF2 AAA40459, 235 amino acids a tumor necrosis factor house mouse). The designed analogue is 20 amino acids in length, and MW 2.5 kDa)

(c) MV-T5 (based on the NM-T5 protein AAC55050 483 amino acids from Myxoma virus). The designed analogue is 18 amino acids in length, and MW 2.3 kDa.

(d) A negative control peptide (C1) - with no active frequencies - was also designed using the RRM. The designed sequence CVLQDCVLQDCVIQDCVLQDCV is 22 aa; MW 2.45 kDa). The short peptides were commercially synthesized by Auspep Pty Ltd, Australia

Cell cultures and growth conditions

The mouse skin melanoma cell line (B16-F0) used to assess the toxicity of the RRM peptides was donated by Dr. Glen Boyle (QIMR, Australia). The cell line was grown and maintained in Dulbecco's modified Eagle medium (DMEM; Invitrogen, USA) supplemented with 10% heat-inactivated foetal bovine serum (FBS; Bovogen Biologicals, Australia) at 37°C in a humidified 10% CO_2 incubator. The cell line was regularly screened for *Mycoplasma* contamination using Mycoflor flourscent kit (invitrogen). Cells were seeded (3×10^5 cells per well) and grown to 90-95% confluency in 24 well plates before treatment. Controls include cells that were not exposed to any of the peptides as well as cells treated with chicken ovalbumin (Sigma-Aldrich, USA) and cells treated with the C1 control peptide.

Treatment with peptide analogues

For the cellular toxicity assessment fresh stocks of the peptide analogues were prepared. Each analogue was dissolved in DMEM and then added to the 95% confluent cell culture in final concentrations of (100 ng/ml; 200ng/ml; 400ng/ml; and 800ng/ml). Similar concentrations of the inert protein ovalbumin were used as negative controls for this assay, in addition to a non treated cell culture (blank). The cell cultures were similarly further incubated in for 3h, 6h and 16h before they were checked for the presence of any cellular changes, cell death and detachment. Morphological changes in cell culture growth were compared with both the negative and blank controls using microscopic examination. All samples were tested in duplicates and each test was repeated at least three times (before and after washing following peptide treatment.

Microscopy

Microscopic examination of cell cultures was carried out before and after treatment to detect visible changes in the confluent growth. Cell cultures were viewed with Olympus IX51 inverted microscope (Olympus, USA) equipped with light illumination, using 10×, 20× and 40× objectives. Each treatment was observed for cellular death, detachment and morphological changes. Cell images were then acquired with an Olympus CCD digital camera. Confocal imaging of cell cultures was carried out with Nikon Eclipse Ti-E A1 laser-scanning confocal system (Nikon Instruments Inc, USA), using the 10×, 20× and 40× objectives. Cell images captured were analysed with the NIS-Element imaging software.

RESULTS

Protein activation by external electromagnetic radiation

We applied the RRM model for the analysis of 32 LDH protein sequences. A multiple cross-spectral analysis was performed that resulted in two characteristic frequencies identified to be at f_1=0.1688±0.004 and f_2=0.2392±0.004 (Figure 2). These frequencies correspond to EMR wavelengths of 1191 ± 15 nm and 846 ± 15 nm, respectively.

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 3. The effects of light exposures on the LDH activity are measured as the rate of change of the NADH concentration per second. There is the evident increase in the LDH activity after irradiation by visible light at the particular wavelengths: 829 nm and 595 nm (Figure 3). 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 among LDH samples, which were 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 +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. The results of the t-test showed that the difference between radiated vs. non-radiated samples is significant ($p < 0.001$). It is important to note that computationally predicted activation frequency of **846±15** nm for the LDH enzyme is the same (within the calculation/measurement error) as the second experimentally defined frequency of 829 nm. The results clearly show that LDH activity could be modulated by external EMR. The key results from that study are shown in Figure 3, from which one can see *that the experimentally observed maxima induced by EMR coincide with the wavelengths/frequencies of the maxima predicted from the RRM.* These findings are very exciting because they raise the possibility of developing a novel methodology for altering the activity of selective biological processes.

The same experimental procedure was used to irradiate the LDH sample with the IR light in a range of 1140-1200

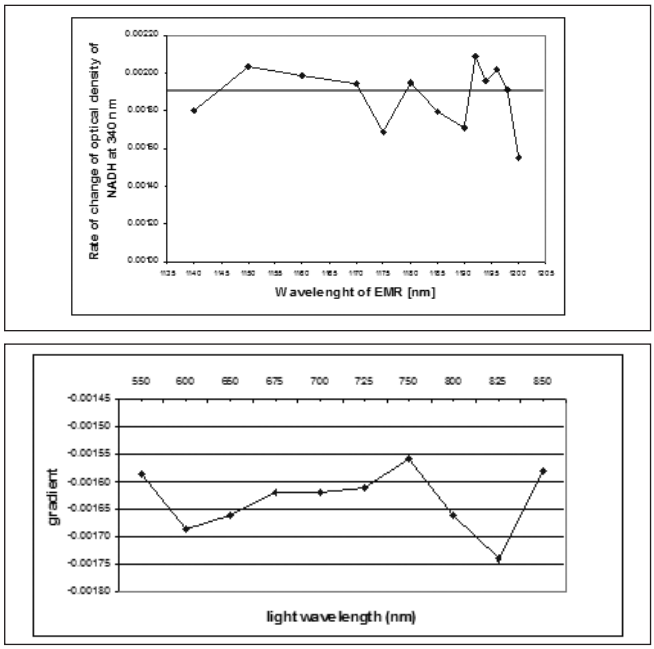

Figure. 4 *Gradient of change in absorbance of NADH upon LDH irradiation with a) IR and b) visible light*

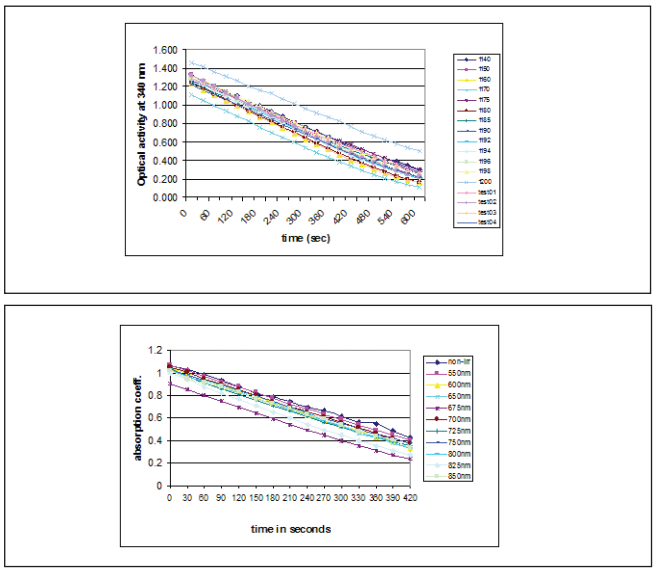

Figure. 5*. Changes of absorption coefficient values (at 340 nm) in time upon LDH irradiation with with a) IR and b) visible light*

nm. The results obtained have clearly shown the change of absorbance of NADH samples under the influence of irradiated LDH. From Figures 4, 5 we can observe that maximum optical density of NADH is achieved at the wavelengths 1192 and 1200 nm as was predicted by the RRM. Hence, the fact that LDH activity can be modulated by EMR at the specific frequencies predicted by the RRM, can strongly support the main RRM concept that protein activity is based on resonant energy transfer between the bio-molecules at the specific frequencies.

Biological examination of the designed IL-12, TNF-2 and MV-T5 bioactive peptides

Visible effects including morphological changes in cell shape, and cell culture detachment were initially evaluated by inverted light microscopy. The peptide analogues (IL12; TNF2; and MV-T5) were toxic to the mouse melanoma cells as variable concentrations of the peptide analogues affected the growth of the B16F0 cancer cell line causing apoptosis

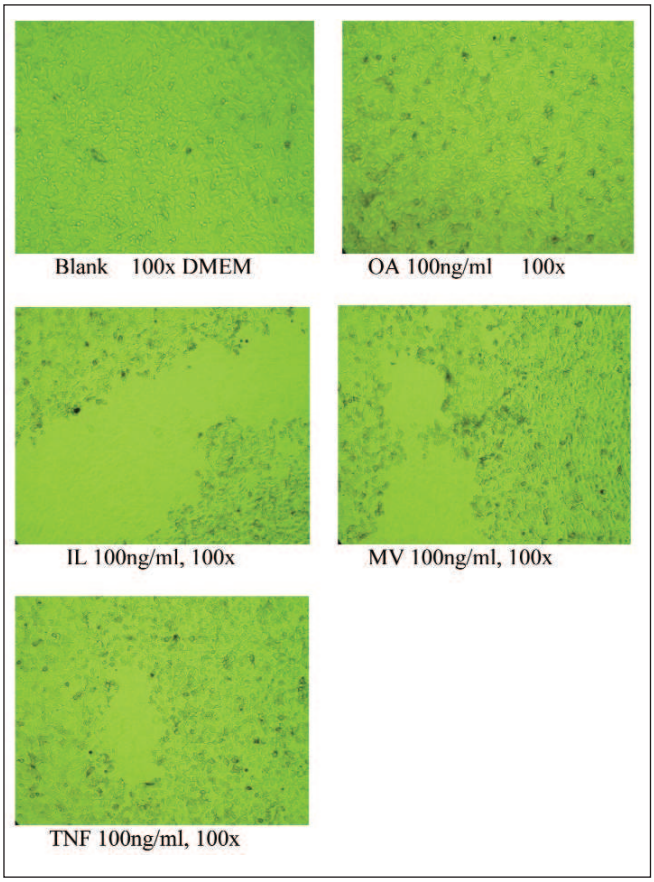

Figure 6. *Phase contrast micrographs showing cellular detachment of the melanoma B16 F0 confluent growth after 16 hours treatment with RRM peptide analogues*

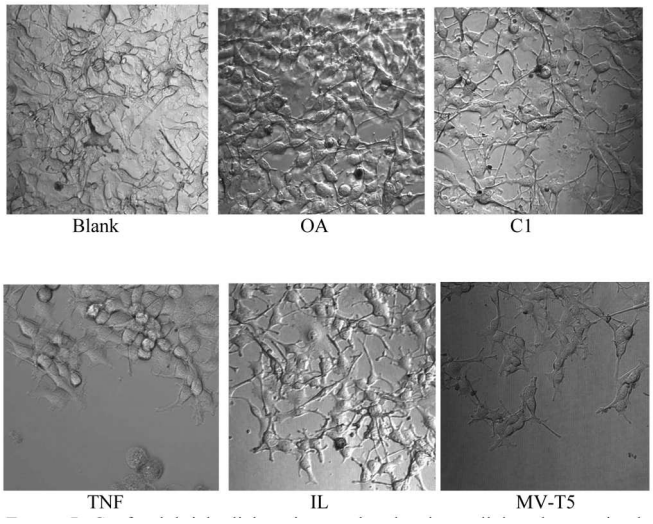

Figure 7. Confocal bright light micrographs showing cellular changes in the B16 melanoma cell line after 3 hours treatment with RRM peptide analogues (800ng/ml, 400X)

and cell detachment of the confluent layer (Figure 6). This toxic effect was dose dependent as morphological changes (including cell death and detachment) caused by peptide concentration of 400ng/ml and 800ng/ml were visible within 3h of incubation (Figure 7), while lower concentrations needed longer incubation periods (up to 16 hours) to achieve visible toxic effects on the cancer cells. Ovalbumin did not produce any visible toxic effect on the cancer cells, even at the highest concentrations used for the length of 16 hours incubation, when compared with similar concentrations/ incubation times used for the peptide analogues. Similarly

there was no change observed in the growth mode and cellular morphology of cell cultures incubated in DMEM only (blank) for up to 16h, as shown in the phase contrast and the bright light confocal microphotographs (Figures 6 & 7 respectively). These initial results indicate that the RRM short peptide analogues, which were designed from full protein sequences of known prospective tumour therapeutic peptides, have toxic effects on cancer cells and leading to cellular degeneration. This toxic effect will be further investigated and evaluated on other cancer cell lines in addition to normal, non-cancerous cells.

CONCLUSION

These reported studies present an attempt to shed a new light on possible deeper physical grounds which lead to understanding of protein interactions. The results obtained reveal that the frequencies obtained for Dehydrogenase enzymes f_1=0.1688±0.004 and less prominent at f_2=0.2392±0.004 (corresponding to 1191+15 nm and 846+15 nm respectively) using the RRM approach can be directly related to the resonances in bio-molecules. Based on the RRM spectral characteristic we can calculate the wavelength of electromagnetic energy that can be used to modulate the protein activity hence giving rise to an innovative efficient methodology to program, predict, design and modify proteins and their bioactivity. This could have major implications in drug design, medicine, agriculture, pharmacology and biotechnology. In addition, we showed that the tested *de novo* designed peptide analogues produced a toxic effect on the B16F0 melanoma mouse cancer cells. Importantly, their anti-tumor activity is dose and time dependent, taking into consideration the calculated estimated half-life and the instability index of the synthetic peptides. There is also a clear indication on the enhancement of the tolerance of the skin cancer cells to the toxic effect of the peptide analogues, when they are grown in the presence of non-heat treated FBS, which is a subject of further investigation. The fact that using this novel approach, non-homologous, biologically active peptides can be designed solely from the computationally determined characteristic frequencies, demonstrate that these frequencies are in fact the critical parameters for protein biological functions. Rational *de novo* design of a variety of peptides and proteins would have a significant impact on pharmaceutical industry.

Apstrakt

Jedan od nerešenih problema u analizi proteinskih sekvenci je način na koji je biološka funcija upisana u DNA i proteinskim sekvencama. Biološki procesi u svakom živom organizmu su bazirani na selektivnoj interakciji specifičnih za tu reakciju bio-molekula. Međutim pravila koja određuju zapis biološke funkcije ili predispozicije za selektivnu interakciju sa nekim drugim proteinom još nisu definisana.

"Resonat Recognition Model" (RRM) je jedan od pokušaja da se ustanovi selektivnost proteina na osnovu poznavanja njegove primarne strukture.

Takođe sa svakodnevnim povećavanjem broja proteinskih sekvenci pojavljuje se potreba za razvijanjem metodologije za analiziranje proteinske srtukura-funkcija relacije.

RRM metodologija polazi od pretpostavke da su interakcije proteina u suštini elektromagnetske interakcije i da je seletivna priroda tih interakcija bazirana na rezonantnom energetskom transportu između interaktivnih molekula. Taj transport se odvija samo na određenim frekvencijama elektromagnetskog polja. Ukoliko se te frekvencije mogu proračunati dobila bi se mogućnost predikcije funkcionalno važnih "ključnih" aminokiselina. Isto tako bilo bi moguće dizajnirati sintetičke proteine sa željenom biološkom funkcijom.

U ovom radu je predstavljana hipoteza da je selektivna priroda proteinske aktivnosti bazirana na elektromagnetnoj rezonantnoj interakciji proteina. Ta ideje je obrađena na oba načina teoretski i eksperimentalno. Dati su brojni eksperimentalni rezultati u kojima se potvrđuje aktivacija proteina sa spoljašnjim elektromagnetskim zračenjem na teoretski proračunatim frekvencijama.

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The paper was accepted on on 24.11.2010.