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Use of infrared and visible light radiation as modulator of protein activity

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Abstract. In this study we discuss the possibility of modulating protein activity using infrared and visible light radiation based on the concepts of protein activation incorporated in the resonant recognition model (RRM). Application of the RRM approach includes prediction of the functional "key" amino acids in the protein molecule, prediction of the protein active site, design of *de-novo* peptides with the desired function and determination of the specific electromagnetic radiation frequency that may activate protein sequence. The theoretical basis behind the RRM model expounds a potential interaction mechanism between electromagnetic radiation and proteins as well as protein–protein interactions. Here the RRM hypothesis of protein activation is experimentally validated via irradiation of the L-lactate dehydrogenase enzyme by the electromagnetic field exposures in the range of 1140–1200 nm. In this paper we also present an application of the RRM to bioactive peptide design, and explore theoretically if proteins or DNA molecules can be activated by much lower frequencies, particularly in the microwave range (from 10^9 to 10^{10} Hz).

Key words: electromagnetic radiation, protein function, peptide design, characteristic frequency, digital signal processing.

1. INTRODUCTION

It has been shown that light-activated changes in protein energy states can induce or modulate biological processes. For instance, light-activated excitation of rhodopsin (bacteriorhodopsin) molecules, involved in the hyperpolarization process of the cell membrane, can either generate nerve impulses, ATP synthesis, or regulate embryogenesis [1–5]. It has been also suggested that cytochrome c oxidase and certain dehydrogenases may play a key role in the photoreception process, particularly in the near infrared (NIR) frequency range [5]. Recent studies into effects of low-intensity non-thermal light irradiation on eukaryotic

and prokaryotic cells, both pulsed and continuous, have reported the accelerated proliferation rate in yeast and mammalian cells upon irradiation by He-Ne laser light [6,7] and increased *E. coli* proliferation rate by argon laser light exposures [5,7]. The increased proliferation rate has also been observed in various bacterial cultures, irradiated by laser light with radiant exposures of 1–50 J/cm^2 at wavelengths of 630 and 810 nm [8]. Several studies have reported a change in the activity of human erythrocytes after low-intensity light radiation at 810 nm. Various up-to-date methodologies that incorporate low-intensity light into therapeutic procedures have been integrated into modern medicine.

Here we have studied experimentally the hypothesis of the resonant recognition model (RRM) that selectivity of protein activities is based on specific resonant electromagnetic interactions [1,9]. The RRM theory proposes that an external electromagnetic field at a particular activation frequency would produce resonant effects on protein biological activity. In our previous study [9] we have investigated the effects of visible light radiation in a range of 550–850 nm on enzyme kinetics of the LDH enzyme. In this study we have tested the influence of electromagnetic radiation (EMR) on biological activity of the LDH enzyme in the wavelengths ranging from 1140 up to 1200 nm. In addition, we examined theoretically the possibility that lower frequencies, in the microwaves range, can also activate long macromolecules such as proteins and DNA. The RRM procedure for bioactive peptide design is also presented using the example of oncogene proteins.

2. THE RESONANT RECOGNITION MODEL

2.1. Protein activation frequency

The RRM is designed for the analysis of protein (DNA) interactions and their interaction with EMR [1,9]. Protein primary structures are linear sequences of their constitutive elements, i.e. amino acids. The RRM model interprets this linear information using digital signal analysis methods that include 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 the protein function (interaction) [1]. In our previous work a relationship between the RRM spectra of some protein groups and their interaction with visible light was established. The RRM theory states that an external electromagnetic field at a particular activation frequency would produce resonant effects on the protein biological activity [1,9]. 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 the protein biological function [10]. Furthermore, it was also shown that proteins and their targets share a characteristic frequency. Thus it can be further postulated that RRM frequencies characterize not only a general function but also recognition/interaction between

particular proteins and their target at a distance [10,11]. Thus protein interactions can be viewed as a resonant energy transfer between the interacting molecules. This energy can be transferred through oscillations of a physical field, possibly electromagnetic in nature [1].

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 [1,12]. The frequency range of this field depends on a charge velocity estimated to be 7.87×10^5 m/s and on the distance between amino acids in a protein molecule, which is 3.8 Å. Therefore the maximum frequency due to the exciton transfer is estimated to be $F_{\max} < V/(2d)$ $< 1 \times 10^{15}$ Hz, ($L_{\min} > 330$ nm). The minimum frequency depends on the total length L of the protein and is estimated to be around 10^{13} Hz (at 30 000 nm) for a protein of about 200 amino acids in length [1,2]. The range from 30 000 to 300 nm is very wide, from far infrared through the visible to the ultraviolet region of the spectrum. For larger structures (e.g. longer proteins, DNA, protein clusters, membrane proteins and membrane channels) the relevant bioactivity frequency range can be estimated to start in the high microwave range of 10^{10} to 10^{11} Hz. The frequency range predicted for protein interactions is from 10^{13} to 10^{15} Hz. This estimated range includes infrared (IR), visible and ultraviolet (UV) light.

The estimated electromagnetic energy levels were initially investigated by the comparison of the absorption spectra of some groups of chromophore-bearing proteins with their corresponding RRM characteristic frequencies (Table 1, Fig. 1). As can be seen from Table 1 and Fig. 1, a strong linear correlation exists between the predicted and experimentally determined frequencies. 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 exposed proteins [9]. These computational predictions were confirmed by comparison of absorption characteristics of light-absorbing proteins and their characteristic RRM frequencies, frequency selective light effects on cell growth and characteristic RRM frequencies of growth factors, and activation of enzymes by laser radiation [1,13].

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 spectra of proteins and their RRM spectra with a regression coefficient of $K = 201$ has been established. Using RRM postulates, a computationally identified characteristic frequency for a protein functional group can be used to calculate the wavelength of applied irradiation, λ, which assumingly would activate this protein sequence and modify its bioactivity

$$\lambda = K / f_{\mathrm{RRM}}.$$

Table 1. The RRM frequencies and characteristic absorption frequencies of different visible light-absorbing protein groups and their scaling factor, *K* [1,9]

Protein	nm	rrm	cm^{-1}	*K*
cyt c	415	0.473	24 096.39	196
blue	430	0.475	23 255.81	204
green	540	0.355	18 518.52	191
red	570	0.346	17 543.86	197
hem.	14 770	0.02	677.0481	295
purple	860	0.281	11 627.91	241
flavodoxin	470	0.379	21 276.6	178
igf	400	0.492	25 000	196
fgf	441.6	0.453	22 644.93	200
insulin	552	0.344	18 115.94	189
growth f.	633	0.293	15 797.79	185
	650	0.293	15 384.62	190
pdgf	830	0.242	12 048.19	200
chymotr.	851	0.236	11 750.88	200
calculative	400	0.5	25 000	200

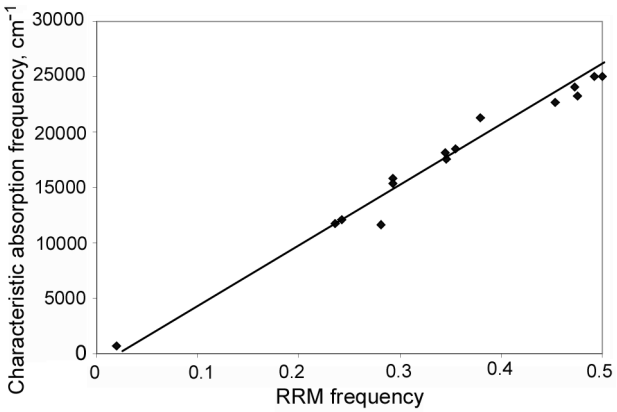

Fig. 1. Linear correlation between RRM frequencies and corresponding absorption frequencies of different visible light-absorbing protein groups.

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

2.2. Irradiation of the L-lactate dehydrogenase enzyme

Enzymes are proteins crucial in accelerating metabolic reactions in the living organism. Dehydrogenase enzymes catalyse a variety of oxidation-reduction reactions within the cells. As the protein example we have chosen the L-lactate dehydrogenase (rabbit muscle). This enzyme has been selected on the basis of its commercial availability, simplicity of the assay, and the possibility of measuring

their bioactivity using the standard well accepted procedure, i.e. Continuous Spectrophotometric Rate Determination. As a source of IR and visible light we have used a 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 analysed enzyme solutions we use an Ocean Optics USB2000 spectrometer coupled to a CCD array, which can detect in the 190–870 nm range.

LDH rabbit muscle EC1.1.1.27 catalyses the inter-conversion of the L-lactate into pyruvate with the nicotinamide adenine dinucleotide oxidized 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 contrary to the NAD, which is inactive at this frequency. Due to different optical characteristics of 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 experimental procedure is presented below.

1. The samples are irradiated for 10 min using Monochromator SpectraPro 2150i (Acton Research Corporation) set at the activation wavelength identified computationally using the RRM approach.
2. These irradiated samples are added to the already prepared solution of NADH and pyruvate.
3. The optical density of NADH is measured at 340 nm.
4. The values of the rate of change in absorbance of NADH and changes of absorption coefficient values (at 340 nm) in time are collected and presented graphically.

2.3. Bioactive peptide design

The ability to predict the functions and three-dimensional 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 biomolecule could be modelled using computational tools. These tools can allow a drug molecule to be constructed using knowledge of its structure and the nature of its active site. 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. Here we present the results of our computational analysis of oncogene and proto-oncogene proteins and the rational design of bioactive peptide analogues having the oncogenic or proto-oncogeneic-like activity.

The RRM presents a completely new engineering approach to the analysis of proteins and DNA. This model is based on the finding that the distribution of delocalized electron energies along the protein amino acid sequence correlates with the protein biological function [1,10]. 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 [14,15]:

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

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

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

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^* 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. The presence of a peak

frequency with significant signal-to-noise ratio in a multiple-cross-spectral function implies that all of the analysed sequences within the group have one frequency component in common. From previous studies [1,10,11] the fundamental conclusion was drawn: one RRM peak frequency characterizes one particular biological function or interaction. Therefore, those peaks were named as RRM characteristic frequencies.

Once the RRM characteristic frequencies and corresponding phases for particular biological functions are determined, it is possible then to design amino acid sequences having those spectral characteristics only. It is expected the designed peptide will exhibit the desired biological activity. The strategy for the design of such defined peptides is as follows [16]:

- Within the multiple cross-spectral analysis of the group of protein sequences sharing the corresponding biological function, determine the unique RRM frequency that characterizes this specific biological function or interaction.
- Define the characteristic phases at the characteristic frequencies for the particular protein that is chosen as the parent for agonist or antagonist peptide design.
- From the known characteristic frequencies and phases derive a numerical sequence. This can be done by summing sinusoids of the particular frequencies, amplitudes and phases. The length of the numerical sequence is defined by the appropriate frequency resolution and the required peptide length.
- To determine the amino acids that correspond to each element of the new numerical sequence. It can be achieved by the tabulated EIIP or other appropriate amino acid parameters.

Earlier we determined the characteristic frequencies of forty six oncogene and fifteen proto-oncogene proteins, that characterize their common biological activity, i.e. the ability to promote uncontrolled cell proliferation, in case of the oncogene proteins, and normal cell growth for proto-oncogenes [10]. This study emphasizes the *de novo* design of peptide analogues only on the basis of the frequencies and phases, predicted computationally. Ultimately, these designed peptides would exhibit the desired oncogene or proto-oncogenic-like activity as their parental protein.

3. RESULTS AND DISCUSSION

3.1. Enzyme activation by infrared light radiation of defined wavelength

The RRM approach has been used here to identify the activation frequency of the LDH enzyme. The experimental measurements of its activity upon electromagnetic field exposures of defined wavelengths were performed. A computational analysis using the RRM was carried out resulting in two characteristic frequencies identified at $f_1 = 0.1688 \pm 0.004$ and less prominent at $f_2 = 0.2392 \pm 0.004$ (Fig. 2). These frequencies are related to the biological activity of

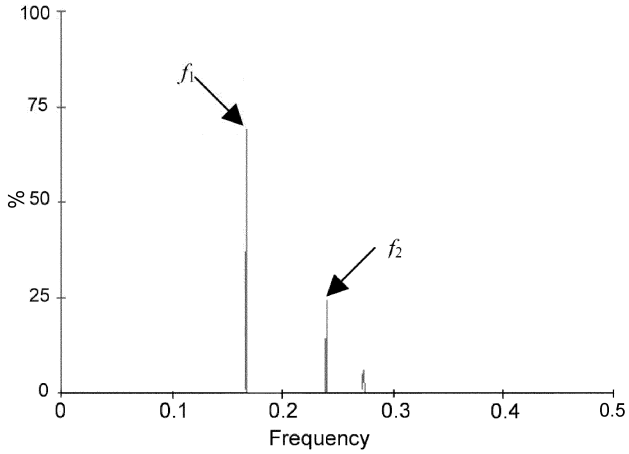

Fig. 2. Multiple cross-spectral function of dehydrogenase proteins (32 sequences). The prominent peak(s) denote common frequency components. The abscissa represents RRM frequencies, and the ordinate is the normalized intensity.

the LDH as it was found in our previous investigations [9]. Based on the characteristic frequencies determined for the whole dehydrogenase functional group, we have calculated the wavelength of irradiation, $\lambda = 201/f_{\mathrm{RRM}}$, which assumingly would activate dehydrogenase sequences and modify their bio-activity. Thus the wavelengths of the electromagnetic exposure required for dehydrogenase enzymes activation would be at 1191 ± 15 nm and 846 ± 15 nm. In our previous study we investigated the effects of EMR (550–850 nm) on the LDH kinetics [9]. Here we have studied the changes in the LDH activity upon irradiation in a range of 1140–1200 nm.

3.2. Measurement of the NADH absorbance

We have diluted the stock coenzyme solution with the 0.003 M potassium phosphate assay buffer. Using the properly diluted coenzyme solution, we have measured the NDAH absorbance upon irradiation at 1140–1200 nm with the interval of 2–5 nm. The spectrophotometer is set to 100% transmittance (zero absorbance) at each wavelength using the 0.003 M K_2HPO_4 assay buffer blank. The results obtained have shown that NADH concentration corresponds to the maximum absorbance of 1.6 at 340 nm. Figure 3 shows how NADH sample absorbance is affected by the applied radiation of the defined wavelength.

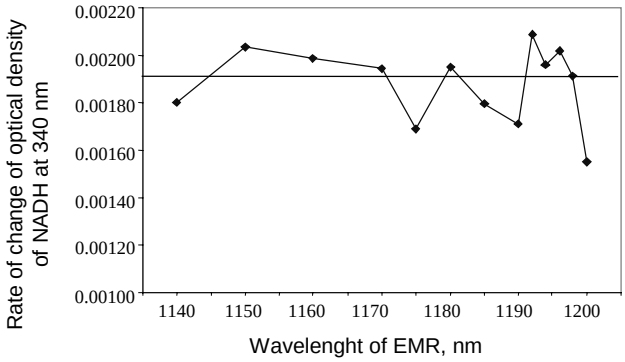

Fig. 3. Gradient of change in the NADH absorbance.

3.3. Measurement of the LDH activity

The 2.5 ml cuvettes are filled with the following components:

- 0.1 ml of 0.0027 M sodium pyruvate (BioWhittaker);
- 0.1 ml NADH; disodium salt (C21H27N7O14P2Na2 Roche);
- 0.005 M phosphate buffered saline (SIGMA);
- 1.5 ml of deionized water; 0.3 ml LDH diluted in 2.5 μg/ml of phosphate buffered saline with BSA (SIGMA).

The experiments were performed at room temperature 27°C (Temperature controller Quantum Northwest). The cuvettes were filled with 0.3 ml of the LDH samples. The samples were previously irradiated with the light of different wavelengths (1140–1200 nm) for 600 sec. These irradiated samples were added then to the already prepared solution of NADH and pyruvate. The optical density of NADH was measured at 340 nm for each irradiating wavelength. These values are collected and presented in Table 2 and Fig. 4. The results obtained have revealed the change of the NADH absorbance under the influence of irradiated LDH. Thus, the LDH activity has changed upon radiation, resulting in accelerating the reaction

$$\text{pyruvate} + \text{NADH} \rightarrow \text{lactate} + \text{NAD}^+ + \text{H}^+.$$

From Figs. 3 and 4 we can observe that maximum optical density of the NADH is achieved at the wavelengths 1192 and 1200 nm ($f_1 = 0.1688 \pm 0.004$) as was predicted by the RRM as the possible activation frequency of the dehydrogenase enzymes. Hence, the results suggest that this specific biological process can be modulated by irradiation with defined frequencies strongly supporting the main concept of the RRM methodology. The possibility to calculate the frequencies with the following use of IR and visible light to produce the desired biological mutations and alterations in proteins would benefit the development of new biomaterials, non-invasive treatments and advanced technologies.

Table 2. Absorbance and wavelength values of the NADH sample

t, sec	Wavelength of irradiating light, nm													
	1140	1150	1160	1170	1175	1180	1185	1190	1192	1194	1196	1198	1200	test01
0	1.311	1.318	1.223	1.104	1.276	1.235	1.243	1.261	1.286	1.285	1.296	1.307	1.456	1.283
30	1.257	1.255	1.161	1.048	1.231	1.186	1.191	1.218	1.228	1.217	1.233	1.268	1.411	1.238
60	1.196	1.196	1.104	0.989	1.169	1.115	1.132	1.168	1.184	1.153	1.185	1.205	1.353	1.176
90	1.138	1.131	1.049	0.938	1.121	1.061	1.082	1.117	1.113	1.089	1.116	1.146	1.307	1.125
120	1.096	1.071	0.986	0.878	1.067	1.000	1.029	1.058	1.053	1.028	1.055	1.088	1.260	1.074
150	1.031	1.010	0.931	0.824	1.016	0.936	0.975	1.012	0.987	0.970	1.004	1.037	1.201	1.014
180	0.983	0.947	0.866	0.758	0.962	0.877	0.913	0.956	0.927	0.914	0.942	0.978	1.158	0.960
210	0.926	0.891	0.806	0.696	0.915	0.823	0.863	0.912	0.871	0.859	0.882	0.918	1.120	0.904
240	0.873	0.831	0.752	0.649	0.853	0.755	0.811	0.858	0.811	0.797	0.827	0.859	1.066	0.850
270	0.813	0.772	0.689	0.596	0.808	0.703	0.762	0.805	0.751	0.741	0.771	0.810	1.011	0.787
300	0.761	0.714	0.629	0.538	0.754	0.644	0.706	0.755	0.694	0.681	0.712	0.752	0.961	0.732
330	0.711	0.661	0.572	0.488	0.704	0.589	0.659	0.705	0.636	0.622	0.657	0.695	0.913	0.678
360	0.656	0.608	0.515	0.436	0.656	0.530	0.603	0.653	0.579	0.571	0.606	0.643	0.868	0.625
390	0.601	0.547	0.458	0.385	0.613	0.480	0.561	0.596	0.524	0.512	0.545	0.583	0.823	0.575
420	0.550	0.494	0.404	0.336	0.566	0.429	0.513	0.535	0.469	0.455	0.495	0.530	0.769	0.537
450	0.509	0.435	0.354	0.290	0.518	0.374	0.464	0.488	0.413	0.404	0.442	0.482	0.708	0.496
480	0.403	0.403	0.304	0.244	0.470	0.324	0.426	0.444	0.366	0.358	0.392	0.435	0.666	0.440
510	0.420	0.341	0.259	0.205	0.427	0.279	0.379	0.394	0.319	0.314	0.340	0.396	0.620	0.387
540	0.392	0.300	0.221	0.170	0.373	0.234	0.338	0.358	0.276	0.276	0.292	0.346	0.576	0.348
570	0.348	0.260	0.189	0.137	0.335	0.198	0.302	0.314	0.238	0.243	0.255	0.300	0.538	0.301
600	0.301	0.225	0.160	0.110	0.294	0.162	0.259	0.275	0.207	0.211	0.223	0.267	0.498	0.267
Grad.	0.0018	0.0020	0.0020	0.0019	0.0017	0.0020	0.0018	0.0017	0.0021	0.0020	0.0020	0.0019	0.0016	0.0018

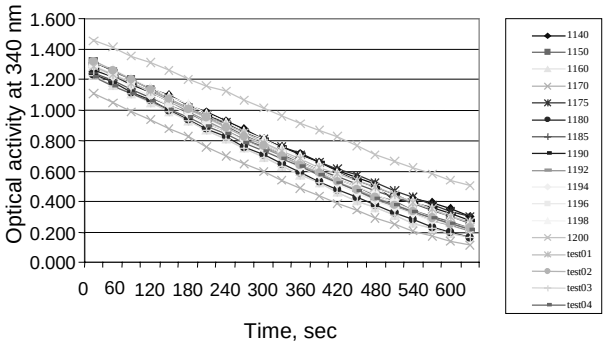

Fig. 4. Changes of absorption coefficient values (at 340 nm) in time upon irradiation.

3.4. Bioactive peptide design: oncogene/proto-oncogene analogy

In this study we applied the RRM approach to design bioactive peptides having oncogene-like and proto-oncogene-like activities. The oncogene proteins are generally involved in regulation of cell proliferation through regulation of DNA transcription. The Ha-*ras* oncogene proteins (p21 proteins) are known to function *in vitro* as GTP binding proteins involved in signal transduction pathways, as well as in control of DNA synthesis, cell transformation, proliferation and differentiation [10]. In our previous studies a group of forty six oncogene proteins were analysed and their RRM characteristic frequencies were identified at $f_1 = 0.0322 \pm 0.004$, $S/N = 297.29$ and $f_2 = 0.0537 \pm 0.004$, $S/N = 77.25$. As is evident from Fig. 5a, both identified frequencies with significantly different amplitude ratios are observed in the cross-spectral function of all oncogene proteins. The prominent frequency at $f_1 = 0.0322 \pm 0.004$, $S/N = 297.29$ characterizes a common biological behaviour of this oncogene product, i.e. an ability to promote an uncontrolled cell growth and proliferation. These two peaks identified for oncogene proteins reveal that oncogenes are multifunctional proteins, i.e. they can be involved in different biological processes (interact with other proteins). The same analysis was carried out on fifteen proto-oncogene sequences (Fig. 5b). As can be observed from Fig. 5b, there is a prominent peak at $f_2 = 0.0537 \pm 0.004$, $S/N = 199.74$, which represents a characteristic feature of all proto-oncogene sequences and corresponds to their common biological function, i.e. normal cell growth. It is noteworthy that the same frequency $f_2 = 0.0537 \pm 0.004$, $S/N = 77.25$ was identified as the less significant peak existing in multiple cross-spectral function of oncogene proteins (Fig. 5a).

Here the RRM has been applied to Ha-*ras* p21 (Harvey Murine sarcoma virus) protein to design the peptide that exhibits *ras*-like activity, i.e. ability to transform cells. The design of six *de novo* peptides A-F was based on two characteristic frequencies and phases determined for the entire functional group of oncogene proteins $f_1 = 0.0322$, $\varphi_1 = 1.641$ and $f_2 = 0.0537$, $\varphi_2 = 2.460$. Each peptide has either one or both frequencies with the same or opposite phases at these frequencies as presented in Fig. 6.

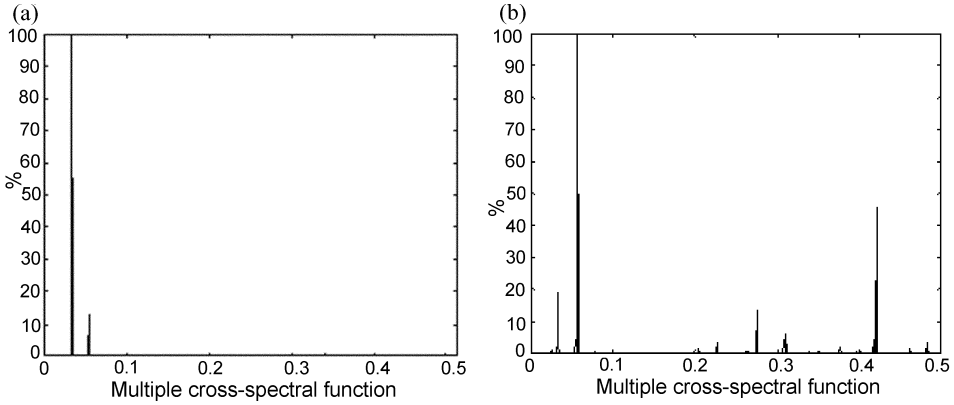

Fig. 5. Multiple cross-spectral function of oncogene (a) and proto-oncogene (b) proteins.

Peptide/frequency	f_1	f_2	φ_1	φ_2
A	*	*	–	–
B	*		–	
C		*		–
D	*	*	+	+
E	*		+	
F		*		+

Fig. 6. Schematic presentation of the designed peptides [1,16].

Original protein:

Transforming protein (Ha-*ras*) – Harvey murine sarcoma virus 241 a.a.

MPAARAAPAADEPMRDPVAPVRAPALPRPAPGAVAPASGGA
RAPGLAAPVEAMTEYKLVVVGARGVGKSALTIQLIQNHFVDE
YDPTIEDSYRKQVVIDGETCLLDILDTTGQEEYSAMRDQYMRT
GEGFLCVFAINNTKSFEDIHQYREQIKRVKDSDDVPMVLVGNK
CDLAGRTVESRQAQDLARSYGIPYIETSAKTRQGVEDAFYTLVR
EIRQHKLRKLNPPDESGPGCMSCKCVLS

The designed peptides (18 amino acids):

1) $f_1 = 0.0322,\ \varphi_1 = -1.641,\ f_2 = 0.0537,\ \varphi_2 = -2.460$

A: NLNEPAWQTRDDDDDRFM

2) $f_1 = 0.0322,\ \varphi_1 = -1.641$

B: LNEPHAYWQCRRDDDDDD

3) $f_2 = 0.0537,\ \varphi_2 = -2.460$

C: EILEPAWQRDDDDDRQWK

4) $f_1 = 0.0322,\ \varphi_1 = 1.641,\ f_2 = 0.0537,\ \varphi_2 = 2.460$

D: DRMWKPEILGPHKYYWWY

5) $f_1 = 0.0322,\ \varphi_1 = 1.641$

E: DDDRFMQWAAPPEILINV

6) $f_2 = 0.0537,\ \varphi_2 = 2.460$

F: CWAHEILEPAWQRDDDDD

Proto-oncogene proteins are the products of proto-oncogenes. Generally, they do not have oncogenic or transforming properties but are involved in the normal regulation or differentiation of cell growth. However, proto-oncogenes can promote cancer development only if they acquire new properties as a result of mutations at which point they are known as oncogenes. Most common cancers involve modification of certain proto-oncogenes. Determination of two distinct characteristic frequencies, which correspond to two different functions, i.e. normal cell growth (proto-oncogenes) and uncontrolled cell transformation (oncogenes), reveal that the RRM approach can assist in distinguishing between the oncogene and proto-oncogene activity of oncogene proteins. Thus, these results can lead to the conclusion that the RRM approach is capable of identifying "cancerous" feature (frequency) within the protein primary structures of the studied proteins. In addition, the *de novo* peptides designed on the basis of the oncogenic (cancerous) and proto-oncogenic (normal) frequencies determined within the RRM might be used in the development of new testing and treatment procedures for cancer.

3.5. Macromolecular activation by microwave radiation

In addition, we were interested to explore theoretically if proteins or DNA molecules can be activated by much lower frequencies, particularly by the frequencies in the microwave range (from 10^9 to 10^{10} Hz). Our preliminary results have shown that activation of proteins can occur in the range of 10^{13} to 10^{15} Hz, where the lower frequency is determined by the length of the protein, which was supposed to be 200 amino acids. As the relation between the lower frequency and the length of the protein is linear, it follows that when aiming to activate proteins by microwaves using the above proposed mechanism, the protein length needs to be in the order of 200 000 amino acids. The largest known proteins are the titins, a component of the muscle sarcomere, with a molecular mass of almost 3000 kDa and a total length of almost 27 000 amino acids [16]. Thus the minimum frequency that can activate the titin proteins according to the RRM would be about 10^{11} Hz.

However, DNA sequences are much longer and thus may be considered as good candidates for activation by microwaves. The analogous RRM calculations of activation frequencies that we use for proteins can also be applied to DNA sequences. The EIIP values of amino acids in protein and nucleotides in DNA are presented in Table 3. We may assume that the charge is able to travel through the DNA backbone, with a velocity similar to the charge velocity in proteins:

$$V < 7.87 \times 10^5 \text{ m/sec.}$$

Table 3. Chromosome and DNA length variation between different organisms

Organism	Chromo-some	Chromosome length, kbp	Organism	Length of mitochondrial DNA
Homo sapiens	20	90 000	Homo sapiens	16K
Homo sapiens	19	100 000	Great Indian Rhino	16 829
Homo sapiens	17	125 000	Donkey	16 670
Homo sapiens	6	225 000	Atlantic cod	16 696
Dros. melanogaster	4	1 750	Locust (L. migratoria)	15 722
Dros. melanogaster	3	50 000		
Dros. melanogaster	2	51 000		
Mus musculus	11	45 000		

If the charge is able to pass different energy levels as was calculated using the EIIP for different nucleotides [17], then the only factor that can influence the frequency is the distance between the nucleotides, which is defined as $d = 3.4$ Å.

This distance is close to the distance $d = 3.8$ Å, which was used for calculations for amino acids in the protein sequence. Therefore, we propose that the maximum frequency that could be emitted during the electron transfer is practically the same:

$$F_{\max} < V/2d,$$

$$F_{\max} < 1\times10^{15} \text{ Hz}.$$

The minimum frequency that could be emitted depends on the total length of the DNA sequence

$$F_{\min} = 2F_{\max}/N,$$

where N is the total number of nucleotides in the DNA sequence. For example, for DNA of 200 nucleotides in length, the minimum frequency is defined as

$$F_{\min} < 1\times10^{13} \text{ Hz}.$$

However, DNA length is much longer, especially if we take into account the entire chromosome (45 000 000 bp to 300 000 000 bp). DNA contour length ranges from approximately 50 to 2700 nm, and could be affected by the electro-magnetic radiation in the microwave frequency range from 10^{15} all the way down to 10^{7}.

A chromosome in a mammalian cell is made up of a single DNA molecule about 5 cm long, which is effectively compacted into each chromosome. A study of the DNA lengths in each chromosome in several organisms using the Entrez Swiss database shows that the base pair lengths in each chromosome of several evolutionary distinct organisms can be quite different. Some of this data is shown in Table 3. Interestingly, if we consider the mitochondrial DNA, we observe much greater homogeneity in the DNA length (Table 3). This is most likely

explained by the theory that mitochondria were once separate organisms that were incorporated into the cellular structure of eukaryotic cells. The slight differences in mitochondrial DNA length are due to differences in the length of the control regions of the mitochondrial DNA. The suggestion that the same mechanisms are applicable for lower frequencies' interaction with macromolecules would imply that activation would be possible only for longer macromolecules, i.e. for extremely large proteins and DNA.

4. CONCLUSIONS

In this study we have irradiated the LDH enzyme with the light of defined wavelength determined within the RRM. The results obtained have shown that the RRM frequencies, identified for the dehydrogenase enzymes at $f_1 = 0.1688 \pm 0.004$ and less prominent at $f_2 = 0.2392 \pm 0.004$ (corresponding to 1191 ± 15 and 846 ± 15 nm respectively) can be directly related to the resonances in electron differential scattering cross-section of these macromolecules. Thus, we conclude that the RRM spectral characteristic and their corresponding wavelengths of electromagnetic energy 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.

We have shown previously, using the RRM concepts, that digital signal processing methods can be used to analyse linear sequences of amino acids to reveal the informational content of proteins and determine functionally significant amino acids of the analysed proteins. In this study we have shown that the RRM is capable of identifying the difference between oncogenes and proto-oncogenes and thus, identifying general "cancerous" feature within oncogene protein primary structures. If such feature can be identified then it would be possible to validate unknown or modified proteins as well as relevant DNA sequences for their possible cancerous activity. In addition, as shown here, it is possible to design peptides which would have only this "cancerous" characteristic. Such peptides are predicted to carry the common characteristic of all oncogenes and thus, they can be used for development of a vaccine, which could be then preventive for most kind of cancers. Thus, the RRM provides a new strategy for a wide variety of protein and peptide structural manipulations, e.g., protein engineering by recombinant techniques could be undertaken based on the RRM predictions for the redesign of specific protein or peptide variants with modified biological properties.

In this work we have also examined the possibility of activation of large macromolecules such as proteins and DNA by lower frequencies in the range of microwaves. Based on the RRM postulates it has been shown that activation of macromolecules by microwaves can be only achieved for linear macromolecules in excess of 200,000 residues/nucleotides. Thus, it is unlikely that microwaves would be able to activate proteins, based on the resonant energy transfer as was

proposed in the RRM as their length does not exceed the length required. However, DNA sequences are much longer and there is a possibility that microwaves can activate DNA through the resonant energy transfer as it is suggested in the RRM model in a wide frequency range of 10^7–10^{15} Hz.

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Infrapunase ja nähtava valguse kiirguse kasutamine valkude aktiveerimise modulaatorina

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On uuritud võimalust moduleerida valkude toimimist, kasutades infrapunast ja nähtavat kiirgust, lähtudes resonantsi tuvastamise mudeli kontseptsioonist valkude aktiveerimisel. Resonantsmudelil põhineva lähenemise kasutamine võimaldab saada infot valgu struktuurist, koostada soovitud funktsiooniga uute peptiidide struktuuri ja määrata elektromagnetkiirguse sagedusi, mis võivad valgu sekventse aktiveerida. Resonantsmudeli teoreetiliseks aluseks on elektromagnetkiirguse ja valkude, aga ka valkude omavahelise vastastikuse mõju skeem. Töös on resonatsmudeli hüpoteesi valkude aktiveerimisest eksperimentaalselt kontrollitud, kiiritades LDH-ensüüme elektromagnetkiirgusega vahemikus 1140–1200 nm. Ka on resonantsmudelit kasutatud bioaktiivsete peptiidide konstrueerimisel ja teoreetiliselt uuritud valkude ning DNA-molekulide võimalikku aktiveerimist palju madalamate sagedustega, eriti mikrolainete diapasoonis 10^9–10^{10} Hz.