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STUDY OF SPECTRAL CHARACTERISTICS OF BOVINE SERUM ALBUMIN UNDER THE EFFECT OF MILLIMETER RANGE ELECTROMAGNETIC WAVES

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The study of the effect of millimeter range electromagnetic waves (MM EMW) on bovine serum albumin (BSA) structure at the frequencies both resonant (51.8 *GHz*) and non-resonant (41.8 *GHz*) for water has been conducted. The absorption and fluorescence spectra of non-irradiated and irradiated samples of physiological solution and BSA with exposition 60 *min* were obtained. It was revealed that the spectral characteristics of physiological solution did not change under the effect of MM EMW, while the analogous characteristics of BSA change relevantly. It was also shown that the absorption spectra of the irradiated samples significantly differ from those of non-irradiated protein.

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***Keywords*:** millimeter range electromagnetic waves, irradiation, resonant and non-resonant frequency, absorption and fluorescence spectrum.

Introduction. Nowadays electromagnetic irradiation of anthropogenic origin was added to the physical ecological factors of the environment. Among different ranges of this factor, the portion of millimeter range electromagnetic waves (MM EMW) increases in an interval of GHz-frequency region [1–3]. Electromagnetic irradiation is a superposition of electrical and magnetic fields, spread through water, with which these fields interact. Living organisms evolved under the influence of natural fields, having different wavelengths. Anthropogenic fields are unusual for organisms that is why the effect of such factors demands circumstantial examination [4–7].

In the mentioned diapason the resonant absorption of MM EMW by water has a prominent value. This fact is important from the point of view that water is a medium for activity expression of biological macromolecules, including proteins and nucleic acids. Among biomacromolecules blood serum albumin plays an important role, which realizes transport of different compounds in the constitution of blood plasma [8–10].

Details of albumin conformational changes on the molecular level under the effect of various physical and chemical factors are an important key problems for all processes, in which these serum proteins take part. It was shown that albumin can be in several stable conformations, depending on physicochemical factors of solvent.

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It is obvious that the study of the effect of various factors on the conformation or conformational changes of this protein has an important biological value [8–10].

The work is aimed at the study of fluorescence and absorption characteristics of albumin under the effect of MM EMW.

Materials and Methods. Bovine serum albumin (BSA, “Sigma”, USA), physiological solution, bi-distilled water were used in experiments. Concentration of BSA was determined spectrophotometrically, using the following coefficient of extinction: $\varepsilon_{280} = 43824\ M^{-1}\cdot cm^{-1}$ for BSA. Data averaging was carried out from 5 values. The measurements were conducted in thermostating cells, using quartz cuvettes with optic pathway length 1 *cm*, volume 3 *mL* and hermetically closing caps. In all experiments the error does not exceed 5–10%.

BSA solution was irradiated by MM EMW with frequencies 41.8 *GHz* and 51.8 *GHz* for 60 *min*. The frequency 41.8 *GHz* significantly affects on biological systems, while the frequency 51.8 *GHz* is a resonant one for water. Irradiation exposure was equal to 1 *h*, which turns to be optimal, according to the results of our early investigations.

Generator G4-141 served as a source of MM EMW irradiation (“Istok”, Fryazino, RA), with power flux density $\approx 5\ \mu Wt/cm^2$ and power at the end 5 *mWt*. The value of the power flux density was registered on the sample. Generator output power was measured by thermistor head M5-49 and wattmeter M3-10A (“Istok”, Fryazino, RA). Generator stability frequency in the constant wave regime was equal to ±15 *MHz*. Irradiation of the sample solutions was carried out in the far zone of cylinder-shaped antenna, having a hole with sizes 32×32 *mm* on the distance 250 *mm* from the plane of antenna irradiation. Electromagnetic field was homogeneous. The sample was irradiated from the above side of glassy container with diameter 6 *cm*, where the solution thickness was 1 *mm* that in turn permits rays entirely penetrating into solution volume. Irradiation of the sample was conducted in Petri dishes, closed with thin, permeable layer, which interferes water evaporation during the irradiation process.

Absorption and fluorescence spectra were registered on the spectrophotometer UV-VIS Perkin Elmer Lambda 365 (USA) and spectrofluorometer Agilent Cary Eclipse (USA). Excitation of the protein was done at the wavelength 280 *nm*. Fluorescence spectra were registered in the interval $230 \le \lambda \le 500\ nm$.

Results and Discussion. Albumin is a key transport protein of blood, which deponates and transports the main part of endogenous and exogenous compounds in the blood [11–14]. Obviously, the structural state of this protein is determining for the realization of its biological role. From this point of view, the studies of various physicochemical factors on albumin structure in model systems are actual. One of the physical factors, affecting the structure of biomacromolecules, is electromagnetic irradiation, especially non-thermal waves that can both immediately and in mediated way influence biosystems. Such factor is MM EMW which can be resonantly absorbed by water and affect through water medium of biosystems or directly influence the cell or organism. In the last case plasmatic membrane, animal skin or external coverage of plants become the main targets. Meanwhile, water is the most important substrate for absorption of MM EMW, that is why the studies of this factor on biomacromolecules in water and water-saline solutions, i.e. in media, close to natural ones, are more informative. In this aspect, we studied the effect of MM EMW on albumin structure.

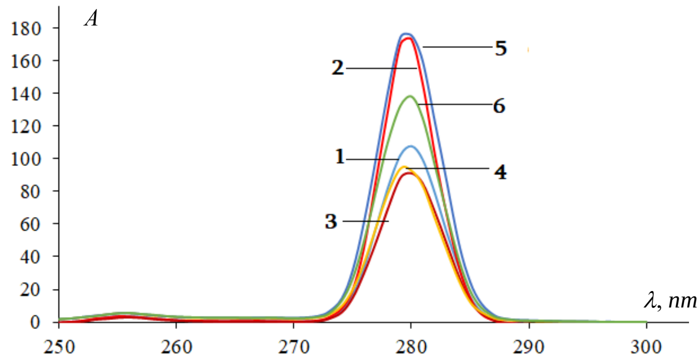

Fig. 1. Absorption spectra of non-irradiated (curve 1) and irradiated (curves 2–6) samples of albumin. Curve 2 corresponds to the irradiated sample by water non-resonant freuency 41.8 *GHz*; curve 3 – by non-resonant freuqency 41.8 *GHz*, obtained after 24 *h* of the irradiation. Curve 4 corresponds to the irradiated sample by resonant freuquency 51.8 *GHz*, obtained immediately after the irradiation; curve 5 and curve 6 – after 24 *h* and 48 *h* of the irradiation, respectively.

In the Fig. 1 the absorption spectra of non-irradiated and irradiated albumin samples were presented.

As it is obvious from Fig. 1, comparing to the non-irradiated albumin, the absorption of irradiated sample by 41.8 *GHz*, measured immediately after the irradiation, sharply increases (curve 2). But after 24 *h* after the irradiation by the same frequency, the albumin absorption sharply decreases (curve 3). At the irradiation by the resonant frequency for water – 51.8 *GHz* the albumin absorption, measured immediately after the irradiation, increase, after 24 *h* after the irradiation it sharply increases, then after 48 *h* – decreases, remaining higher, than that of non-irradiated albumin. We assume that these changes, taking place especially at the water resonant frequency, can result from the alterations of hydration degree around protein tryptophan residues or from the changes of the protein structure, or from both reasons simultaneously. It is remarkable that both resonant and non-resonant frequencies have comprehensive effect. The absorption of molecules reflects oscillation force and is higher, when chromophore group (tryptophan residues) owns relatively high freedom degree. From this point of view, we assume that MM EMW invoke such changes due to which oscillation force of tryptophan residues (albumin absorption results from these aminoacidic residues) increases. As it is obvious from the obtained data at the resonant frequency a little decrease of albumin absorption is observed, which can be the result of water molecule structuration, contacting with the protein. It, in turn, can affect the letter’s folding, establishing more compact structure, due to which mobility and consequently the oscillation force of amino-acidic residues decrease. Obviously, it should result in reverse effects, at the decrease or absence of MM EMW effect. In other words, it is reflected after 24 *h* and 48 *h* after the irradiation.

Based on this, we assume that under the effect of MM EMW dynamic change of the protein structure change takes place. This protein contains two tryptophan residues that absorb light energy in ultraviolet region ($\lambda \approx 280$ *nm*) [15]. It is obvious that the irradiation induces such changes due to which the oscillation force of tryptophan residues sharply increases, since these residues acquire a high lability.

Most apparently, the irradiation results in relevant change of 3D folding of albumin and it should not be excluded that a partial or entire loss of tertiary structure occurs, proceeding from the fact that the frequency 41.8 *GHz* is not resonant for water and affects 3D structure of the protein as destabilizing factors (particularly, high temperature or pH).

Earlier it was shown that at the irradiation of water-saline solutions by water resonant frequencies a change of water structuration takes place. Most apparently, the same occurs in water-saline solutions of biomacromolecules which may lead to hydration alterations of the latters, due to which BSA folding 3D change takes place [16].

Fluorescent characteristics of solved biomacromolecules are more sensitive to environment changes [17]. In the Fig. 2 the fluorescence spectra of non-irradiated and irradiated samples of albumin are presented.

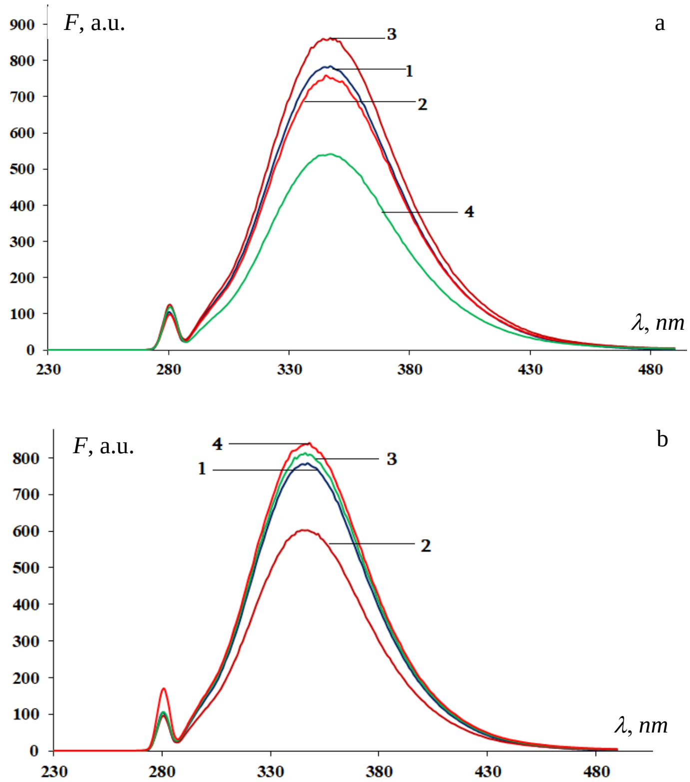

Fig. 2. Fluorescence spectra of the non-irradiated (curve 1) and irradiated samples by the frequencies 41.8 *GHz* (a) and 51.8 *GHz* (b) (curves 2–4). Curve 2 corresponds to the fluorescence spectra, obtained immedietly after the irradiation; curve 3 and curve 4 – after 24 *h* and 48 *h* of the irradiation, respectively.

It is obvious from Fig. 2, a that the fluorescence intensity of the irradiated albumin by the frequency 41.8 *GHz* takes a little decrease (curve 2) immediately after the irradiation, a scompared to non-irradiated sample. Though, after 24 *h* after the irradiation the fluorescence intensity increases (curve 3), becoming higher, than the intensity of non-irradiated albumin. In water solutions the hyperchromism of the fluorescence spectra of hydrophobic molecules indicates that these molecules are in more non-polar environment [17]. Consequently, after 24 *h* from the irradiation under the effect of MM EMW the structure of albumin takes such changes, at which it is not excluded the shift of tryptophan residues to more hydrophobic environment. We assume that the non-resonant frequency for water 41.8 *GHz* induces changes in water-saline solution, as a result of which inter-molecular reconstructions occur and the protein conformation changes resulting in shifting of tryptophan residues to more hydrophobic environment to avoid unpleasant contacts with water; this affects their spectral characteristics: the absorption intensity decreases, the fluorescence intensity increases. From this point of view, it is unexpected that after 48 *h* after the irradiation by the frequency 41.8 *GHz*, the fluorescence intensity of albumin significantly decreases. Most apparently, after 48 *h* after the irradiation, the changes in water-saline solution under the effect of MM EMW either relevantly is recovered, or these changes condition such intramolecular reconstructions in protein globule, due to which the fluorescence of tryptophan residues decreases (possibly quenching or scattering of excitation energy).

Another scenario is observed at the irradiation of albumin water-saline solution by the frequency 51.8 *GHz*. Immediately after the irradiation the prostein fluorescence intensity sharply decreases (Fig. 2, b, curve 2). We assume that the protein structural reconstructions under the effect of irradiation by resonant frequency are more pronounced, since the hypochromic effect is much more significant, that by the non-resonant frequency. This effect is maintained by the abovementioned arguments. After 24 *h* and 48 *h* the fluorescence intensity of the irradiated samples by the resonant frequency increases (curves 3 and 4, respectively), which indicates that the irradiation effect on the protein water-saline solution decreases during the next days, which results in such reconstructions of albumin structure that the hydrophobicity degree in the immediate environment of tryptophan residues remains higher, as compared to that of non-irradiated protein.

Conclusion. Thus, the studies on the MM EMW effect on the absorption and fluorescence characteristics of BSA reveal that this factor has a significant effect on the protein structure. The obtained data reveal that MM EMW relevantly affects the water solution of biomacromolecules, which results in the certain structural reconstructions, due to which their some optic characteristics change. On the other hand, it is obvious from the obtained data that MM EMW effect is weakened after 48 *h*, due to which the recovery of the original structure is observe, while irradiating by water resonant frequency. The same does not occur at the irradiation by non-resonant frequency, since after 24 *h* and 48 *h* after the irradiation the differing from the original structure is established. We assume that MM EMW irradiation results in such changes of water medium of the albumin solutions, due to which a significant change of the protein folding takes place, which is reflected on the optic characteristics of tryptophan residues of the protein. This fact has a valuable importance and may lie at the basis for practical application of MM EMW in biological and medical spheres.

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Ս. Վ. ԳՐԻԳՈՐՅԱՆ

ՑՈՒԼԻ ՇԻՃՈՒԿԱՅԻՆ ԱԼԲՈՒՄԻՆԻ ՍՊԵԿՏՐԱԼ ԲՆՈՒԹԱԳՐԵՐԻ ՀԵՏԱԶՈՏՈՒԹՅՈՒՆԸ ՄԻԼԻՄԵՏՐԱՅԻՆ ՏԻՐՈՒՅԹԻ ԷԼԵԿՏՐԱՄԱԳՆԻՍԱԿԱՆ ԱԼԻՔՆԵՐԻ ԱԶԴԵՑՈՒԹՅԱՆ ՆԵՐՔՈ

Ուսումնասիրվել է միլիմետրային տիրույթի էլեկտրամագնիսական ալիքների (ՄՄ ԷՄԱ) ազդեցության հետազոտությունը ցուլի շիճուկային ալբումինի (ՑՇԱ) կառուցվածքի վրա ջրի համար ռեզոնանսային՝ 51,8 *ԳՀց* և ոչ ռեզոնանսային՝ 41,8 *ԳՀց* հաճախություններով ճառագայթահարելու դեպքում: Ստացվել են 60 *րոպե* ընթացքում ճառագայթահարված և չճառագայթահարված ֆիզիոլոգիական լուծույթի և ՑՇԱ-ի կլանման և ֆլուորեսցենտային սպեկտրները: Բացահայտվել է, որ ֆիզիկական լուծույթի սպեկտրալ բնութագրերը փոփոխության չեն ենթարկվել ՄՄ ԷՄԱ ազդեցության ներքո, մինչդեռ ՑՇԱ-ի համանման պարամետրերը էականորեն փոփոխվել են: Ցույց է տրվել, որ ճառագայթահարված նմուշների կլանման սպեկտրները էականորեն տարբերվում են չճառագայթահարված սպիտակուցնի նույն պարամետրերից:

С. В. ГРИГОРЯН

ИССЛЕДОВАНИЕ СПЕКТРАЛЬНЫХ ХАРАКТЕРИСТИК БЫЧЬЕГО СЫВОРОТОЧНОГО АЛЬБУМИНА ПОД ВЛИЯНИЕМ ЭЛЕКТРОМАГНИТНЫХ ВОЛН МИЛЛИМЕТРОВОГО ДИАПАЗОНА

Проведено исследование влияния электромагнитных волн миллиметрового (ММ ЭМВ) диапазона на структуру бычьего сывороточного альбумина (БСА) при резонансной для воды частоте 51,8 *ГГц* и нерезонансной частоте 41,8 *ГГц*. Получены спектры поглощения и флуоресценции необлученного и облученных в течение 60 *мин* образцов физ. раствора и БСА. Выявлено, что спектральные характеристики физ. раствора не претерпевают изменений под влиянием ММ ЭМВ, в то время как аналогичные характеристики БСА значительно изменяются. Выявлено, что спектры поглощения облученных образцов существенно различаются от таковых необлученного белка.