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COMPARISON OF INTERACTION OF METHYL VIOLET AND METHYLENE BLUE WITH HUMAN SERUM ALBUMIN

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In this work the study of the effect of millimeter range electromagnetic waves (MM EMW) on the complexes of human serum albumin (HSA) with methyl violet (MV) has been carried out at the different frequencies, using the methods of fluorescence and thermodenaturation. It was shown that MV binds to HSA, forming a complex, and increasing of the intensity of the protein own fluorescence takes place, meanwhile the stabilization of HSA increases. MM EMW irradiation of HSA by various frequencies invokes additional changes in the protein structure. Thus, at the protein irradiation with the frequency 41.8 GHz the protein stabilization increases as well as hydrophobicity around the tryptophan rises, while at the irradiation with 51.8 GHz the stabilization is expressed less and the hydrophobicity enhancement around the tryptophan can be neglected.

Human serum albumin – methyl violet – irradiation – denaturation parameters – fluorescence spectra

Աշխատանքում իրականացվել է միլիմետրային տիրույթի էլեկտրամագնիսական ալիքների (ՄՄ ԷՄԱ) ազդեցության ուսումնասիրություն մարդու շիճուկային ալբումինի (ՄՇԱ) հետ մեթիլ մանուշակագույնի (ՄՄ) կոմպլեքսների վրա տարբեր հաճախությունների ազդեցության դեպքում՝ կիրառելով ֆլուորեսցենցիայի և ջերմադենատուրացիայի մեթոդները: Ցույց է տրվել, որ ՄՄ-ն կապվում է ՄՇԱ-ի հետ՝ առաջացնելով կոմպլեքս, ընդ որում տեղի է ունենում սպիտակուցի սեփական ֆլուորեսցենցիայի ինտենսիվության աճ, իսկ սպիտակուցի կայունացումը կապման դեպքում աճում է: ՄՇԱ-ի ՄՄ ԷՄԱ ճառագայթահարումը տարբեր հաճախություններով լրացուցիչ փոփոխություններ է առաջացնում սպիտակուցի կառուցվածքում: Այսպես, 41.8 ԳՀց հաճախությամբ սպիտակուցի ճառագայթահարման դեպքում սպիտակուցի կայունացումն աճում է, ինչպես նաև աճում է հիդրոֆոբությունը տրիպտոֆանի շուրջը այն դեպքում, երբ 51.8 ԳՀց հաճախությամբ ճառագայթահարելիս կայունացումն ավելի քիչ է արտահայտված, իսկ տրիպտոֆանի շուրջը հիդրոֆոբության աճի էֆեկտը կարելի է անտեսել:

Մարդու շիճուկային ալբումին – մեթիլ մանուշակագույն – ճառագայթահարում – դենատուրացիայի պարամետրեր – ֆլուորեսցենցիայի սպեկտրներ

В работе исследовано влияние электромагнитных волн миллиметрового диапазона (ММ ЭМВ) на комплексы сывороточного альбумина человека (САЧ) с метильным фиолетовым (МФ) при различных частотах воздействия, с использованием методов флуоресценции и термоденатурации. Показано, что МФ связывается с САЧ, образуя с ним комплекс, при этом происходит увеличение интенсивности собственной флуоресценции белка, а стабилизация белка при связывании растет. Облучение САЧ ММ ЭМВ разными частотами вызывает дополнительные изменения в структуре белка. Так, при облучении белка частотой 41.8 ГГц стабилизация белка растет, а также увеличивается гидрофобность вокруг трип-

тофана, в то время как при облучении частотой 51.8 ГГц стабилизация выражена меньше, а увеличением гидрофобности вокруг триптофана можно пренебречь.

Сывороточный альбумин человека – метильный фиолетовый – облучение – параметры денатурации – спектры флуоресценции

Human serum albumin (HSA) is known as one of the most spread and well-studied proteins in the circulatory system. HSA in the blood has various important functions, including transport of endogenous and exogenous compounds [1-5]. Its role in establishment of osmotic pressure is not big, but it also exists. Albumin is known as a chief transport protein for drug preparations that binding to HSA attain to their destination point. Crystallographic studies revealed that this protein contains 585 amino-acids and consists of three homologous domains, possessing α-helical structure. Though, HSA contains only one tryptophan residue (Trp-214) [6-9]. HSA, having the aforementioned structure, reversibly binds to many low-molecular and bioactive compounds and contributes to their transport by circulatory system. It should be mentioned that HSA binds to hydrophilic as well as hydrophobic compounds by different binding centers that is why a necessity of study of formed complexes emerges.

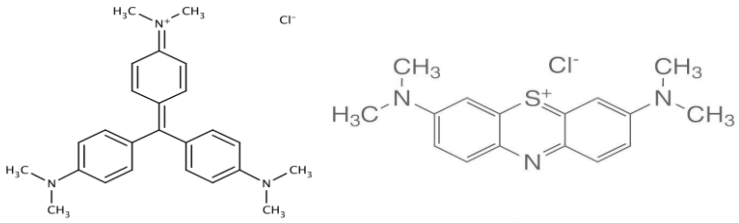

Fig. 1. Chemical structures of methyl violet (a) and methylene blue (b).

From this point of view the interaction of different drugs with HSA is important, because they change a structure and function of HSA differently, provoking alterations of its physical properties [5]. In the present work methyl violet (MV) was chosen as a sample of low-molecular compound, since MV is also known as a crystal violet and it is an external drug preparation for skin diseases (fig. 1a) [10-12]. However, MV possesses a good sterilization, low toxicity and hormesis.

Nowadays, the effect of various ecological external factors on biological systems, being on different levels of organization should be mentioned. One of these factors is an electromagnetic background, accompanying all living material on The Earth. Millimeter range electromagnetic waves (MM EMW) are separated on the scale and their intensity directly increases, which is connected to scientific-technical progress. MM EMW takes the interval of 30-300 GHz and has wavelength 1-10 mm. MM EMW affects biological objects, being on various levels of organization [13-15].

In the presented work the effect of MM EMW on HSA complexes with MV was studied at different frequencies of the influence, applying the methods of fluorescence that is a powerful tool to study the complexes, formed between biomacromolecule and low-molecular compound. The results were compared to those for methylene blue (MB) complexes with HSA, obtained in our laboratory earlier [15].

Materials and methods. Human serum albumin solution (1 %) and methyl violet ("Sigma", USA) were used in experiments. MV concentration was determined spectrophotometrically, using the following value of extinction coefficient for MV ε_{590}=87000 $M^{-1}cm^{-1}$.

1 %-solution of HSA was irradiated by MM EMW frequencies 41.8 GHz and 51.8 GHz. Generator G4-141 ("Istok", Russia) served as a source of MM EMW with working intervals of frequencies 37.5-53.5 GHz and power flux density 64 $\mu Vt/cm^2$. The sample is on the distance 180 mm from waveguide and the electromagnetic field was homogeneous. Irradiation of the samples was carried out in Petri dishes, covered by thin transparent cellophane layer to avoid water evaporation during the irradiation. Width of the irradiating solution was ~1 mm. Samples were irradiated during 60 min. Temperature of the irradiated samples does not change due to the low specific absorption power.

Thermo-denaturation of the complexes was carried out using PYE Unicam SP-8-100 spectrophotometer (England) with automatic heating of thermostating cells with rate 0.5^0C/min. Heating was realized by program device Temperature Programme Controller SP 876 Series 2. Values of temperature and absorption at each increment were taken on PC monitor in program medium LabVIEW 6.0. After obtaining of data the denaturation curves were constructed by the method, described in [15, 16]. From the denaturation curves the denaturation temperature and width of the denaturation interval were determined. The concentration ratio MV/albumin was equal to 1/10.

Fluorescence spectra of HSA and its complexes with MV were obtained by spectrofluorometer Cary Eclipse (Australia). Excitation of the samples was carried out at the wavelength 280 nm and the spectrum was registered in the interval $300 \leq \lambda \leq 500$ nm. After obtaining the fluorescence spectrum of the pure albumin, the titration of HSA solution by the solution of MV was carried out from the concentration ratio 1/2 up to 1/10. Measurement error was equal to 5-10 %.

Results and Discussion. Interaction of MV with HSA was studied using the method of thermal denaturation. Denaturation parameters, i.e. denaturation temperature and denaturation interval width were presented in tab. 1. As it is obvious from the tab. 1, binding of MV with HSA results in protein stabilization, which is indicated by the shift of denaturation curve toward higher temperatures. Meanwhile, at the irradiation of albumin solutions by MM EMW with 41.8 GHz a destabilization of the complexes of HSA-MV occurs, as compared with the non-irradiated complex. At the irradiation by MM EMW with the frequency 51.8 GHz a destabilization strengthens, moreover, as compared to the denaturation curve of pure protein, the stabilization effect is preserved.

Thus, it is revealed from the tab. 1 that the irradiation leads to the fact that a destabilization of the HSA-MV complex takes place, as compared to the non-irradiated complex. For the comparison the denaturation temperature and denaturation interval width of the complexes of HSA with methylene blue (MB) at the irradiation by 41.8 GHz and 51.8 GHz are presented in tab. 2 [15]. As it is obvious from tab. 1 and 2 in the case of MB the irradiation of HSA leads to the counter-effect, the stabilization strengthens in spite of HSA-MV complexes, that is the binding of MV and MB to HSA differs. Irradiation of HSA at the binding of MV leads to decreasing of the complex stabilization, as compared to the non-irradiated protein; at the binding of MB to the irradiated HSA an increase of stabilization occurs in comparison to the non-irradiated protein complex with MB.

From the presented data in tab. 1 it is shown that the complex-formation of HSA with MV results in complex stabilization, since an enhancement of both the denaturation temperature by 6.0^0C and the denaturation interval width by 2.1^0C is observed.

The study of the complexes HSA-MV at the irradiation by MM EMW with different frequencies becomes interesting. At the irradiation of HSA with 41.8 GHz frequency the stabilization effect of MV on HSA weakens, as compared to the non-irradiated complex, but it is preserved comparing to pure HSA. This is indicated by the thermo-denaturation data.

Table 1. Values of temperature and interval width of denaturation of HSA and its complexes with MV in both presence and absence of MM EMW irradiation

	Denaturation temperature, ^{0}C	Interval width of denaturation, ^{0}C
HSA	80.1±0.3	9.5±0.5
HSA-MV	86.1±0.2	11.6±0.5
HSA-MV (at the irradiation MM EMW with 41.8 GHz)	83.9±0.3	11.7±0.4
HSA-MV (at the irradiation MM EMW with 51.8 GHz)	81.5±0.2	11.2±0.5

Denaturation temperature at MM EMW effect with the frequency 41.8 GHz is equal to 83.9^{0}C, while the denaturation interval width does not relevantly differ from that of the non-irradiated complex. At MM EMW irradiation of HSA by the frequency 51.8 GHz the weakening effect of MM EMW strengthens, as the denaturation temperature decreases more, than at the irradiation by 41.8 GHz. In this case the denaturation temperature is equal to 81.5^{0}C and the denaturation interval width – 11.2^{0}C. Proceeding from the data, presented in tab. 1, MV can play a role of stabilizer, leading to increasing of resistance to thermo-denaturation. MM EMW irradiation, vice versa, leads to decreasing of HSA stability, which is connected to MV. It means that MM EMW irradiation weakens the stabilization effect of MV, while binding to HSA.

Table 2. Values of denaturation temperature and denaturation interval width of MB complexes with non-irradiated and irradiated albumin [15]

	T_m, ^{0}C	ΔT, ^{0}C
MB-albumin	76.7±0.2	4.0±0.4
MB-albumin (41.8 GHz)	78.4±0.2	5.2±0.4
MB-albumin (51.8 GHz)	79.7±0.4	5.5±0.5

To reveal the complex-formation peculiarities of HSA-MV, the studies were carried out by the fluorescence spectroscopy method as well. Fluorescence spectra of the albumin and its complexes with MV were obtained and they are not presented here. It is obvious from tab. 3 that the increase of fluorescence intensity occurs at the HSA solution titration by MV solution. This increase may indicate a conformational change of HSA, though the shift of the wavelength, corresponding to fluorescence maximum is not observed (354 nm). But in any case the rise of fluorescence maximum means that the hydrophobicity increase around the fluorescing tryptophan takes place, as a result of which its fluorescence intensity increases. Moreover, at the irradiation of the protein the fluorescence intensity increasing takes some variations.

For both non-irradiated and irradiated HSA the fluorescence intensity was equal to 480 a.u. From the tab. 3 it can be considered that the difference between intensity maxima of HSA-MV complex in the end of the experiment and pure protein is equal to 65 a.u., at the HSA irradiation by 41.8 GHz this difference is equal to 94 a.u. The fact is interesting that at the MM EMW irradiation with 51.8 GHz the aforementioned difference is equal to 21 a.u.

This experimentally obtained result indicates that the protein irradiation by MM EMW with the frequency 41.8 GHz leads to more pronounced increasing of HSA fluorescence intensity that is bound to MV. Based on this fact one can note that the irradiation of HSA by 41.8 GHz leads to such changes of the protein structure that HSA becomes more available to MV molecules that increase the hydrophobicity around the

tryptophan and the fluorescence intensity raises by 94 a.u. In contrast, the protein irradiation with 51.8 GHz leads to less change of the fluorescence intensity – by 21 a.u. This result indicates that HSA irradiation with 51.8 GHz leads to the fluorescence intensity irrelevant change, which means that the protein screening by water molecules occurs in such way that after MV binding to HSA the intensity does not change sharply.

Table 3. Maximal values of the fluorescence intensities in the fluorescence spectra of HSA-MV complexes in the presence and absence of the irradiation (maxima are observed at 354 nm)

	Fluorescence maxima (a.u.)
HSA	480
HSA-MV	545
HSA-MV at the MM EMW irradiation by 41.8 GHz	574
HSA-MV at the MM EMW irradiation by 51.8 GHz	501

Thus, generalizing the obtained results of thermo-denaturation and fluorescence spectra, one can conclude that the binding of MV to HSA results in changing of HSA structure, meanwhile a stabilization of the protein is observed, which is indicated by the denaturation temperature values. On the other hand, it is obvious from the fluorescence spectra that the intensity of the formed complex enhances, as compared to the pure protein, which means that MV binding to HSA leads to the change of the protein in such way that the fluorescing tryptophan enters deeply into hydrophobic environment. At MM EMW irradiation of the protein with 41.8 GHz the following scene is observed: the denaturation curve of the complex irradiated protein-MV is shifted to lower temperature region comparing to that of non-irradiated protein-MV, meanwhile compared to the pure protein the stabilization takes place. According to the fluorescence spectroscopy data the fluorescence intensity of the irradiated protein enhances along with titration of ligand, by about ~94 a.u. From these data one can conclude that the irradiation of HSA with 41.8 GHz invokes the protein structure compacting and at the further binding to MV, the changes result in hydrophobicity increasing around the tryptophan due to which the protein fluorescence rises. At the protein irradiation with 51.8 GHz the complex stabilization decreases, remaining higher, than at the non-irradiated pure protein. Moreover, the fluorescence intensity does not increase relevantly, based on which one can insist that MV binding to the irradiated HSA with 51.8 GHz invokes such structural changes, which means that the protein screening by water molecules surely protects itself from alterations caused by MV. It can have a biologically important value for binding and transferring of drug preparations, since the irradiation results in some destabilization of HSA-MV complexes.

Resulting from the obtained data one can conclude that MV binds to HSA, forming a complex and increasing the own fluorescence intensity of the protein, on the other hand, the protein stabilization rises. MM EMW irradiation of HSA with different frequencies induces additional changes in the protein structure. Thus, at the protein irradiation with 41.8 GHz its stabilization enhances as well as a relevant hydrophobicity increase around the tryptophan occurs, while at the irradiation with 51.8 GHz the stabilization is expressed less and the hydrophobicity enhancement around the tryptophan can be neglected.

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