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THE HYDROPHOBIC-HYDROPHILIC BALANCE IN WATER SOLUTIONS OF PROTEINS AS THE POSSIBLE TARGET FOR EXTREMELY LOW FREQUENCY MAGNETIC FIELDS

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1. INTRODUCTION

Alexander Gurwitsch was the first to demonstrate the presence of extremely low photon radiation (mitogenetic radiation) in the living organisms that plays a regulatory role. Together with his follower Gleb Frank, he attributed this radiation to the ultraviolet diapason of electromagnetic scale[1,2,3].

Numerous further studies showed meanwhile that the spectral range of low intensity radiation in the living organisms is significantly wider, including the visible and infrared waves. During past decades, the radiation of the living organism in the extremely high radiofrequency range (millimeter waves) was also revealed[4]. The interaction of these electromagnetic waves with living organisms seems to proceed via resonance mechanisms. Low intensive millimeter waves are biologically very active, therefore the millimeter waves are now widely used for therapeutic treatments in medicine[4].

A biological activity of the extremely low frequency magnetic fields has been also revealed at the end of past century[5]. The ideas about regulatory and informational role of extremely low frequency magnetic fields in biosphere were developed by B.Vladimirsky and N.Temuryants[6].

Although a high sensitivity of living organisms to the action of weak magnetic fields of the natural and artificial origin is reliably established, their primary physical-chemical mechanisms remain unclear. In this paper, we propose that the biological effects of electromagnetic fields are mediated by the changes in hydrophobic-hydrophilic balance of water-colloidal systems[7].

Hydrophobic interactions are known to play an important role in stabilization of protein and nucleic acids structure and in the regulation of their conformation changes, as well as in stabilization of biological membranes via regulating phase transitions in bilipid layers. The hydrophobic interactions are also important for

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binding specific and non-specific ligands (hormones, vitamins, intracellular messengers, pharmacological substances, etc.) by enzymes, receptors and signaling proteins, as well as for extracellular, membrane, and intracellular transportation of hydrophobic substances. It is possible to expect that slight changes in hydrophobic-hydrophilic balance will result in appreciable biological effects on the molecular, cellular, and systemic level. However, this assumption must be verified on simple experimental models of hydrophobic interactions in biological structures. Therefore, the investigation of the influence of ecologically significant low frequency magnetic field upon hydrophobic interaction with proteins was carried out.

2. THE EXPOSURE OF EXPERIMENTAL SAMPLES TO EXTREMELY LOW FREQUENCY MAGNETIC FIELD (ELFMF)

In our experiments, we have used rectangular and different polarity magnetic field impulses with 8 Hz frequency and 5 or 25 μT induction generated by Helmholtz coils. Such a frequency was selected because of its ecological and geophysical importance and high biological activity[6,8]. Induction vector of magnetic field was parallel to the direction of geomagnetic field vector. Exposure time varied from 1 to 24 hours.

Experiments with sham exposure were carried out to estimate the possible influence of background magnetic fields in the samples' locations. In these cases, experimental samples were located in Helmholtz coils but magnetic field was not generated. A usual noise ELFMF intensity was in 500-1000 time lower than that of magnetic fields in Helmholtz coils.

3. INFLUENCE OF ELFMF UPON THE BINDING OF LOW MOLECULAR NON-POLAR SUBSTANCES WITH HYDROPHOBIC CAVITIES IN PROTEIN MOLECULES

3.1. Basic Experimental Model

The phenomenon of saturation of protein solutions by low-molecular non-polar substances described in Ref. 9 was used as the basis for our experiments. Non-polar substances are known to have an extremely low water solubility because the dissolution of such compounds in water is exothermal. Their dissolution proceeds with increase of temperature and dissipation of heat energy indicating the decrease of enthalpy of the system after mixing up non-polar substances with water ($\Delta H<0$). But the enthalpy decrease is compensated by a decrease of entropy ($\Delta S<0$) due to formation of ordered crystal-hydrates in which non-polar substances are located. Therefore, a hydrophobic dissolution of substances is accompanied by a small increase of free energy of the system ($\Delta G>0$).

Thus, the absolute value of enthalpy decrease is smaller than the entropy decrease, and as a consequence the dissolution of non-polar compounds in water is thermodynamically disadvantageous: $\Delta G = \Delta H - T\Delta S > 0$. The consequence

of the big contribution of entropy component into change of free energy of the system is the repulsion of non-polar molecules by water molecules and its thermodynamically advantageous interaction with each other. Such effect of repulsion of non-polar substances by water molecules is named “hydrophobic interactions”. The increase of concentration of non-polar substances in the water phase results to increase of van der Waals interaction between dissolved molecules and also to formation of associates “covered” with ordered layer of water on its hydrophobic surface. This phenomenon is the cause of division of the system to two phases - the phase that consist of molecules of solvent (water) and the phase of hydrophobic substance (Fig. 1). Thus, the primary factor in dissolution of non-polar substances on the hydrophobic mechanism is the ability of molecules of water to form ordered crystal-hydride structure around molecules of non-polar substance that result to decrease entropy of the system. Any factors that destroy or change the ordered structure of water change to some extent the solubility of hydrophobic compounds in water phase.

If the water solution contains large colloidal particles with hydrophobic cavities, for example protein molecules, then part of non-polar molecules bind with these cavities on the hydrophobic mechanism (Fig. 2). Such binding of the hydrophobic substances with proteins can be studied by means of different quantitative or semi-quantitative methods.

In these studies the chloroform and benzol (Fig. 2) were used as the non-polar substances that non-specifically bind with proteins on hydrophobic mechanism. The saturation of water and water protein solutions by non-polar substance made by means of layering of 3 ml protein solution on 1.5 ml organic phase of non-polar substance and incubation of samples under room temperature during 1-24 hours in dependence of the chosen experimental protocol.

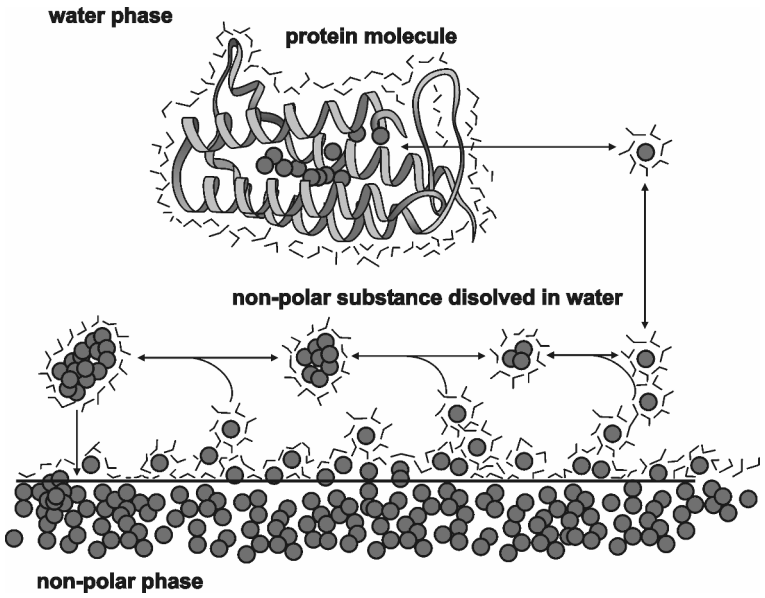

Figure 1. Experimental model of non-specific binding of non-polar ligands with protein molecules.

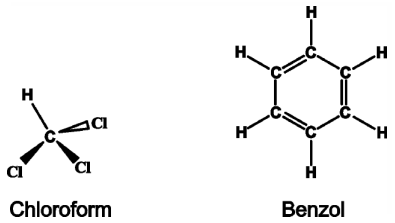

Figure 2. Non-polar ligands used in the experiments.

3.2. The Refractometric Study of Influence of ELFMF on Binding of Benzol with Serum Albumin

3.2.1. Method

The refractometric method of quantitative estimation of hydrocarbons, bound with protein, is based on dissolution of hydrocarbons on hydrophobic mechanism in water-protein solutions that results to the increase of refraction of experimental solutions up to its saturation (Fig. 3). The usage of aromatic hydrocarbons in such model systems is most convenient because their refraction strongly differ from the refraction of water systems that allows to increase the precision and sensitivity of this method.

The volume of the dissolved hydrocarbon in water and water solution of proteins is determined on the base of the rule of additivity of specific refraction[9]. Quantity of the hydrocarbon bound with molecules of protein is calculated as $\mathbf{V_{prot} = V_{prot.\ solution} - V_{water}}$ (ml).

The serum albumin with concentration 0.5% was used in this experimental series.

The rectangular and different polarity magnetic impulses with frequency 8 Hz and induction 5 μT were used in these studies. The ELFMF influenced during saturation of experimental solutions for 5 hours.

The statistical significance of the differences between experimental series was assessed by 5% level Student T test.

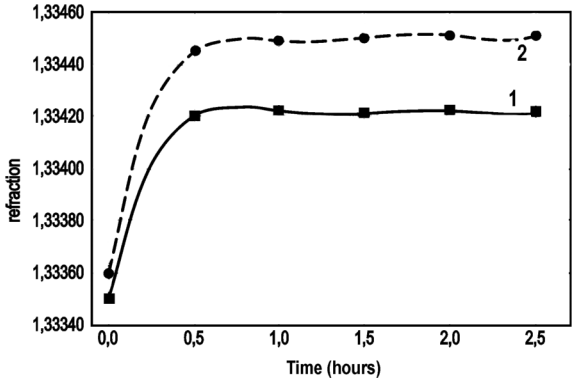

Figure 3. Time dynamics of refraction coefficient of water (1) and protein solution (2) under its saturation by benzol.

3.2.2. Results

Results of refractometric study on binding of benzol with serum albumin testified on significant increase of binding index ($\mathbf{V_{prot}}$) under the influence ELFMF (Fig. 4). This statistically significant increase has been revealed already after 1 hour of MF exposure. Simultaneously with the increase of binding of benzol with protein the solubility of used hydrocarbon in the water statistically significant decreased (Fig. 5).

So, these experimental results testify on the following features of influence of ELFMF on hydrophobic interactions. In the used model conditions, the influence of impulse 8 Hz magnetic field changes the dynamical balance in the system *benzol : water : protein* that resulted in pushing out of benzol from water to hydrocarbon phase, on one hand, and on the other, pushing out of benzol to non-polar phase of hydrophobic cavities of protein molecules.

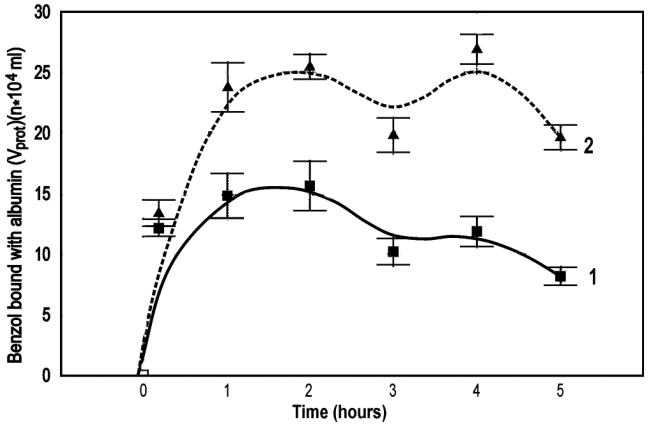

Figure 4. Dynamics of binding of benzol by serum albumin (V_{prot} = $V_{prot.\ solution}$ − V_{water} (ml)) in control samples (1) and under the influence of 8 Hz 5 μT magnetic field (2).

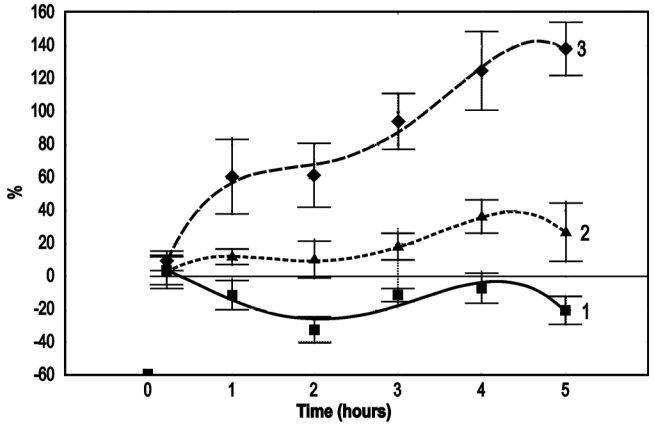

Figure 5. Influence of 8 Hz 5 μT magnetic field on benzol solubility in water (1) and in 0.5% solution of serum albumin (2) and also on binding of benzol by albumin (3) in ±% relative to control samples.

3.3. The Spectrophotometric Study of Influence of ELFMF on Binding of Chloroform with Serum Albumin

3.3.1. Method

The spectrophotometric method of studying of non-specific liganding of non-polar substances with biopolymers is based on registration of changes of light absorption spectra in biopolymers caused owing to changes of polarity of chromophore surrounding. The spectral changes can be caused both by the influence of hydrocarbon substances on chromophores and influence of solvent (water molecules), accessibility to chromophores of which can be raised, or reduced owing to liganding of non-polar substances or/and owing to ligand-induced conformation changes of macromolecule.

Thus, this method does not allow to obtain information about absolute amount of non-polar molecules that are bound with biopolymers, but allows to investigate qualitatively and semi-quantitatively the process of liganding and also the features of influence of non-polar and non-specific ligands on conformation state of macromolecules. With the purpose to increase the informational capacity of this method, the integral absorption spectera and also differential spectera were used for analysis. Differential spectera were obtained

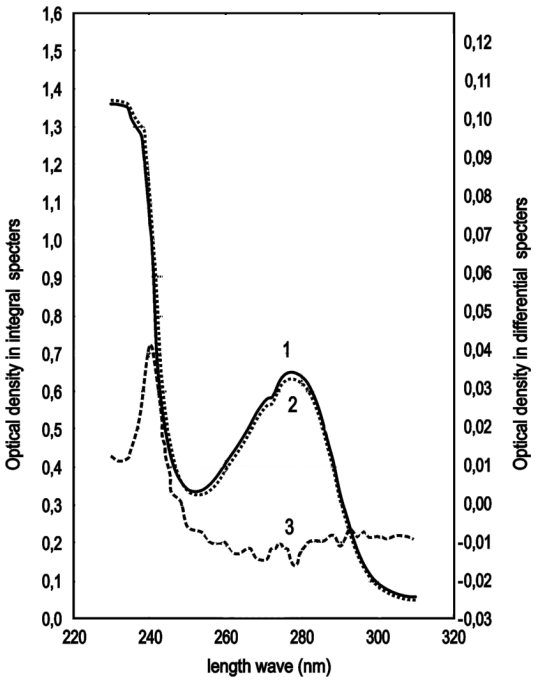

Figure 6. Integral (1, 2) and differential (3) spectera of 0.1% solutions of serum albumin under its saturation by chloroform. 1 - integral specterum of native *serum albumin*; 2 - integral specterum of *serum albumin* saturated by chloroform; 3 - differential spectera as difference between spectra of saturated and native proteins.

by subtraction of the absorption spectera of native protein of the integral spectrum of ligand-loaded protein (Fig. 6) or by means of direct instrumental registration using differential scheme.

The concentration of serum albumin in this experiment was 0.1%.

The rectangular and different polarity impulses with frequency 8 Hz and induction 25 μT used in these studies. The ELFMF influenced during saturation of experimental solutions for 24 hours.

The statistical significance of the differences between experimental series was assessed by 5% level Student T test.

3.3.2. Results

The differential spectera of 0.1% solutions of serum albumin after their incubation with chloroform are shown in Figure 7. The saturation of protein by chloroform realized in two spectral shifts: the long-wave shift (red-shift) in the range of 235-250 nm and also weak short-wave shift (blue-shift) in range of 260-300 nm. The formation of red-shift under the saturation protein molecules of proteins by chloroform testifies about the decrease of polarity of surrounding around the peptide bounds and aminoacid radicals located on protein surface and contacted with molecules of water.

At the same time the cause of the formation of blue-shift is the increase polarity in the internal hydrophobic cavities of molecules of albumin where majority on non-polar aromatic aminoacid radicals is located. The chloroform is considered as the non-polar substance, but dipole moment (μ) of this molecules is about μ=1.06 D (for comparison μ_{water} = 1.84 D; $\mu_{benzol} \approx \mu_{heptan} \approx 0$). Therefore the presence of this substances in hydrophobic cavities induces the spectral shift in blue range.

The influence of ELFMF has statistically significantly ($p<0.05$) increased the amplitude of red shift but not changed the characteristics of weak blue shift (Fig. 7). There were no changes of differential spectera in the experiments with sham exposure. So, the increase of amplitude of red shift in range of 235-250 nm testifies on the MF-induced increase of binding of chloroform on protein surface.

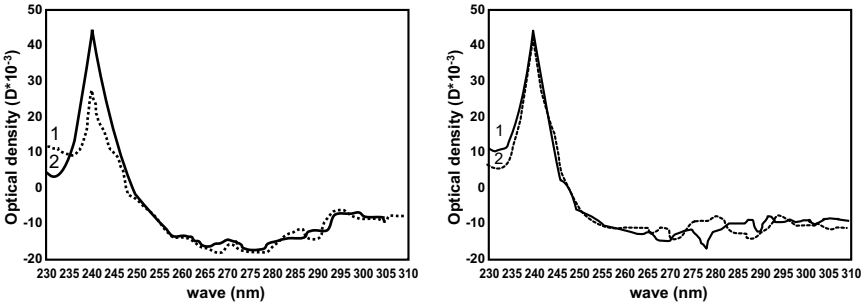

Figure 7. Differential spectera of 0,1% solutions of serum albumin after their saturation by chloroform in experiments with influence of 8 Hz 25 μT magnetic field (left figure) and sham exposure (right figure). The presented spectral lines are the average for 2, 4, and 24 hour exposure to ELFMF. Specters were obtained relative to protein solution without chloroform saturation. 1-control samples; 2 - magnetic field of sham exposure.

Being based on the literature data about the acceleration of coagulation colloidal particles under the magnetic field treatment and also about the increase of adsorption of colloidal particles on surface of solid bodies and air cavities on water[10] it can be supposed that this increase of binding of chloroform with protein molecules can stimulate the reversible formation of the protein associates by means of hydrophobic mechanisms that additionally amplifies the red shift in absorption spectera of studied protein.

3.4. The Spectrophotometric Study of Influence of ELFMF on Binding of Chloroform with Cytochrome *c*

3.4.1. Methods

The binding of chloroform with cytochrome *c* was studied on changes of optical absorption spectera of this protein in Soret-peak wavelength range 350-440 nm. The saturation of 0.05% solutions of cytochrome ***c*** has been resulted in formation of blue shift of Soret-peak (Fig. 8). The value of this shift is depended on time of incubation of protein with chloroform. The differential spectera were obtained by measurement of optical density of the chloroform-saturated protein solutions relativity to the protein solutions that do not contain chloroform.

The enzymatic activity of cytochrome c was studied by measurement of speed of rise of optical density on λ=550 during reduction of enzyme by ascorbic acid.

The concentration of cytochrome *c* in this experiment was 0.05%.

The rectangular and different polarity magnetic impulses with frequency 8 Hz and induction 25 μT were used in these studies. The ELFMF influenced during saturation of experimental solutions for 24 hours.

The statistical significance of the differences between experimental series was assessed by 5% level Student T test.

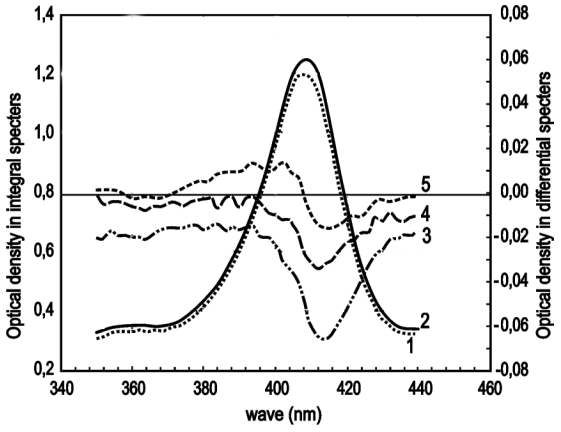

Figure 8. Integral (1, 2) and differential (3-5) spectera of 0.05% solutions of cytochrome ***c*** (oxidized form) under its saturation by chloroform. (1 - integral specterum of native cytochrome ***c***; 2 - integral specter of *cytochrome* ***c*** saturated by chloroform; 3-5 – differential spectera as difference between spectra of saturated and native proteins after 2, 4, and 24 hours of saturation.

3.4.2. Results

The analysis of differential spectera has shown that the influence of ELFMF was more significant during the first hours of saturation of cytochrome c by chloroform. ELFMF has increased the formation of blue shift (Fig. 9). The maximum of differential spectera and the difference λ_{min}-λ_{max} were spectral parameters that more sensitive to influence of studied physical factor (table 1). The statistical increase of D_{max}-D_{min} was revealed after daily exposure of chloroform-saturated solution of cytochrome ***c*** to magnetic field. This parameter characterizes the quantity of bound non-polar substance too[11] that also allows us to conclude about the increase of binding of chloroform with cytochrome c under the influence of ELFMF.

The study of enzymatic activity of cytochrome ***c*** has shown the small but statistically significant ($p<0.05$) inhibitory effect of the slow saturation of protein solution by chloroform on 10% after 2 hours. This fact of presence of the relatively high enzymatic activity testifies on insignificant conformational changes of studied protein under such soft and slow procedure of saturation.

The experiments with influence of ELFMF showed the accelerated inhibition of enzymatic activity due to MF-induced increase of binding of chloroform with protein. The statistically significant decrease ($p<0.05$) of enzymatic activity on 15-20% was shown already after 1 hour of incubation of protein solution with chloroform.

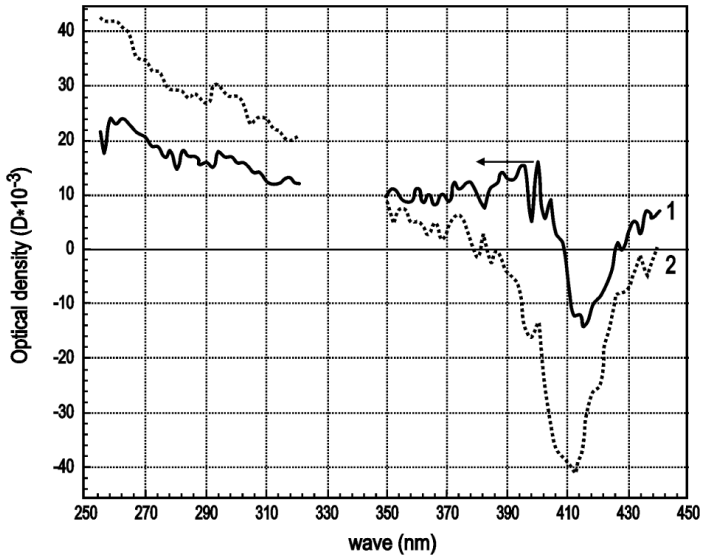

Figure 9. Differential spectera of 0.05% solutions of cytochrome ***c*** after their saturation by choloroform during 2 hours in control samples (1) and under the 8 Hz 25 μT magnetic field influence (2). Spectera were obtained relatively to protein solution without chloroform saturation. 1 – control samples; 2 - ELF MF of sham exposure.

Table 1. The basic characteristics of differential spectera of cytochrome *c*

Expo-sure time (hours)	Parameters	Control samples	Sham exposure	ELF MF influence	Changes relative to control samples (%)	Changes relative to sham exposed samples (%)
2	λ_{max}, nm	397.3 ± 1.9	394.0 ± 3,8	387.8 ± 5.0*	-2.9	-1.6
4	λ_{max},, nm	391.5 ± 3.0	3988 ± 1.6	390.3 ± 43**	-0.6	-2.1
24	λ_{max}, nm	393.2 ± 18	395.8 ± 2.2	391.0 ± 5.1	-1.1	-1.2
2	λ_{min}, nm	413.9 ± 0,5	415.0 ± 1.3	413.0 ± 0.9	-0.2	-0.5
4	λ_{min}, nm	412.7 ± 0.7	414.3 ± 2.6	413.9 ± 0.7	0.3	-0.1
24	λ_{min}, nm	414.4 ± 0.4	415.2 ± 0.4	415.0 ± 1.0	0.0	0.0
2	λ_{min}-λ_{max}, nm	16.7 ± 1.7	21.0 ± 3.4	25.2 ± 4.3*	76.2	20.0
4	λ_{min}-λ_{max}, nm	21.3 ± 2.9	15.5 ± 2.9	23.6 ± 3.7**	24.0	52.3
24	λ_{min}-λ_{max}, nm	21.2 ± 1.9	19.4 ± 2.4	24.0 ± 4.1	32.7	23.7
2	D_{max}-D_{min}, o.d.u.	0.034 ± 0.002	0.038 ± 0.007	0.038 ± 0.006	12.6	0.0
4	D_{max}-D_{min}, o.d.u.	0.032 ± 0.002	0.034 ± 0,004	0.033 ± 0.003	2.8	-4.4
24	D_{max}-D_{min}, o.d.u.	0.0463 ± 0.003	0.046 ± 0.004	0.055 ± 0.002*,**	18.3	20.1

* Statistically significant changes relative to control samples; ** statistically significant changes relatively to sham exposed samples.

3.5. The Spectrophotometric Study of Influence of ELFMF on Binding of Chloroform and Benzol with Methemoglobin

3.5.1. Method

The binding of chloroform and benzol with methemoglobin was studied on changes of optical absorption spectera of this protein in Soret-peak wavelength range 350-450 nm. The saturation of 0.02% solutions of methemoglobin by chloroform or benzol realized to formation of red shift of Soret-peak (Fig. 10). The value of this shift depended on time of incubation of protein with non-polar substances. The integral absorption spectera were registered in this experimental series. The differential spectera were calculated as the difference between integral spectera of saturated protein solution and non-saturated one.

The concentration of methemoglobin in this experiment was 0.02%.

The rectangular and different polarity impulses with frequency 8 Hz and induction 25 μT used in these studies. The ELFMF influenced during saturation of experimental solutions for 24 hours.

The statistical significance of the differences between experimental series was assessed by 5% level Student T test.

3.5.2. Results

The analysis of spectera of methemoglobin in the Soret's peak sowed the maximum absorption in 404 nm. It is necessary to note that the specter of native hemoglobin significantly differs from specter of methemoglobin. The maximum of Soret's peak of native hemoglobin is in the range 412-415 nm. The cause of such differences is the different conformation state of these protein molecules where hem of methemoglobin is more accessible to polar molecules of water, thereof maximum of Soret's peak of methemoglobin significantly shifted in blue range.

The spectral data testify on red shift to 405 nm during saturation of protein solution by chloroform (Table 2.3). In our opinion this fact testifies on binding of chloroform with cavities containing hem and also on decrease of accessibility of water molecules to this chromophore.

The analysis of the absorption specter of methemoglobin in Soret's peak has showed the statistically significant influence of ELFMF on absorption spectera of this protein (Table 2). The influence of ELFMF realized to increase of red shift caused by chloroform binding up to 5-6 nm. The maximal changes were revealed after two hour of exposure to magnetic field but mean value of effect of ELFMF for all exposure time was 1.5 nm (Table 2). The cause of these effects of ELFMF, to our mind, is the increased binding of chloroform with methemoglobin. Such changes were absent in the experiments with sham exposure (Table 3).

The analysis of differential spectera additionally testifies on the statistically significant influence of ELFMF on binding of chloroform with methemoglobin. In particular, the influence of ELFMF has changed also the form of Soret's peak that has realized in dynamical changes of value of maximum and minimum in the differential spectera (Table 4). At the same time the amplitude of differential spectera (D_{max}-D_{min}) was insensitive to the influence of studied physical factors. The statistically significant changes were not revealed in experiments with sham exposure (Table 4).

The saturation of methemoglobin solution by benzole realize to more weak spectral changes than in the experiments with chloroform (Tables 5 and 6). The

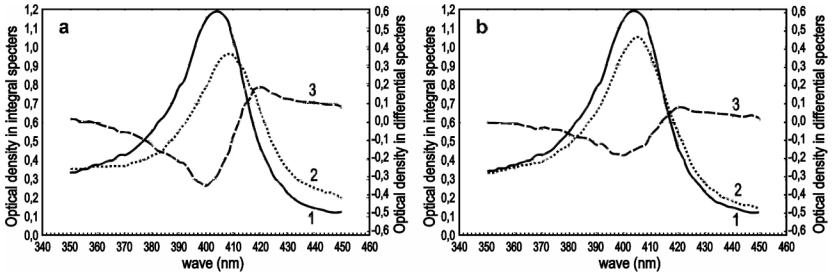

Figure 10. Integral (1, 2) and differential (3) spectera of 0.02% solutions of methemoglobin under its saturation by chloroform (a) and benzol (b) during 1 hour. 1 - integral specter of methemoglobin; 2 - integral specter of *met-hemoglobin* saturated by chloroform; 3-5 – differential spectera as difference between spectra of saturated and native proteins after 2, 4, and 24 hours of saturation.

Table 2. The basic characteristics of integral spectera in range 350-450 nm of met-hemoglobin after its saturation by chloroform and ELF MF influence

Exposure time (hours)	Parameters	Control samples	MF influence	Saturation by chloroform	Saturation by chloroform and MF influence	MF-induced spectral shift relative to samples saturated by chloroform (nm)
1	λ_{max}, nm	404.0±0.3	403.8±0.2	408.0±0.5*	409.3±05*	+1.3
2	λ_{max}, nm	403.8±0.2	404.8±0,3	406,8±0.3*	409.3±0,6*,**	+2.5
4	$\lambda_{max,,}$ nm	404.0±0.3	404.2±0.2	407.3±0.9*	408.2±0.4*	+0.9
24	λ_{max}, nm	405.0±0.5	404.6±0.3	407.6±0.6*	409.1±0.7*	+1.2
1 - 24	$\Delta\lambda_{mean,}$ nm					+1,47±0,35 p<0.05
1	D_{max}, o.d.u.	1.196±0.070	1.219±0.065	0.978±0.072*	0.967±0.071*	
2	D_{max}, o.d.u.	1.254±0.062	1.230±0.055	0.957±0.057*	0.934±0.060*	
4	D_{max}, o.d.u.	1.193±0,058	1.229±0.057	0.947±0.053*	0.985±0.054*	
24	D_{max}, o.d.u.	1.206±0.062	1.185±0.085	0.849±0.090*	0.853±0058*	

* Statistically significant changes relative to control samples; ** statistically significant changes relatively to samples saturated by chloroform.

Table 3. The basic characteristics of integral spectera in range 350-450 nm of methemoglobin after its saturation by chloroform and sham influence

Exposure time (hours)	Parameters	Control samples	Sham influence	Saturation by chloroform	Saturation by chloroform and sham influence	Spectral ship relative to samples saturated by chloroform (nm)
1	λ_{max}, nm	404.4±0.3	404.2±0.2	408.5±04*	409.1±03*	+0.6
2	λ_{max}, nm	404.2±0.2	404.0±0.0	407.8±0.5*	408.4±04*	+0.6
4	$\lambda_{max,,}$ nm	404.4±0,4	404.0±0.0	407.6±0.4*	407.7±0.4*	+0.1
24	λ_{max}, nm	404.4±0.3	404.0±0.0	408.6±0.3*	408.2±0.2*	-0.4
1 - 24	$\Delta\lambda_{mean,}$ nm					+0.22±0.23
1	D_{max}, o.d.u.	1.207±0,020	1.197±0,020	0.991±0.057*	1.006±0.046*	
2	D_{max}, o.d.u.	1.208±0.022	1.150±0.051	0.989±0.044*	0.992±0.043*	
4	D_{max}, o.d.u.	1.239±0.017	1.218±0.085	0.909±0.045*	0.958±0.056*	
24	D_{max}, o.d.u.	1.280±0.062	1.258±0.086	0.919±0.110*	0.946±0.097*	

* Statistically significant changes relative to control samples; ** statistically significant changes relatively to samples saturated by chloroform.

possible cause of smaller values of red shift is the lager size of molecules of benzol that prevent their penetration to hydrophobic caviars containing hem. Therefore the using of optical method for estimation of ELFMF influence on interaction of benzol with methemoglobin is not so effective and statistically significant changes were not revealed after separated exposure. But there was a stabile tendency to increase the red shift in integral spectera on 0,6 nm during all time of exposure to magnetic fields (Table 5) and, in general, this fact does not contradict the results obtained in experiments with chloroform (Table 2.3). On the other hand, there were no any changes or tendencies in experiments with sham exposure (Table 6).

The analysis of differential spectera has confirmed the statistically significant influence of ELFMF on spectral characteristics of methemoglobin loaded by benzol (Table 7). The influence of ELFMF has changed the form of

Table 4. The basic characteristics of differential spectera of methemoglobin after its saturation by chloroform

Exposure time (hours)	Parameters	Control samples	Sham exposure	ELF MF influence	Changes relatively to control samples (%)	Changes relatively to sham exposed samples (%)
1	λ_{max}, nm	419.8±0.7	419.4±1.1	419.4±0.7	-0.1	0.0
2	λ_{max}, nm	419.2±0.8	419.0±1.5	418.7±1.1	-0.1	-0.1
4	λ_{max}, nm	421.2±1.3	421.8±1.4	419.8±1.1	-0.3	-0.5
24	λ_{max}, nm	422.7±1.6	424.0±2.0	**417.9±1.0 *,****	**-1.1**	**-1.4**
1	λ_{min}, nm	399.1±0.7	400.0±0.8	**395.7±1.4 *,****	**-0.9**	**-1.1**
2	λ_{min}, nm	398.7±0.5	398.4±0.9	398.8±0.6	0.0	+0.1
4	λ_{min}, nm	397.9±1.23	399.2±0.7	398.6±0.4	+0.2	-0.2
24	λ_{min}, nm	398.9±0.9	397.8±1.7	398.0±0.7	-0.2	+0.1
1	λ_{min}-λ_{max}, nm	20.7±0.6	19.1±0.9	**23.7±1.8 *,****	**+14.5**	**+22.2**
2	λ_{min}-λ_{max}, nm	20.5±0.9	20.6±1.6	19.9±1.2	-2.9	-3.4
4	λ_{min}-λ_{max}, nm	23.3±1.1	22.6±0.9	21.2±1.1	-9.0	-6.2
24	λ_{min}-λ_{max}, nm	23.8±1.4	26.2±2.0	19.9±1.4 **	-15.3	-24.0
1	D_{max}-D_{min}, o.d.u.	0.589±0.036	0.670±0.049	0.639±0.055	+8.5	-4.6
2	D_{max}-D_{min}, o.d.u.	0.543±0.032	0.552±0.055	0.592±0.038	+9.0	+7.2
4	D_{max}-D_{min}, o.d.u.	0.590±0.029	0.591±0.034	0.546±0.036	-7.5	-7.6
24	D_{max}-D_{min}, o.d.u.	0.602±0.028	0.558±0.039	0.594±0.058	-1.3	+6.5

* Statistically significant changes relative to control samples; ** statistically significant changes relative to sham exposed samples.

Soret's peak more significantly that resulted in statistically significant decrease of value of minimums (λ_{min}) and increase amplitudes (D_{max}-D_{min}) in the differential spectera in the first hours of exposure to magnetic fields (Table 7). The statistically significant changes were not revealed in the experiments with sham exposure (Table 7).

Table 5. The basic characteristics of integral spectera in range 350-450 nm of methemoglobin after its saturation by benzol and ELF MF influence

Exposure time (hours)	Parameters	Control samples	MF influence	Saturation by benzol	Satutation by benzol and MF influence	MF-induced wave shift (nm)
1	λ_{max}, nm	404.0±0.3	403.8±0.2	404.6±0.7	405.6±0.7	+1.0
2	λ_{max}, nm	403.8±0.2	404.8±0.3	404.8±0.3	405.6±0.6	+0.8
4	λ_{max},, nm	404±0.3	404.2±0.2	405.0±0.3	405.3±0.3	+0.3
24	λ_{max}, nm	405.0±0.5	404.6±0.3	405.4±0.5	405.6±0.4	+0.2
1 - 24	$\Delta\lambda_{mean,}$ nm					**+0.57±0.19** p<0.05
1	D_{max}, o.d.u.	1.196±0.070	1.219±0.065	1.175±0.044	1.183±0.039	
2	D_{max}, o.d.u.	1.254±0.062	1.230±0.055	1.132±0.037	1.173±0.019	
4	D_{max}, o.d.u.	1.193±0.058	1.229±0.057	1.150±0.036	1.174±0.024	
24	D_{max}, o.d.u.	1.206±0.062	1.185±0.085	1.106±0.041	1.009±0.063	

* Statistically significant changes relative to control samples; ** statistically significant changes relative to samples saturated by chloroform.

Table 6. The basic characteristics of integral spectera in range 350-450 nm of *met-hemoglobin* after its saturation by benzol and sham influence

Exposure time (hours)	Parameters	Control samples	Sham influence	Saturation by benzol	Satutation by benzol and sham influence	Wave shift (nm)
1	λ_{max}, nm	404.4±0.3	404.2±0.2	405.6±0.6	405.4±0.4	-0.2
2	λ_{max}, nm	404.2±0.2	404.0±0.0	405.2±0,3*	405.6±0.4*	+0.4
4	λ_{max},, nm	404.4±0.4	404.0±0.0	405.8±0,4*	405.4±0.3	-0.4
24	λ_{max}, nm	404.4±0.3	404.0±0.0	407.0±0.6*	406.0±0.3*	-1.0
1 - 24	$\Delta\lambda_{mean,}$ nm					-0.30±0.28
1	D_{max}, o.d.u.	1.207±0.020	1.197±0.020	1.077±0.058	1.090±0.051	
2	D_{max}, o.d.u.	1.208±0.022	1.150±0.051	1.116±0.033	1.118±0.038	
4	D_{max}, o.d.u.	1.239±0.017	1.218±0.085	1.105±0.025	1.107±0.036	
24	D_{max}, o.d.u.	1.280±0.062	1.258±0.086	1.110±0.112	1.054±0.054	

* Statistically significant changes relative to control samples; ** statistically significant changes relatively to samples saturated by chloroform.

Table 7. The basic characteristics of differential spectera of *met-hemoglobin* after its saturation by benzol

Expo-sure time (hours)	Parameters	Control samples	Sham exposure	ELF MF influence	Changes relative to control samples (in %)	Changes relative to sham exposed samples (in %)
1	λ_{max}, nm	422.3±1.8	420.2±1.6	419.4±1.4	-0.7	-0.2
2	λ_{max}, nm	421.5±1.5	423.0±1.7	421.9±1.4	+0.1	-0.3
4	λ_{max},, nm	419.9±1.3	422.0±1.2	419.1±1.3	-0.2	-0.7
24	λ_{max}, nm	422.1±0.9	424.0±1.6	417.9±1.0	-1.0	-1.4
1	λ_{min}, nm	398.1±0.8	400.2±1.3	**394.0±1.6 *,****	**-1.0**	**-1.5**
2	λ_{min}, nm	399.4±1.0	397.3±0.7	**394.5±1.7 ***	**-1.2**	**-0.7**
4	λ_{min}, nm	399.3±1.6	399.8±0.6	397.4±1.0	-0.5	-0.6
24	λ_{min}, nm	397.9±1.2	398.5±2.0	398.9±1.1	+0.3	+0.1
1	λ_{min}-λ_{max}, nm	24.2±1.8	20.0±1.8	**25.4±1.7****	+5.0	**+27.0**
2	λ_{min}-λ_{max}, nm	22.1±1.5	25.7±1.7	**27.4±2.3***	**+24.0**	+6.6
4	λ_{min}-λ_{max}, nm	20.6±1.7	22.2±1.9	21.7±1.7	+5.3	-0.17
24	λ_{min}-λ_{max}, nm	24.2±1.6	25.5±1.9	**19.0±0.9*,****	**-21.5**	**-25.5**
1	D_{max}-D_{min}, o.d.u.	0.344±0.032	0.344±0.042	0.338±0.051	-1.7	-1.7
2	D_{max}-D_{min}, o.d.u.	0.283±0.023	0.275±0.028	**0.365±0.029*,****	**+29.0**	**+32.7**
4	D_{max}-D_{min}, o.d.u.	0.341±0.020	0.278±0.037	**0.382±0.025 ****	+12.0	**+37.4**
24	D_{max}-D_{min}, o.d.u.	0.461±0.026	0.474±0.038	0.430±0.036	-6.7	-9.2

* Statistically significant changes relative to control samples; ** statistically significant changes relative to sham exposed samples.

4. DISCUSSION

The analysis of results that were obtained in the different experimental models and by means of different methods testifies to the binding of low-molecular hydrophobic substances with protein molecules. Is this binding specific or not? On our opinion, the binding of substances on hydrophobic mechanism in general is non-specific because it takes place in different colloidal systems[9]. At the same time, the sorption capacity of biomacromolecules can strongly depend on specific structural-functional properties. For example, the basic function of serum albumin is the binding and transportation of hydrophobic compounds such as fatty acids, bilirubin and others.

The next important question is about conformational changes induced by filling of hydrophobic cavities in protein molecules. According to[9], the

saturation of proteins by hydrocarbons not results in significant changes of secondary structure of protein. Researchers speculatively concluded that tertiary conformation does not change too. But, in our opinion these conclusions were hasty. Our experiments showed that the saturation of protein solutions by non-polar substances is the dynamical and fluctuative process (fig. 4 & 5; table 1) accompanied by spontaneous cooperative and synchronous conformational transition in protein molecules. Such dynamical behavior of proteins in solution is known for a long time[12, 13]. At the same time it is known for a long time that interaction of ligands with proteins induces specific and non-specific conformational changes in protein molecules[14]. On our opinion the non-specific binding of non-polar substances by proteins induces conformational changes too. Therefore the "blue" shift in the absorption spectera of oxidized cytochrome ***c***, caused by binding of chloroform, can be caused by two reasons - by introduction of molecules of chloroform with nonzero dipole momentum (μ=1,06 D) in hydrophobic cavities of protein or/and by conformational changes macromolecule that increase availability of chromophore (gem) to strongly polar molecules of water (μ=1,84 D). It is necessary to note that similar effect was revealed in our experiments with native oxygenated hemoglobin[15]. But the features of conformation of methemoglobin are those that initially gem more strongly interacts with molecules of water. This realizes to shift of Soret peak in the absorption spectrum to short-wave range ("blue" shift) on 8-10 nm (λ_{max} = 404 nm) in comparison to absorption spectrum of native hemoglobin (λ_{max}=412-414 nm). Therefore the saturation of molecules of methemoglobin by chloroform results in the decrease of polarity of around gem and shift of maximum in absorption spectrum to the red range (fig.10). It is important to note that slow saturation of protein solution by non-polar low-molecular substances without active hashing not results in strong denaturation of proteins. The experimental results on high enzymatic activity of cytochrome *c*[16] and on absence of significant changes of electrophoretic mobility of serum albumin[17] after saturation by chloroform testify to it.

The carried out researches do not show statistically significant influence of ELF MF on properties of native proteins. At the same time, all statistically significant effects of ELF MF were revealed in the experiments with saturation of proteins by non-polar substances. So, it can be assumed that the "conformation stressing" of proteins, i.e. its structural modification, caused by loading by low-molecular non-specific ligands, is the factor which increase the sensitivity of protein molecules to the influence of ELF MF. The acceleration and increase of binding of hydrophobic substances by protein molecules is one of the features of the influence of ELF MF.

What are possible mechanisms of influence of ELF MF that result in increase of sorption capacity of protein molecules? Probably, the main cause of it is the decrease of solubility of hydrophobic substances in water. The obtained by refractometric methods experimental data testify to it (see part 2.2). The decrease of solubility, as a consequence, resulted in to moving of non-polar from water to basic volume of the same substance and also to the hydrophobic cavities of protein molecules dissolved in water. So, ELFMF changes the dynamical balance in the system *non-polar substance : water : protein*. The cause of this shift is connected with the changes of hydrophobic interactions in the protein

solutions that directed to increase of hydrophobicity of low-molecular substances. As it is noted above, the properties of water is the basic factor that determines the dissolution of substances on hydrophobic mechanism. The dissolution of non-polar substances results in structurization of water and formation of crystal-hydrates covering hydrophobic molecules. Thus, it is possible to assume, that ELFMF influence on stability of such crystal-hydrates that results in sticking of non-polar molecules to each other and their raised adsorption in hydrophobic cavities of protein molecules. Analysis of literature data[10] confirms the revealed in our experiments facts of changes of solubility of substances under the treatment by magnetic field. So, all these facts confirm the hypothesis that water is primary acceptor of influence of ELFMF, but according to our data the MF-induced changes of properties of water first of all results in changes of hydrophobic interactions in the water-colloidal systems.

It is necessary to note one important circumstance. The mechanism of dissolution of gases in water media is similar to dissolution of hydrophobic compounds. The dissolution of gases is accompanied by structurization of water, too, and also by formation of clatrate cavities inside which molecules of gases are located[18,19]. Therefore it is possible to expect the influence of ELFMF on solubility of biologically important gases. On the other hand the MF-induced changes of structural-dynamical properties of water and its ability to form clatrate structures can be one of the causes of changes of binding of Ca^{2+} with proteins that are revealed in numerous studies[20].

The changes in the hydrophobic interactions caused by the influence of ELFMF can result in far-reaching biological consequences. It is known that the binding of hormones and other biologically active non-polar substances with receptors, enzymes and transport proteins and also reversible interactions of proteins with each other and with biological membranes is realized on hydrophobic mechanisms. The hydrophobic interactions play an important role in structural-functional organization and regulation of biological macromolecules and membranes[21]. Therefore, we can assume that the any small changes in hydrophobic-hydrophilic balance finally should result in shifts of activity of various molecular, cellular and system processes and changes of time organization of biological processes. These system changes should strongly depend on the functional specialization of cells and biological tissues.

Thus, the results of the researches that have been carried out by us at different times on base of the different protein models and the methods of investigations, allows us to make the following conclusions:

1. The saturation of protein solutions by low molecular hydrophobic substances results in non-specific binding of these substances with proteins on hydrophobic mechanisms accompanied by changes of conformation in molecules of protein.
2. ELFMF does not significantly influence on the structure of native proteins but changes the structure of proteins with modified conformation.
3. The influence of ELFMF increases the non-specific binding of non-polar substances with proteins and amplifies the conformation changes induced by such changes.

4. The influence of ELFMF decreases the solubility of low-molecular non-polar substances in water as through raising their non-polarity. More probably, the cause of this phenomenon is the changes of structural-dynamical properties of water that result in shifts of hydrophobic-hydrophilic balance in water-colloidal systems.

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