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INTRINSIC FLUORESCENCE OF HUMAN BLOOD SERUM UNDER THE INFLUENCE OF 50 HZ HIGH TENSION ELECTRIC FIELD

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Abstract- The paper investigates the intrinsic fluorescence of human blood serum proteins exposed to the electric field of 50 Hz high tension 20 kV/m, exposure time of 30 minutes. The intensity of total fluorescence (λ_{ex} = 280 nm, λ_{em} = 332 nm) of the experimental samples was reduced by ~8%, and fluorescence of tryptophan's (λ_{ex} = 295 nm, λ_{em} = 334 nm) decreased by ~15%, in all measurements the maxima are slightly shifted to the left relative to the control. Negative picks of second derivatives of these fluorescence spectra some places showed decreased from ~21% to 200% and the some places increased from ~7% to 88%. In general, the intensity of some maximums changed relative to the control, without changed the position. Peaks on the left relative to the central maximum (332–334 nm) show a slight shift to the left, and the maxima on the right show a slight shift to the right. The results obtained that 30-minute exposure to HVEF 50 Hz on blood serum causes structural disturbances in blood serum proteins molecules, in the form of increase or decrease of the hydrophobic areas, which is reflected changes the intensity and shifts of fluorescence maxima in the range of 300-400 nm. These results can be informative for determination structural and functional changes occurring at the molecular level, and may be used as direct indicator/marker to assess the adverse effects of 50 Hz high voltage EF.

Keywords: 50 Hz High Voltage EF, Blood Serum Proteins, Intrinsic Fluorescence, Protein Fluorescence.

1. INTRODUCTION

Electromagnetic fields (EMF) of industrial frequency (50/60 Hz) created by high-voltage power transmission lines (HVPL) and various electrical installations exceed many times average level of natural EMF intensity and these parameters go beyond the adaptive abilities of organisms on earth [1]. Influences of the low intensity EMF including HVPL on the person's health has be studied long times, but its biological effects and mechanisms of influence on the health of human have not revealed up to now, what led to a long-time debatable the hazards of EMF of HVPL in science world. Currently, the scientific literature has accumulated numerous epidemiological, experimental and clinical information indicating the negative impact of this frequency of EMF on the health of man, people are more likely to have various disorders, including cardiovascular, nervous and immunological systems [1, 2].

For example, Celik et al., studied the influences of EMF of HVPL on hearth of the humans [3], Dasdag et al., discussed the influences of microwaves and ELF magnetic fields on the phagocytic activity of variously macrophages in variously treated rats [4]. Li et al., studied the influences of electromagnetic irradiation of HVPL on the cell proliferation, features of blood, the rheology properties and the electromagnetic properties of biological tissue as well as the features of molecular structure of myoglobin in the rats and mice [5].

Detected changes the activity of some blood serum enzymes - catalase, glutationperoxidase, superoxidedismutase, trypsine, and enzymes associated with cell proliferation and cancer progression, for example, EMF stimulated the production of ornithine decarboxylase enzyme which ultimately can affect cell growth and differentiation and so promote cancer [2, 6, 7, 8, 9, 10, 11]. EMF also affects the cellular structures of the blood, for example, red blood cells are very sensitive to electromagnetic fields due to the fact that they contain more than 80% iron of the body and have 2-4% paramagnetic methemoglobin molecules. From our previous studies, it is known that when the antioxidant defence system is weakened, an increase in the amount of methemoglobin, an oxidized form of hemoglobin in red blood cells, accelerates its penetration into the depth of the membrane, which leads to a further deepening of oxidative processes in the cell membrane [12].

Science has not quantitatively confirmed a direct relationship between the level of low-frequency electromagnetic fields and oncological or other types morbidity, but peoples often face countless health problems, generally with unknown etiologies, i.e., qualitatively this relationship can be traced [13, 14]. This happens for the reason, that in evolutionary terms, such a short time, human or other living organisms are not able to adapt to such rapidly changing conditions of the surrounding environment [1, 2, 14].

People cannot refuse to use electricity it makes their life comfortable. However, every day number of electrical devices is increase and with an increasing their number impact of the ELF on biological objects growths proportionally. The population of cities is more susceptible to the effects of EMF, because in the limited spaces of metropoles a large amount of electrical equipment is accumulated. All these equipment, except for the assigned range of action, create many side (noise) EM fields and emissions, which further enhances the EM pollution of the environment [1, 2, 13, 14].

Therefore, it is relevant and socially significant to protect the people from anthropogenic electromagnetic influences, for which is important to understand the mechanisms of structural and functional changes occurring in living systems, to clarify the cause-and-effect relationships of the influence of low-frequency electromagnetic fields and emissions [2, 13]. This means that science world must investigate deeply and widely the biological effects and properties of EMF including HVTL.

One of the methods used to study the action of external environmental factors is fluorescent spectroscopy. This method is widely used in biophysical research as one of the most accessible, non-invasive and highly sensitive methods, to determine changes in the secondary structure of proteins under the influence of external factors, to assess oxidative damage to proteins, to study protein denaturation [15, 16, 17]. The purpose of this work was to study the intrinsic fluorescence of serum proteins, to obtain information about the structural changes occurring in the serum proteins structures of human blood under the influence of a high-voltage electric field with a frequency of 50 Hz.

2. MATERIAL AND METHODS

All used reagents were of analytical grade. Healthy donors blood samples (n = 25) were taken from the Research Institute of Hematology and Transfusion (Baku). Human blood serum was exposed to high tension EF 50 Hz. High tension electrical fields (EF) with a strength of 20 kV/m were formed using a laboratory transformer I-50 (Russia). Blood serum samples were exposed for 30 minutes, this is the normative permissible operating time (stay) of people under 20 kV/m [19].

Fluorescence of proteins is very sensitive to environmental changes, which makes it very useful for studying the structural, physicochemical and functional properties of proteins, under the influence of external factors [15, 16]. It can reveal even minor information about the state of protein molecules, aggregation, changes in the acid-base balance, the exposure of amino acid side chains to quenchers, etc. Blood, with its protein structures, is a dynamic system, that carry out many different biological functions and is very responsive to the effects of the environment [17]. Any change in the environment, including the effects of EM fields and emission, primarily affects the structural and functional state of blood elements, including protein properties [18]. In the structure of proteins aromatic amino acids, tyrosine (T_{yr}), tryptophan (T_{rp}) and phenylalanine (Phe), which have in their structure systems of conjugate double bonds, have property of intrinsic fluorescence [16].

Fluorescence, which occurs in protein solutions, when excited λ_{ex} = 280 nm, is associated with the residues of T_{rp} and T_{yr} and emission at 350 nm the indole group of T_{rp} is considered dominant. Moreover, in native proteins T_{yr} emission a lot of time suppressed through its interaction with the peptide chain or through the energy transfer of T_{rp}. When λ_{ex} = 295 nm is excited, only fluorescence of T_{rp} is manifested, and in these two excitation waves, the fluorescence of Phe does not noticeable itself at all [20].

Serum proteins fluorescence measurements carried out using the Fluo Time 300 Spectro fluorimeter (Germany). To determine possible qualitative changes in the structure of serum proteins, the fluorescence spectra were measured of human blood serum diluted in the 0.01 mM phosphate buffer solution (pH 7.4), till spectrometric absorption value of A = 0.05 for each excitation wave, with an optical path length 1 cm. For excitation of fluorescence were used wavelengths 280 and 295 nm, emission spectra were recorded in the first case from 295 nm and in the second from 310 to 400 nm. All the graphs were making using the OriginPro 8.5.

Analyzed the maximums and second derivatives calculated by Fourier transformation. Secondary derivatives of fluorescence spectra allow obtaining more detailed information about the state of the microenvironment of fluorophore amino acid residues of proteins. The use of secondary derivatives of fluorescence spectra in most cases also allow the separate of tyrosine and tryptophan components in the total protein spectrum, which is due to a much larger half-width of the fluorescence spectrum of tryptophan compared to the fluorescence spectrum of tyrosine. This prevents the exact determination of the positions of the maxima of fluorophores from the initial data of the fluorescence spectrum [18].

All measurements were carried out with threefold repetition. Differences between control and experiment blood serum samples were established using t-Student. To verify the statistical significance of the means (M) of the parameters, the 95% confidence intervals of triplicate samples ± SD were defined. This study followed the Guidelines in the Declaration of Helsinki in all stages and all mandatory laboratory health and safety procedures have been complied with in the course of conducting this study.

3. RESULTS AND DISCUSSIONS

The Figures 1 and 2 show the typical spectra of intrinsic fluorescence and second derivatives of human blood serum during excitation of λ_{ex} = 280 nm and tryptophan fluorescence obtained by excitation λ_{ex} = 295 nm. As can be seen from the Figure 1, the maximum intensity of total fluorescence (λ_{ex} = 280 nm) is observed for experimental samples at λ_{em} = 332 nm, and control at λ_{em} = 332.5 nm. i.e., the peak is slightly shifted to the short-wave region, while showing a decrease in intensity by 8.12±1.40%. In addition, the Tyr fluorescence

intensity is lower than an order of magnitude that of tryptophan. If take into account the amount of Tyr residues in proteins, the spectra should show a second peak in the form of a "shoulder" in the short-wavelength part of the T_{rp} fluorescence band [15]. But we observe only spectral asymmetry, indicating the participation of Tyr amino acid residues in formation of the total fluorescence spectrum.

As indicated above, at a wavelength of $\lambda_{ex} \geq 295$ nm (Figure 2), emission is mainly associated with amino acid residues of T_{rp}, the maximums of which in the aquatic environment are observed at λ_{em} = 350 nm, and this emission is extremely sensitive to the polarity of the environment. The emission maximums of proteins reflect the average availability of their tryptophan residues in the aqueous phase [16, 20].

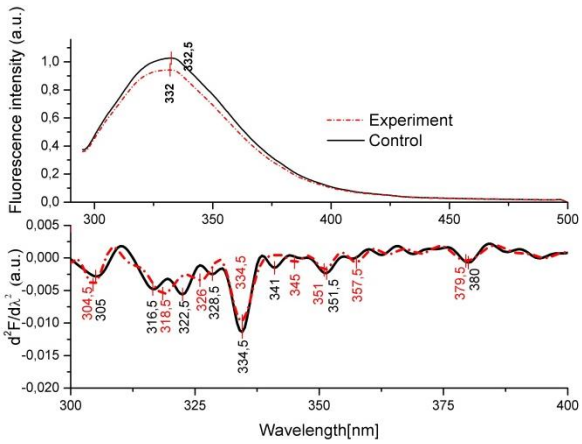

Figure 1. Spectro and second derivatives of human blood serum intrinsic fluorescence during excitation of λ_{ex} = 280 nm after exposure to EF 20 kV/m for 30 minutes

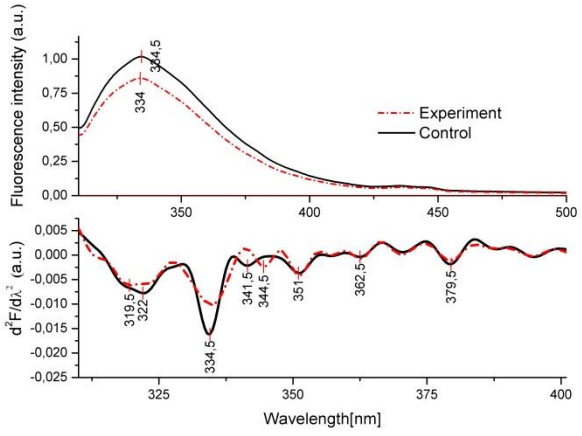

Figure 2. Spectro and second derivatives of human blood serum intrinsic fluorescence during excitation of λ_{ex} = 295 nm after exposure to EF 20 kV/m for 30 minutes

At excitation λ_{ex} = 295 nm on the fluorescence spectrum (Fig. 2), the maximum T_{rp} under the action of EF decreased by 15.07±1.73%, and 0.5 nm moved to the left (λ_{em} = 334 nm), relative to the control at 334.5 nm. If look at the fluorescent spectra of blood serum, can see that peak in the fluorescence spectra of 280 and 295 nm are observed in different places. In our opinion, this difference can be explained for two reasons, firstly at λ_{ex} = 295 nm, trirosine does not affect the fluorescence of tryptophan, and secondly by the fact that blood serum consists mixture of proteins and other compounds, which not permit fluorophores to fully manifest themselves.

The shift of the maximum at 295 nm to the right (344 nm), can be explained by the removal of tryptophan residues from the range of action of tyrosines. This is evidenced by the intensity decrease by another 7.22±1.07%. This decrease indicates that the distance between Tyr and T_{rp} is small enough for energy transfer. There is another interpretation in the literature - the mechanism for reducing T_{rp} fluorescence is explained by the processes of photochemical decomposition occurring in the protein molecule before denaturation [15, 18].

We think that this factor is a secondary cause of the extinction of fluorescence. The first reason for the decrease in fluorescence may be the movement of T_{rp} into a more hydrophobic environment as a result of dimerization of neighboring monomers, which can reduce the internal stress in the protein molecule arising under the influence of an external field. Investigated human serum albumin (HSA) by fluorescence quenching, in the presence of EMF obtained that the microenvironment around T_{rp} of the protein became modified, more hydrophobic after presence of EMF, and quenching was considered to be a static quenching mechanism corresponding Stern-Volmer shifts [8].

According to E.E. Tekutskaya, et al. quenching tryptophan fluorescence of plasma proteins shows a one-stage nature of denaturation - loosening of protein globules. The second stage, the complete unfolding of the amino acid chain of the protein under the action of EMF frequency from 5 to 50 Hz, does not occur [21].

The presence in protein molecules of several, different residues of tryptophan and tyrosine, as well as other factors affecting fluorescence, create certain difficulties in interpreting the fluorescence spectra of proteins. That is, only drawback of the method may be considered a strong overlap of the maximums of individual residues of Try and Tyr, due to the large half-width of fluorescent maximums of Try, unlike Tyr [16]. This does not allow in the primary spectrum of fluorescence to accurately determine the locations of each amino acid residue located in a various microenvironment. These difficulties are to some extent be overcome by obtaining the second degree of differentiation of the primary fluorescence spectrum. The second derivatives of fluorescent spectra allow obtaining more detailed information about the state of the microenvironment of amino acid residues, improving precision of determining spectral shifts, as well as separating the components of tyrosine and tryptophan in the general fluorescence spectrum of proteins [22].

Analysis second derivatives of the fluorescence spectra λ_{ex} = 280 nm (Figure 1), shows that the tyrosine peak in the control sample (305 nm) is shifted to the blue side, with an increase of 45.08±2.29%. According to the literature, a distinctive feature of this fluorescence from the luminescence of tryptophan is loyalty to the position of the maximum fluorescence of tyrosine to

conformational changes in the protein macromolecules, in other words, tyrosine maintains its peak position in the spectrum of proteins and upon denaturation. Denaturation of proteins usually enhances the emission of tyrosine, its pKa is very much reduced upon excitation, and ionization can occur in excited state [18, 20]. Since the maximum fluorescence of tyrosine in the aquatic medium is observed around 303 nm, and changes polarity of the medium have little effect on its spectral properties, the observed changes in the spectra may be due to the influence of EF on conformational and structural changes blood serum proteins.

The other maxima are distributed as follows, 2 peaks of the shortwave position in the control at 316.5 and 322.5 nm show a blue shift of 4 nm in the experiment, exhibiting at positions 312.5 and 318.5 nm, with decrease of 78.43±6.37 and 2,51±0.04%, respectively, which indicates the tryptophanils moving to the more hydrophobic environment [16, 17]. The maxima in the control sample at 328.5 nm after 30 minutes of exposure in the experiment observed transition to a maximum of 326 nm with a shift of 2.5 nm and decreases of partial accessibility of tryptophan residues to the aqueous environment by 15.48±2.63%.

The maximum 341 nm of the control samples shows a shift on 4 nm and appears in the experimental sample at 345 with a decrease of 57.26±4.05%, the maximum of 351 nm in the experimental sample counter replaces the maximum of 351.5 nm in the control and shows a blue shift on 0.5 nm with a decrease 20.53±2.86%. Pik 356.5 nm in the control pass into 357.5 nm with a decrease of 40.33±4.81%. Such multidirectional shifts and changes in the intensities of the maxima are obviously associated with structural changes in the protein components of the human blood serum [17, 18].

At excitation λ_{ex} < 290 nm in the blood serum fluorescence spectrum in the areas 300-400 nm, the fluorescence is determined mainly by the emission of human serum albumins and globulins. Of the non-protein components of blood plasma: bilirubin, coenzymes - NAD, and their derivatives and a number of other substances, only indicant emission can be manifested in the form of a low-intensity shoulder at 385 nm [18]. In our case (Figure 2), the fluorescence around the 380 nm maximum changes by 40.63±3.87%, which indicates that the non-protein structures of the blood serum also undergo changes from the high-intensity EF. Analysis of the fluorescence spectra at λ_{ex} = 295 nm show that two maxima demonstrate a red shift (1 nm) at 322 nm to 323 nm and decreased of 64.30±5.28%, and peak 341.5 nm moves to 344.5 nm (3 nm) and an increase of 7.23±2.65%. The remaining maxima relative to the control changes in intensity, but does not change the location.

Thus, the maxima of the experiment samples at 313.5 nm, 319.5 nm, 334.5 nm and 379.5 nm showed 200.50±19.32%, 21.21±3.70%, 37.16±4.30%, 40.11±3.24% decrease, respectively. The maxima at 351 nm and 362.5 nm showed 11.13±2.80% and 88±5.08% increases, respectively. In the available literature many works devoted to the study different aspects of protein fluorescence including serum proteins, but there is nearly no works to study of the fluorescence of proteins under the influence of electromagnetic fields and emission.

Zhang et al., to investigate the relationship between the fluorescence spectra of serum and the brain injury effect, the alteration of fluorescence emission in serum was collected by fluorescence spectroscopy and the results showed that the fluorescence intensity of 400 kV groups were higher than 200 kV groups at different time points after exposure to pulse EMF [26].

According to the literature, all T_{rp} fluorescence maxima in proteins can be describes as discrete superpositions of main spectra outgoing from five different local media: 1- without exciplexes (λ_{em} = 308 nm), 2- with exciplex 1:1 (λ_{em} = 316 nm), 3- 2:1 exciplex inside (λ_{em} = 331 nm), 4- exciplex on the surface in contact with the structure water (λ_{em} = 340-342 nm), and 5- exciplex on the surface in contact with mobile water (λ_{em} = 350-353 nm) [23].

Other authors dispute this opinion and write, that the consistent pattern is that there is no pattern, each tryptophan residue has different properties. In other words, the spectroscopic properties of T_{rp} are complex with respect to the simpler fluorophore T_{rp}, in particular, as high sensitivity to the local environment and the presence of two different fluorescence lifetimes [24, 25]. At present, emission in the $1L_a$ excited state is considered, except for cases when the immediate environment is completely hydrophobic. The polarity of the medium strongly affects the T_{rp} fluorescence, facilitating the emission in both the $1L_a$ and $1L_b$ states. In addition, $1L_a$ in an excited state is more sensitive to hydrogen bonds and has a higher dipole moment than an excited $1L_b$ [16].

We believe that the strong, external influence of EFHT 50 Hz further enhances this polarity, forcing the fluorophores to shift to the less energetic side. Since the maximum and intensity of T_{rp} fluorescence depend mainly on the polarity of the microenvironment, hydrogen bonds, and other non-covalent interactions, an increase in polarity manifests itself as a shift of the maximum of the emission spectrum from the $1L_a$ position to the right, to red side. In a hydrophobic medium, when the fluorophores enter more non-polar medium, the $1L_b$ state has a lower energy than the $1L_a$ state, which explains the blue shift of the T_{rp} fluorescence spectra [20].

Similar data obtained in the study of the influence of an external electric field on the dynamics of photoexcitation of the chromophores green fluorescent protein (GFP). T. Nakabayashi measured of the field-induced change in fluorescence decay profile indicate that the field-induced decrease in fluorescence intensity results from the field-induced increase in rate of the nonradiative process of the GFP chromophore. The enhancement of the non-radiative process in the presence of ELF may arise from the charge transfer character of the twisted intermediate on the way of the non-radiative process [27].

In general, standard rules are used to interpret the results of fluorescence, nevertheless, the method is very sensitive and in different proteins, even with one tryptophan, the process of quenching of fluorescence does not obey the general kinetics [16]. Therefore, it is difficult to interpret the contribution of each tryptophan residue to the fluorescence spectrum of blood serum, which consists of a heterogeneous mixture of protein molecules containing several different tryptophanes and tyrosines in their structure, the emission spectra of which overlay to some area, therefore fluorescent manifestations of different fluorophore residues in various proteins under the influence of external environment factors cannot interpreted in the same mode, in other words, the material provided can have another interpretation [22].

Biological organisms consist of interconnected and rapidly changing in time composite structural elements, that are in the mode of unstable dynamic equilibrium, therefore, if such system is swing by low-frequency weak signals, the biosystem passes into another unstable dynamic equilibrium state [17]. In our case, observed structural disturbances manifested in the form of increase or decrease in hydrophobic regions fluorophores, which is reflected by an increase or decrease in fluorescence in both sides of the spectrum.

Taking into account our preliminary research to identify the influence of high-voltage power lines on biological structures, including the elements of human blood, we can say that it is necessary to continue research in this direction. Our results show that 30-minute exposure of 50 Hz EF high voltage 20 kV/m to the human blood serum causes structural changes in the molecules of serum proteins in the form of increase or decrease in hydrophobic regions, which is reflected in changes in the fluorescence intensity in range 300-400 nm. These results can be important informative for indicating structural and functional changes occurring at the proteins molecules and can be used as a direct indicator/marker for assessing the adverse effects of high-voltage electromagnetic fields of industrial frequency.

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