Fluorescent and chemiluminescent effects of low-frequency (50 Hz) electromagnetic field on blood proteins

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Abstract: The article investigates the effect of high-voltage power lines on human serum proteins and bovine serum albumin. A high-intensity electromagnetic field is created near these lines, which can cause a potential difference on the surface of the body and internal organs, and this can lead to serious pathologies with prolonged exposure. Experiments with human blood serum have shown that under the influence of EMF 20 kV/m 50 Hz there is a decrease in the fluorescence intensity at excitation 295 nm and an increase in the chemiluminescence intensity. Incubation of BSA samples after EMF exposure, in the Fenton medium, enhanced the EMF effect, in the form of a decrease in fluorescence at excitation of 295 nm and at 325 nm dityrosine fluorescence increased. The obtained results indicate possible disruptions in the secondary structure of proteins under the influence of EMF with a frequency of 50 Hz, as well as the occurrence of oxidative damage. The results of the study highlight the need for further research to better understand the mechanisms of the effects of low-frequency EMFs on living systems and to develop measures aimed at protecting against their negative effects.

Key-Words: Electromagnetic pollution, 50 Hz ELF-EMF, blood serum, protein fluorescence, dityrosine, chemiluminescence.

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1 Introduction

Due to the dynamic growth of sources of electromagnetic fields (EMF) and radiation, their impact on human health has increased significantly. The intensity of EMF is especially high near high-voltage power lines, various electrical, television, and radio stations, radar, and other installations. In large, densely populated cities, the intensity of EMF reaches even higher values due to the accumulation of a large amount of equipment that generates EM radiation. In addition, any electrical device creates a few side (noise) EMF and radiation during operation. As a result of all these processes, in certain places on the globe, the total intensity of EMF increases several thousand times compared to the natural EMF of the Earth [1,2,3,4,5,6].

Modern science does not dispute the thermal effects of high-frequency EMF. However, there is no consensus yet on the biological effects of low-frequency (0 - 300 Hz) EMF [3,7,8]. The literature has put forward many mechanisms and mathematical models regarding the biological effects of low-frequency electromagnetic fields on living beings, for example: biogenic magnetite, eddy currents, magnetohydrodynamic effects, cyclotron and stochastic resonances, phase transitions in magnetic fields, spin chemistry effects, interference of quantum states of ions and molecular groups, metastable states of water, etc. Some of them have received development, but they also stay hypotheses [7,8,9,10,11]. For example, the hypothesis about the role of quantum coherence in the effectiveness of potential-dependent neural channels is questioned, however, such neural characteristics as electromagnetic activity of neurons can increase the duration of quantum effects, and under the influence of weak electromagnetic fields, these quantum processes of neural activity are enhanced [12]. IARS classified low-frequency EMF as a probable carcinogen, which is based primarily on epidemiological studies suggesting a link between exposure to low-frequency EMF and an increase in the incidence of leukaemia in children [5,10,13,14,15]. However, to date, this connection has not been confirmed by experimental studies. This is because the presented evidence of the cause-and-effect relationship of the influence of low-frequency EMF is not entirely convincing [16]. The human body consists of conductive tissues (nervous tissue) and fluids (blood, lymph, intercellular fluid), so it plays the role of a resonator, i.e., an antenna. An electric field can create a potential difference on

the surface of the body and internal organs from few hundred millivolts to more than tens of volts. This potential can interact with internal bioelectric fields and impulses of organs, thereby creating the possibility of disruption of the functioning of certain organs and systems (nervous, cardiovascular systems, etc.) of the body [9,10,15,17,18,19]. These deviations can induce next disruptions of various vital processes, such as the formation of free radicals, changes in the structure of proteins, DNA, gene expression, proliferation, and apoptosis of cells, etc. If these disorders continue for a long time, then cumulative manifestations in the form of serious pathologies are possible, such as diabetes mellitus, leukemia, cancer, neurodegenerative pathologies, etc. [8,10,12,16,17,18,20,21,22,23]. EMFs are also used for therapeutic purposes, for the treatment of epilepsy, Parkinson's disease, cancer, and other diseases [15,17,18,24]. Therefore, the biological effect of low-frequency (0 - 300 Hz) EMFs is an urgent fundamental scientific problem with practical application.

Luminescent methods have high sensitivity and use to obtain information about the structure, function, and behavior of biological objects at the microscopic and molecular levels. They are widely used in biophysical and clinical research to study the effects of external factors on organisms. Fluorescence methods used to detect oxidative-conformational changes in protein molecules are manifested under the influence of external agents [25]. And chemiluminescence (CL) methods are a unique tool for assessing fast-flowing free radical and electron-excited states and properties of biological systems [26].

The presented work studied the effect of high-voltage power lines (50 Hz) on human serum proteins and bovine serum albumin (BSA) using fluorescence and CL methods.

2 Materials and Methods

Methods: All reagents used were chemically pure. Human serum samples were obtained from the blood bank of the Baku Hematology Institute; however, we did not have information about the identity of the blood sample donors. Eleven human serum samples were studied in the experiments. 50 Hz EMF was created using an *И*-50 laboratory transformer (Russia). Blood serum samples and BSA were exposed to 50 Hz EF with an intensity of 20 kV/m for 2 hours. After a 2-hour exposure of BSA to EF with an intensity 20 kV/m, the samples were divided into two parts. Buffer was added to the first part instead of an oxidizing agent, and an oxidizing agent, Fenton's solution, was added to the other, after which both samples were incubated at a temperature of 37 °C for 1 hour. Then, before measuring fluorescence and CL, the samples were kept in the dark for 0.5 hours at room temperature. Fluorescence spectra were recorded using a FluoTime 300 spectrometer (Germany). For fluorescence measurements, the samples were excited at wavelengths of 295, and 325 nm. For each excitation wavelength, the samples were diluted with 0.01 M phosphate buffer solution (pH 7.4) to an optical density of 0.07 on a 1 cm optical path of a quartz cuvette. With excitation at 295 nm, fluorescence emission was recorded in the range of 310–500 nm, and at 325 nm in the range of 340–500 nm. The emission spectra were processed using the Origin 8.5 program; an FFT filter was used to smooth the spectra. In addition, in this study, after exposure to EMF, BSA was exposed to another oxidative stress agent, Fenton's solution (1 hour at 37°C). Then, tryptophan and dityrosine fluorescence were measured [21,27]. The essence of the Fenton reaction is the oxidation of Fe2+ in the presence of H2O2: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\bullet$. CL measurements were recorded using a Lum-5773 chemiluminometer (Russia) with PowerGraph software. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) was used to enhance CL [21,26,27]. All measurements were performed in triplicate. Differences between control and experimental samples were decided using Student's t-test ($p<0.05$).

3 Results and discussions

It is known that blood serum is a mixture of protein and non-protein fluorescent compounds that can affect protein fluorescence. Albumins and globulins make up the main part of blood serum; they perform number of important functions. The presence of only albumins in the reaction medium may allow protein fluorophores exhibit their fluorescent properties more “freely”. Based on this consideration, we studied Trp fluorescence at 295 nm and dityrosine fluorescence at 325 nm in BSA samples after exposure to EF and EF and another oxidizer, Fenton’s solution, which is widely used in biological experiments. BSA is structurally like to HSA. Human albumin contains one tryptophan residue (214) and eighteen tyrosine residues. Bovine albumin contains two tryptophan residues (134 and 213) and twenty-one tyrosine residues.

The results of tryptophan fluorescence are shown in Figure 1. The results show that the

fluorescence intensity of the control and experimental samples exposed to EF without incubation in Fenton's medium differs by an average of 6% ($p<0.05$). The study of the fluorescence of human serum tryptophans upon excitation at $\lambda ex \geq 295$ nm and exposed to EF showed similar results. Thus, compared to the control, the maximum of Trp fluorescence in the experimental samples decreases by approximately 13%. The maximum of the experimental samples is shifted to the left relative to the control, from $\lambda em = 337$ nm to $\lambda em = 334$ nm ($\Delta\lambda em = 3$ nm). It is possible that the observed decrease is a result of the manifestation of structural changes in the protein molecule after interaction with EF.

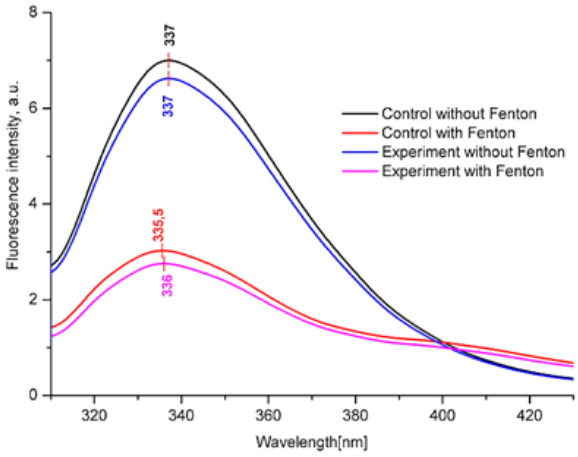

Figure 1. Tryptophan fluorescence intensity (λ ex = 295 nm) of bovine serum albumin samples incubated and not incubated in Fenton medium after exposure to an electric field for two hours.

The observation graph not presented here since it partially repeats Figure 1. Based on the literature data and our previous observations, where we also studied the intrinsic fluorescence ($\lambda ex \geq 280$ nm) of blood serum, it can be stated that such behavior of Trp depends on the polarity of the microenvironment, hydrogen bonds and other non-covalent interactions [28]. And it is also possibly associated with the distance between Tyr and Trp, where energy transfer occurs and when this is absent (at $\lambda ex \geq 295$ nm) an even greater decrease/quenching of fluorescence is observed [25,28]. Similar results were obtained in the work [29], which, studying the effect of external EF on the energy state and photoexcitation dynamics of a mutant of UV-excitable green fluorescent protein (GFPuv5) in a PVA film, came to the conclusion that the observed fluorescence quenching is the result of a field-induced increase in the rate of nonradiative energy transfer.

Samples exposed to EF and incubated in Fenton's solution showed a difference of 9% on average ($p<0.05$). That is, the fluorescence intensity changed by another 3%, with a ΔFWHM difference of 3 nm. And the control and experimental samples, incubated and not incubated in Fenton's medium, demonstrated a difference in intensity of about 58% ($p<0.05$) with a ΔFWHM difference of 2 nm. In other words, Fenton's solution significantly affects blood samples after exposure to EF. The obtained results explain the literature findings that the effect of EF on proteins and other biological molecules has an oxidizing effect on them, which can weaken their molecular structures and make them vulnerable to other stress factors of the environment [21,27,30].

According to the literature, the intensity and position of the Trp fluorescence maxima depend mainly on the polarity of the microenvironment, hydrogen bonds and other non-covalent interactions, and under the influence of EMF with a frequency of 5 - 50 Hz, the main mechanism of decreasing Trp fluorescence is associated with photochemical dissociation, which leads to loosening of protein globules, but complete unfolding of amino acid chains does not occur [31]. This may also be associated with the movement of tryptophan residues to a more hydrophobic environment due to the dimerization of neighboring monomers, such as the formation of dityrosine. We believe that the effect of high voltage additionally enhances the polarity of the microenvironment, causing a shift of the fluorophore toward a lower energy side, which allows for an increase in hydrophobicity, thereby reducing the internal stress of the protein molecule that occurs under the influence of external factors [25,32]. Other authors, studying the relationship between human blood serum fluorescence and brain damage, found that the fluorescence intensity in groups exposed to 400 kV pulsed EMF was higher than in groups exposed to 200 kV pulsed EMF [33]. According to [34], weak combined static (42 μT) and low-frequency alternating (40 nT; 3–5 Hz) magnetic fields change the intensity of the intrinsic fluorescence of some proteins (cytochrome c, bovine serum albumin, horseradish peroxidase, alkaline phosphatase), which may be associated with a change in the conformational state of the protein and this leads to a change in the functional activity of some enzymes (horseradish peroxidase, alkaline phosphatase).

The results of dityrosine fluorescence are shown in Figure 2. At excitation $\lambda ex = 325$ nm, the fluorescence intensity of the control and experimental samples incubated in Fenton's medium differed by approximately 37% from the

fluorescence intensity of the samples not incubated in Fenton's medium. When comparing the control samples with the samples exposed to EF and not incubated in Fenton's solution, an insignificant difference of approximately 5.0% on average was observed. The maxima of the control and experimental samples were at 410.5 and 411 nm, respectively, and ΔFWHM = 2.5 nm and ΔFmax = 4 a.u. In the samples incubated in Fenton's solution, the same indicators averaged 12.0%, i.e. the difference in fluorescence intensity additionally increases by approximately 7.0%. Comparison of the control and experimental samples exposed to EF and incubated in Fenton medium shows that for the control samples the fluorescence maximum falls in the region of λmax = 409 nm and FWHM = 71.07 nm, while for the experimental samples these figures were λmax = 410 nm and FWHM = 72.03 nm. Another peak appears in the spectrum of experimental samples in the region of λmax = 477 nm with FWHM = 54.81 nm. As can be seen from Figure 2, in the spectrum of the remaining samples a similar maximum appears as a shift in the right shoulder of the spectrum but does not stand out from the general background.

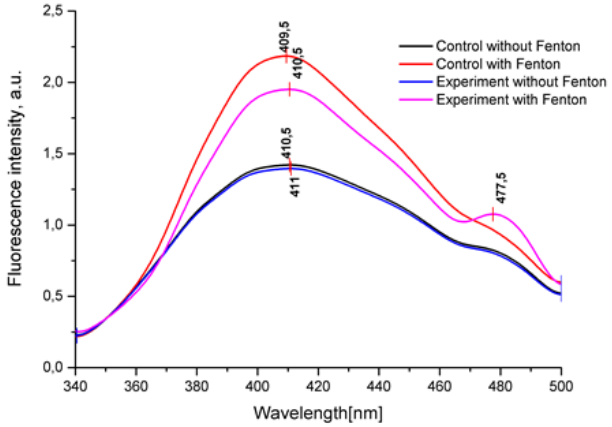

Figure 2. Dityrosine fluorescence intensity (λ ex = 325 nm) of bovine serum albumin samples incubated and not incubated in Fenton medium after exposure to an electric field for two hours

About the maximum λmax = 477 nm Ushijima Y. et al. write that the CL spectrum of tyrosine in the tyrosine-H2O2-horseradish peroxidase system showed two pronounced peaks at 478 nm and 500 nm, and this is similar to the phosphorescence arising from excited tyrosine in the triplet state, which is formed from the tyrosine cation-radical, the precursor of excited tyrosine [35]. This also shows that the oxidative processes do not end after the cessation of external action but continue. Even if we do not consider the indicator of the specified maximum, the situation does not change. In the sense that a significant difference is observed between the experimental samples exposed to EF and Fenton's medium and all other samples. Thus, the fluorescence intensity of the experimental samples exposed to EF and incubated and not incubated in Fenton's medium is Fmax = 189 a.u. and 78 a.u., respectively, which is a difference of >2.4 times. The same indicator for the control samples was >1.8. In other words, the difference is ~60%. In other words, in the protein samples exposed to EF, after incubation in Fenton's solution, the fluorescence intensity of dityrosine increases, which indicates an increase in the oxidized form of tyrosine - dityrosine. It is known that one-electron oxidation of L-Tyr generates a long-lived tyrosyl radical, which, when interacting with the same radical, forms dityrosine cross-links. The formation of dityrosine cross-links due to the interaction of tyrosyl radicals of various polypeptide chains leads to a change in the specific functions of proteins involved in the regulation of cell structure, signaling and various enzymatic processes. Oxidative modification is often accompanied by disruption of biological structures, which leads to aggregation or fragmentation of protein molecules depending on the amino acid composition [12,23,24,31,36]. Elevated levels of dityrosine play a significant role in the pathogenesis of various diseases, such as eye cataracts, atherosclerosis, acute inflammatory processes, Alzheimer's or Parkinson's disease, skin cancer. An increase in the proportion of dimeric and oligomeric forms of albumin can also be the first symptom of the development of tumors or neurodegenerative pathologies, given the fact that these pathologies develop over a long period of time [37,38].

We used the CL method to evaluate the effect of high voltage on the production of reactive oxygen species (ROS). The results are shown in Figure 3. The results show that after 1 and 2 hours of exposure of blood serum to 20 kV/m 50 Hz electric field, at the best dilution (1/200), the CL intensity increased by about 20 and 45% ($p<0.05$) compared to the control samples. That is, in samples exposed to high voltage EMF, an increase in CL is seen, showing the stimulation of free radical processes by high voltage EMF with a frequency of 50 Hz. For example, as indicated above, one-electron oxidation of L-Tyr generates a long-lived tyrosyl radical, which, when interacting with these same radicals, forms dityrosine crosslinks. According to [12], the impact of an electromagnetic field leads to the overproduction of active forms of oxygen, caused a significant decrease in red blood cell count,

hemoglobin concentration, and catalase activity, while white blood cell count, aspartate aminotransferase, alanine aminotransferase, total bilirubin, urea, creatinine, uric acid, and malondialdehyde levels increased significantly [26,28,29,30,31,33,39]. Our fluorescence and CL data are consistent with the literature [19,23,24,25,30,31,39,40].

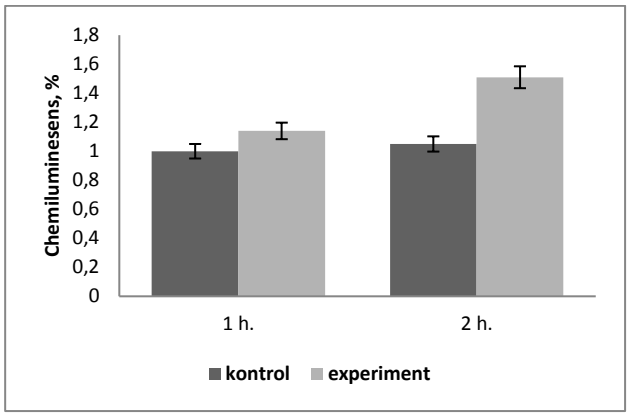

Figure 3. Intensity of chemiluminescence of human blood serum after exposure to EF 20 kV/m 50 Hz for 1 and 2 hours.

4 Conclusion

Considering the literature data on the effect of high-voltage EF on blood serum proteins, as well as our data, it can be said that under certain conditions, depending on the intensity and duration of exposure, the effect can be strong enough to cause structural and/or functional changes in proteins [40,41]. In this case, based on the decrease in fluorescence intensity and increase in CL intensity of serum proteins, it can be said that exposure to high voltage can have a strong enough oxidative-destructive effect to cause structural and/or functional changes in proteins with subsequent consequences.

It is known that EMF is one of the four fundamental forces of nature: gravity, electromagnetism, and strong and weak nuclear interactions. Strong and weak nuclear forces act only at distances comparable to the diameter of the atomic nucleus. And electromagnetic and gravitational forces act over greater distances. It would be logical that gravitational and electromagnetic (including technogeny) forces also act on these nuclear forces [42,43]. In other words, protein molecules and other compounds involved in regulation, signaling, and the implementation of various physiological processes are also subject to these forces.

Therefore, biophysical studies of molecular, structural, and functional changes will help decide the mechanisms of interaction of low-frequency EMFs with living organisms. To solve this problem, it will be useful to consider the quantum-biophysical properties of organisms both at the microquantum level, at the level of atoms and molecules, and at the highest levels of the organization (from microscopic to whole) of organisms - the macroquantum level. Low-frequency artificial EMFs as a stress factor causing deviations from the optimal vital activity of living organisms can have a multidirectional effect depending on the general EM background, the susceptibility of the studied biological objects (the general psychobiophysiological state of the organism), as well as the genetic differences of the tested organisms. Continuation of research in this direction will contribute to the clarification of the mechanisms of the effect of low-frequency electromagnetic fields on humans and other organisms. We believe that with the advent of new research methods, with an increase in their sensitivity, improvement of registration methods, as well as methods of mathematical modeling, new opportunities will open up for solving more unresolved issues related to the impact of ELF-EMF on living systems.

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