The cell response to the effect of heliogeophysical factors and extremely high frequency radiation of low intensity

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Abstract. The aim of the work was to investigate the effect of low-intensive electromagnetic radiation (EMR) of extremely high frequencies (EHF) on the metachromasia reaction of yeast Saccharomyces cerevisiae depending on heliogeomagnetic environment, and to determine the effect of phosphates in the culture medium on the cell response. The culture Saccharomyces cerevisiae Y-517 was grown on the solid nutrient medium made on the base of wort. The media Sabouraud (without phosphates) and Sabouraud+ (with addition of phosphates) were also used. The yeast was reseeded daily, the smears were prepared, stained with methylene blue, the type of color was determined using microscope, color changing indicated metachromasia. Yeast cells were affected by EHF-radiation (120 μW/cm2) at 54, 65, 73 GHz. Heliogeomagnetic activity was controlled using Kp and Ap indices. The cell response to the effect of heliogeophysical factors was observed only when cultivating yeast in the medium with phosphates; it used to change under the influence of EHF-radiation, the third coloration type didn’t appear. We supposed intracellular water to be the unified target for both physical factors. Probably, destabilization of the structured intracellular aqueous medium by EHF-radiation changes the strength of the impact of heliogeophysical factors on cell macromolecules.

1. Introduction

Heliogeophysical factors are a complex of physical factors associated with the solar activity, the Earth rotation, fluctuations of geomagnetic fields and structural features of the atmosphere [1, 2]. All alive organisms are extremely sensitive to the effects of heliogeophysical factors, abrupt changes in which can cause various disorders of the body's vital functions. At the same time, the constant impact of these factors within the limits not exceeding the adaptive capabilities of biological objects is a necessary condition for the existence of life [1-3].

Molecular targets for the action of heliogeophysical factors have not been finally determined. The first scientific data on the effect of heliogeomagnetic activity on a cell were obtained in 1925 by S. Velhover. He noticed that the color of polyphosphate granules of Corynebacteria, when stained with methylene blue, changed from blue to red through violet, depending on the solar activity [4]. This phenomenon was called metachromasia.

The yeast Saccharomyces cerevisiae containing polyphosphate granules was used for long-term monitoring of the metachromasia reaction [5]. Correlations of this reaction with various
heliogeophysical factors (solar activity, geomagnetic activity, solar wind density, cosmic rays) were determined [1, 5]. Since it was proved that the metachromasia reaction is caused by the changes in polyphosphates [6], the authors suggested that polyphosphate granules were a biophysical sensor of the changes in heliogeomagnetic activity [5].

Earlier we studied the metachromasia reaction of *S. cerevisiae* depending on the heliogeomagnetic activity, which was controlled by the values of *K*<sub>p</sub> and *A*<sub>p</sub> indices [7]. The third type of coloration (violet-red) was registered, as a rule, on the second or third day after the geomagnetic disturbance (*K*<sub>p</sub> ≥ 16 and *A*<sub>p</sub> ≥ 18), i.e. the cells response was delayed. An inverse relationship was found between the metachromasia reaction and *A*<sub>p</sub> and *K*<sub>p</sub> indices of geomagnetic disturbance. The obtained data correlated with the results of long-term monitoring of the research group of E.N. Gromozova [1].

The influence of electromagnetic radiation (EMR) of extremely high frequencies (EHF) of low intensity (65 GHz) on the response of cells to heliogeomagnetic disturbances was also studied. Our interest in EHF-radiation is due to its high biological activity and ability to modify the response of biological systems to the action of chemical substances and physical fields [8, 9], as well as the use of this radiation in medical practice [10].

It has been proven that water plays an important role in the perception of EHF-radiation by biological systems [9, 11]. It is not only the environment in which biological membranes and cell macromolecules function, but also their integral structural component. Water largely determines the electrical characteristics of biological fluids and tissues. The frequencies of the rotational motions of water molecules lie in the millimeter and submillimeter ranges, this fact determines the resonant nature of the absorption of EHF-radiation by aqueous media [12].

We have shown [7] that the third type of coloration rarely appears if the yeast is exposed to EMR at a frequency of 65 GHz. It was assumed that the effect of EHF-radiation is associated with destabilization of the structure of the aqueous component of biosystems and, as a consequence, the conformation and activity of biomolecules.

The aim of the work was to investigate the effect of EHF-radiation of low intensity at different frequencies on the metachromasia reaction of the yeast *Saccharomyces cerevisiae* depending on heliogeomagnetic environment, and to determine the influence of phosphates in the culture medium on the cell response.

2. Materials and methods

The yeast culture *Saccharomyces cerevisiae* Y-517 was used in the experiments. The culture was grown on the solid nutrient medium made on the base of wort (6–7°) with agar content of 2 – 2.5 %. There were also used the media Sabouraud and Sabouraud+ (with addition of KH<sub>2</sub>PO<sub>4</sub> 0.5 g/l and Na<sub>2</sub>HPO<sub>4</sub>*12H<sub>2</sub>O 0.9 g/l). Cultivation was provided in thermostat under +28°C during 24 hours. Every day the yeast culture was reseeded.

The generator G4-142 (Russia) was used as a source of EMR of 54, 65, 73 GHz. The density of the radiation current was 120 μW/cm<sup>2</sup>. The yeast culture was exposed to EMR using a pyramidal horn-type antenna with 12 cm length and aperture 42×50 cm<sup>2</sup>, during 30 min, at the temperature 21-22°C. The distance between the antenna and the biological object was 15 cm and wasn’t changed during the experiment. The irradiation of cells was performed after seeding on the solid medium or before a smear preparing.

After 24-hour cultivation the smears were prepared and stained by methylene blue. The color of polyphosphate granules was determined using microscope “Biomed-6”, Russia, (x100) with the visualization system ("Type I" – dark blue; "Type II" – blue-violet; "Type III" – violet-red).

Heliogeomagnetic activity was controlled using the values of *K*<sub>p</sub>-index and *A*<sub>p</sub>-index obtained from the Yu.G. Shafer Institute of Cosmophysical Research and Aeronomy of Siberian Branch of Russian Academy of Sciences, Yakutsk, Russia. Geomagnetic activity is considered to be normal at *K*<sub>p</sub> < 16 and *A*<sub>p</sub> < 18; increased – at *K*<sub>p</sub> ≥ 16 and *A*<sub>p</sub> ≥ 18 [1].
The reliability of the results of the studies was confirmed by 6 parallel experiments with 100% coincidence of the type of cell response, as well as the use of a computer integral image assessment, which confirmed the differences in cell coloration [1].

3. Data and results

The studies were carried out during several years, generally in spring and autumn, when significant fluctuations in heliogeomagnetic activity are observed. Initially, in the experiments low-intensity EMR (120 μW / cm²) with a frequency of 65 GHz was used. There were used three samples of the yeast S. cerevisiae: 1) without exposure to EMR (control); 2) after irradiation at a frequency of 65 GHz; 3) repeatedly irradiated at a frequency of 65 GHz (before each reseeding). Smears were prepared, stained with methylene blue, the type of coloration was determined. The total duration of the experiment was 100 days. For each sample of the yeast culture, the number of smears was summed up and a number of smears with coloration of Type I, Type II and Type III was defined as a percentage. The results are shown in Figure 1.

![Figure 1](image.png)

*Figure 1*. The relation of different coloration types of polyphosphate granules (Type I, Type II, Type III) of the yeast S. cerevisiae: 1 – not exposed to EMR (control); 2 – once irradiated at 65 GHz; 3 – daily irradiated at 65 GHz.

It is seen in Figure 1 that all three types of coloration (the metachromasia reaction) appear in the control sample. It is important to note that the third type of coloration appeared, as in previous studies [7], on the second or the third day after the geomagnetic disturbance. All types of coloring were also determined when using once irradiated cells. The culture, repeatedly irradiated during subculture, did not give the third type of coloring.

Further, we studied the response of cells to heliogeophysical disturbances under the influence of EMR of different frequencies. The samples of the yeast culture were daily exposed to EHF-radiation at 65, 73, 54 GHz, subcultured on standard medium. The smears were prepared every day and stained with methylene blue, the type of coloring was determined. The ratio of the coloration types of smears for each culture sample for the entire duration of the experiment (60 days) is shown in Figure 2.

It was defined that the cells exposed to EHF EMR of the pointed frequencies did not acquire the third type of color. The greatest difference from the control was found in cells after prolonged irradiation at a frequency of 54 GHz, which has a high biological activity [13].

At the next stage, the influence of phosphates added to the nutrient medium on the metachromasia reaction of S. cerevisiae was determined. For this purpose, the yeast culture was grown on the medium Sabouraud (without phosphates) and on the medium Sabouraud+ (with the addition of phosphates). The two samples of the yeast culture were not exposed to EMR, the other two were irradiated at 54
GHz. The ratio of the coloration types of smears for each culture sample for the entire duration of the experiment (60 days) is shown in Figure 3.

**Figure 2.** The relation of different coloration types of polyphosphate granules (Type I, Type II, Type III) of the yeast *S. cerevisiae*: 1 – not exposed to EMR (control); daily irradiated by EMR: 2 – 65 GHz; 3 – 73 GHz, 4 – 54 GHz.

**Figure 3.** The relation of different coloration types of polyphosphate granules (Type I, Type II, Type III) of the yeast *S. cerevisiae*, harvested from the media: 1 – Sabouraud, 2 – Sabouraud+, 3 – Sabouraud, the culture was daily irradiated at 54 GHz, 4 – Sabouraud+, the culture was daily irradiated at 54 GHz.

In Figure 3 it is seen that only cells of the culture *S. cerevisiae* grown on the medium Sabouraud+ exhibit the metachromasia reaction.

4. **Discussion**

*Saccharomyces cerevisiae* is an unicellular eukaryote usually used as a model for the investigation of the effect of electromagnetic fields of different frequency ranges at a cell functioning [14]. In particular, the culture was used for studying nonthermal biological effects of EHF EMR on the division of cells [15]. With the help of this culture a frequency of 54 GHz with a high biological activity was discovered [13]. Influence of radiofrequency of EMR (40.68 MHz) on physiology of development, sensitivity of cells to stress factors, cell cycle kinetics and enzymes activity of a cell was investigated by using this organism as well [14].
The choice of *S. cerevisiae* for this study is due to the presence of specific structural-morphological formations of protoplasm - polyphosphate granules (volutin grains) in the cells [6]. Polyphosphates are an osmotically inert reserve of phosphates and energy, which enables cells to rapidly transition to intensive growth and reproduction under any suitable conditions. In addition, polyphosphates are involved in the processes of genetic and structural regulation, in the transport of substances through membranes.

Cytological detection of polyphosphates in cells is carried out by staining with dyes: toluuidine blue, neutral red or methylene blue. It is known that dye molecules, when binding to polyanion molecules (polyphosphates), acquire an increased ability to dimerize, which leads to a change in the absorption spectrum of the dye. These interactions depend, on one hand, on the polyphosphate chain length, its conformation, on the other hand, on pH, temperature, ionic strength, and the polymer / dye ratio [6]. It has been proved [16] that an increase in the length of the polyphosphate chain shifts the maximum in the absorption spectrum of methylene blue to a shorter region, which leads to a color change from blue to red.

Previously it was determined that polyphosphate granules in *S. cerevisiae* cells responded to heliogeomagnetic disturbances by changing color when the smears were staining by methylene blue (methachromasia) [5,7]. We supposed that heliogeophysical factors as well as EHF-radiation influence at the condition and structure of intracellular water, at ionic interactions that leads to polyphosphates conformational changing and results in methachromasia.

To confirm these assumptions, we studied the effect of heliogeophysical activity in combination with EHF EMR of low intensity on the reaction of metachromasia of *S. cerevisiae* cells. It is known that the EHF-radiation is non-ionizing, the quantum of its energy, for example, for $\lambda = 1 \text{ mm}$, is of the order of $10^{-3} \text{ eV}$, i.e. waves of this range can affect the conformational states of molecules [8, 10]. Although various possible mechanisms of reception of EHF-radiation at the cellular level are discussed in the scientific literature, all the authors agree that its primary molecular target is the intracellular water [9-12]. Thus, the change in the reaction of metachromasia of yeast cells under the influence of EMR may point to the unified target for the action of EHF EMF and heliogeophysical factors.

At first, yeast cells were used in the experiments, once and repeatedly irradiated with EMR at a frequency of 65 GHz. The response of cells to heliogeomagnetic disturbances was compared with the response of non-irradiated cells. The choice of the frequency 65 GHz is due to the results of our previous studies: by using different biological and physical models it was discovered earlier that this EMR destabilize near-surface water structure [11]. Smears were prepared daily and stained with methylene blue. The first two samples of the culture upon staining gave the same type of color (Fig. 1), which depended on the level of heliogeomagnetic activity. Cells exposed to EMR on a daily basis did not have Type III color, i.e. cells did not respond to magnetic storms.

Further, the effect of EMR of different frequencies (65, 54, 73 GHz) on the yeast response to heliogeomagnetic disturbances was studied (Fig. 2). We chose frequencies that, according to scientific literature data, exhibit high biological activity [10], in particular, affect yeast [13]. Long-term irradiation of cells with EMR at the indicated frequencies changed the effect of heliogeophysical factors on cells, which is manifested in the absence of Type III coloration. The smallest variations in the color of polyphosphate granules were observed when the culture was exposed to EMR at a frequency of 54 GHz, which has a high biological activity [13]. Possibly, the higher the level of the impact of EHF-radiation on a cell, the less it responds to the change in heliogeomagnetic activity.

Comparative cultivation of yeast in a medium without phosphates (Sabouraud) and in the same one supplemented with phosphates (Sabouraud+) showed that the metachromasia reaction appears in yeast cells only if there is a sufficient amount of phosphates in them (Figure 3). When using medium without phosphates we observed only the Type I coloration in smears regardless of heliogeomagnetic environment. This proves that the color of the granules depends on the length of the polyphosphate chain. When yeast was grown in a phosphate-free medium, irradiation at 54 GHz resulted in the Type II coloration. This may indicate conformational changes in polyphosphates under the influence of
EMR. When yeast was cultivated in a medium with phosphates, irradiation promoted the appearance of only Type II coloration, regardless of the heliogeomagnetic environment. The Type III coloration did not appear at all.

The research results allow us to confirm our assumption that intracellular water is a single target of the impact of heliogeophysical factors and EHF-radiation. According to the domain-cluster model [17], the intracellular environment is an ordered structure of macromolecules with hydration shells 3-4 nm in size, which is comparable to the size of macromolecules themselves; water inside the cell is organized into clusters and has low entropy. Polyphosphate granules in yeast cells are hydrated macromolecules as well. Since an ordered water system is a good conductor of external signals [17], hydrated polyphosphates should respond to heliogeomagnetic disturbances. EMR destroys water clusters, increasing the entropy of water molecules inside cells, which leads to random fluctuations of polyphosphates. Due to the changes in size and energy, hydrated polyphosphate structures cease to respond to heliogeophysical factors. That is why, no metachromasia reaction is observed in irradiated cells.

Thus, we suppose that exposure of yeast cells to EMR of low intensity at the pointed frequencies (54, 65, and 73 GHz) protects them from the influence of heliogeophysical factors. A similar effect can be expected for other eukaryotic cells.

5. Conclusions
1. The combined effect of heliogeophysical factors and extremely high frequency radiation of low intensity at the metachromasia reaction of the yeast Saccharomyces cerevisiae was studied.
2. It has been found out that the response of cells Saccharomyces cerevisiae to the effect of heliogeophysical factors is changed by the influence of EHF-radiation (54, 65, and 73 GHz). The most noticeable change in the biological response has been determined when using EMR at 54 GHz.
3. The reaction of metachromasia of Saccharomyces cerevisiae has been shown to appear only in the case of phosphates presence in cultivation media, consequently, the type of coloration of polyphosphate granules depends on the length of the polyphosphate chain.
4. We believe that intracellular water is a single target for the impact of heliogeophysical factors and EHF-radiation. Destabilization of the structured intracellular aqueous medium by EHF radiation can change the strength of the impact of other external signals on cell macromolecules, i.e. EHF radiation of certain frequencies (54, 65 and 73 GHz) can protect cells from the effects of heliogeophysical factors.

6. References
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