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THE MODIFICATION OF THE STRUCTURAL STATE IN PLASMATIC MEMBRANES OF YEAST CELLS ON THE UV-B RAYS

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Abstract. By using the method of fluorescent zonde was conducted the estimation of microviscosities of membranes in yeast cells under the influence UV-B radiation at $0,7 \cdot 10^4$ - $4,5 \cdot 10^4$ erq/mm^2-dose. According to the spectra of pyrene fluorescence has been determined the microviscosity of lipid bilayer, zones of protein-lipid contacts and in the zone of annular lipids. Based on data, it was shown that, after the irradiation of yeast cells at doses $0,7 \cdot 10^4$ - $3 \cdot 10^4$ erq/mm^2 there was a change of viscosity of adaptive structural-functional rearrangements. The totality of the obtained data suggests that, adaptation processes at high radiation dose $3 \cdot 10^4$ erq/mm^2- $4,5 \cdot 10^4$ erq/mm^2 in yeast cells, obviously, end at an earlier time.

Keywords: *Fluorescence, UV-B rays, yeast cells, pyrene, microviscosities.*

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1. Introduction

In the past several decades, fluorescence methods have become one of the most significant techniques for studies of biophysical and biochemical processes in biological membranes. Various fluorescence techniques have played key roles in modern membrane science (Kyrychenko, 2015; Freakin, 2016; Roshupkin *et al*., 1988).

Lipids are one of the main components of cellular membranes. Depending on the types of cells, 30–55 % of the cell content are lipids. Both sides lipid composition is different in the membranes and this fact determines asymmetry of the structure of bili-pid layer. It identified that, the condition of membranes, their quality, their quantitative composition and modification under the influence of different factors as well as their interaction with carbohydrate and protein component are of great importance for the functioning of both membranes, cells and the body (Freakin, 2016; Roshupkin *et al*., 1988). Biological membranes form cells and allow separation between outside and inside of an organism, controlling by means of their selective permeability. In addition, they control the pass of messages between cells by sending, receiving and processing information in the form of chemical and electrical signals (Watson, 2015). Ultraviolet-B radiation is an important environmental factor, that regulates gene expression and the reaction to UV-B depends on the nature of the UV-B treatment. (Jenkins G.I., 2019).

However, Takshak and Agrawal (2014) have reported that under the long term supplemental UV-B (3.6 kJ m−2 d−1) influence on *Withania somnifera* (a medicinal plant), in leaves and roots a reduction in biomass and an increasing the activities of all enzymatic antioxidants, malondialdehyde were observed.

The stability of DNA is very significant for proper functioning and existence of all living systems, as it is the prime molecules of the cell. Ultraviolet-B (280–315 nm)

can change the normal state of life by inducing mutagenic and cytotoxic DNA lesions such as 6-4 photoproducts (6-4PPs), cyclobutane-pyrimidine dimmers. (Rastogi *et al.*, 2010).

Tian and Li (2019) were investigated, the physiological effects of the activities and expression profiles of antioxidant enzymes to UV-B radiation stress and on survival rate and egg damage of N. barkeri. Adults of the HTAS strain had lower levels of enzymatic activity of superoxide dismutase (and catalase against oxidative damage and weaker upregulation of superoxide dismutase genes than those of the CS strain.

Under the UV radiation influence the emergence of molecular damage DNA, unresolved (or partially solved) reparative cell systems, also photodestruction of proteins and biomembrane cause the development of enough numerous biological effects (Piniaskina, 2010).

Chen & Lee (2012) were reported that a decreasing of population density of S. cerevisiae cells by irradiation of UV radiation was observed, but they cannot attribute this outcome to being a consequence of ultraviolet radiation exposure. It was a rejection of their null hypothesis. Saccharomyces yeast cells cerevisiae were exposed to near-UV (300-400 nm), their absorption spectra altered slightly within the range 220-300 nm with growing dosage

Photochemical decomposition of ergosterol by UV rays revealed in vivo, although UV radiation also induced a decrease in activity of membrane-bound ATPase and caused membrane function damage. (Arami *et al*., 1997). Takeshita et al (2003) shows that cell membrane damage is observed during exposure to UV-B radiation in Saccharomyces cerevisiae cells.

2. Materials and methods

As the investigation object served yeast cells of *Candida* guilliermondii-916. Yeast cells were grown in the wort-agar in thermostat at 28^0C. The experiments were carried out with the suspension of 2-days culture (1×10^8cell/ml).

As a control served non-irradiated suspension. For the estimation of structural state of membrane was determined the micro viscosity of lipid phase. The determination method based on the ability of a fluorescent pyrene probe to form excimers in a nonpolar medium. The speed of lateral diffusion and pyrene excimerization in the lipid layer of membranes are inversely proportional to the viscosity of the medium.

1 ml suspension, aligned with the protein content, was placed on a magnetic stirrer and 10^{-3}M pyrene solution was added in ethanol: 0,001ml in cell suspension. In a minute (time of wholly solution pyrene excimerization in lipid phase of membrane) was measured the fluorescence of samples on a spectrophotometer-Varian Cary Eclipse 2007 at a maximum wave 334 nm excitation light for the evaluation of microviscosity of lipid bilayer. The peak of fluorescence excime pyrene $F_Э$ was registered at an emission wavelength 470 nm, but the peak of fluorescence monomer F_m at an emission wavelength of 393 nm. The pyrene excimerization coefficient Fe / Fm (334) reflecting the microviscosity of lipid bilayer, expressed by the ratio of the maximum fluorescence value of pyrene excimers Fe (in relative fluorescence units at $\lambda_{emission}$= 470 nm) to the maximum of the fluorescence of monomers pyrene $F_м\lambda_{emission}$=393nm) at excitation λ 334 nm. The ratio of fluorescence intensity of excimers to monomers F_e/F_m is inversely proportional to the microviscosity of lipid bilayer and is directly proportional to its

fluidity. The determination of the polarity of the lipid phase and the zones of protein-lipid contacts of yeast cell membranes.

The cell suspension containing 1mm ethanol pyrene solution (7 microl./ml cell suspension) was fluorimetricated at an excitation light wavelength of 334 nm and at the emission wavelength 372 and 393 nm. The polarity of the menide phase of membranes (F_{372} / F_{393} (334)) was evaluated with respect to the fluorescence intensity of two monomeric forms of $F_m$pyrene at the excitation wavelength 334 nm and at the emission wavelength 372 and 393 nm. The polarity of the protein-lipid contact zones (F_{372} / F_{393} (282) was evaluated with respect to the fluorescence intensity of two monomeric forms of $F_m$in the thin pyrene spectrumat the excitation wavelength of 282 nm and at the emission wavelength 372 and 393 nm (Vladimirov & Dobresov, 1980).

The statistical results were processed using the t-test of the student.

3. Results and discussion

Structural parameters of the biomembrane (in the zone of lipid bilayer and protein-lipid contact in the membrane) were determined by the relative microstructure and polarity.

The obtained results show that (based on the excimerization rate of pyrene) the total lipid layer in the yeast cell membranes exposed to UV-B rays increases depending on the dose (decreases flow). Appropriate results were also obtained in the annular (close to protein) lipid fields (Fiq.1-3).

Based on the indications of the cell membrane microstructure it can be supposed that after the irradiation UV-B rays contribute to the polarity and change of conductivity of lipid bond (Fiq.2). According to the conducted researches, it was determined that as a result of UV-B rays influence the indications characterizing the plasmatic membrane structure of yeast cells change as in the following schedule.

Table 1. The influence of UV-B rays on the physico- chemical peculiarities of the plasmatic membrane in yeast cells

	Microstructure, n.v.		Polarity, n.v.	
Dose	Lipid bilayer	Annular lipid	Lipid bilayer	Annular lipid
Control	0,62±0,03	0,59±0,04	0,37±0,03	0,28±0,03
$0{,}7\cdot10^4$ erq/mm^2	0,65±0,02	0,60±0,03	0,40±0,02	0,32±0,02
$1{,}5\cdot10^4$ erq/mm^2	0,68±0,04	0,63±0,05	0,48±0,03	0,36±0,03
$2{,}2\cdot10^4$ erq/mm^2	0,75±0,04	0,67±0,02	0,55±0,04	0,41±0,01
$3{,}0\cdot10^4$ erq/mm^2	0,80±0,03	0,70±0,02	0,59±0,02	0,45±0,01
$3{,}7\cdot10^4$ erq/mm^2	0,81±0,02	0,72±0,04	0,61±0,01	0,46±0,04
$4{,}5\cdot10^4$ erq/mm^2	0,82±0,05	0,75±0,03	0,63±0,02	0,48±0,03

After exposure to UV-B rays of $3.0 \cdot 10^4$ erq / mm^2, the microstructure of the lipid bilayer is increased by 30% compared to the control, and the microarray of the lipid is increased by 12%. The increase in lipid density and also the microstructure of

anomalous lipids in comparison with the control sample can be explained by changes in the quantitative and qualitative composition of the cell membrane.

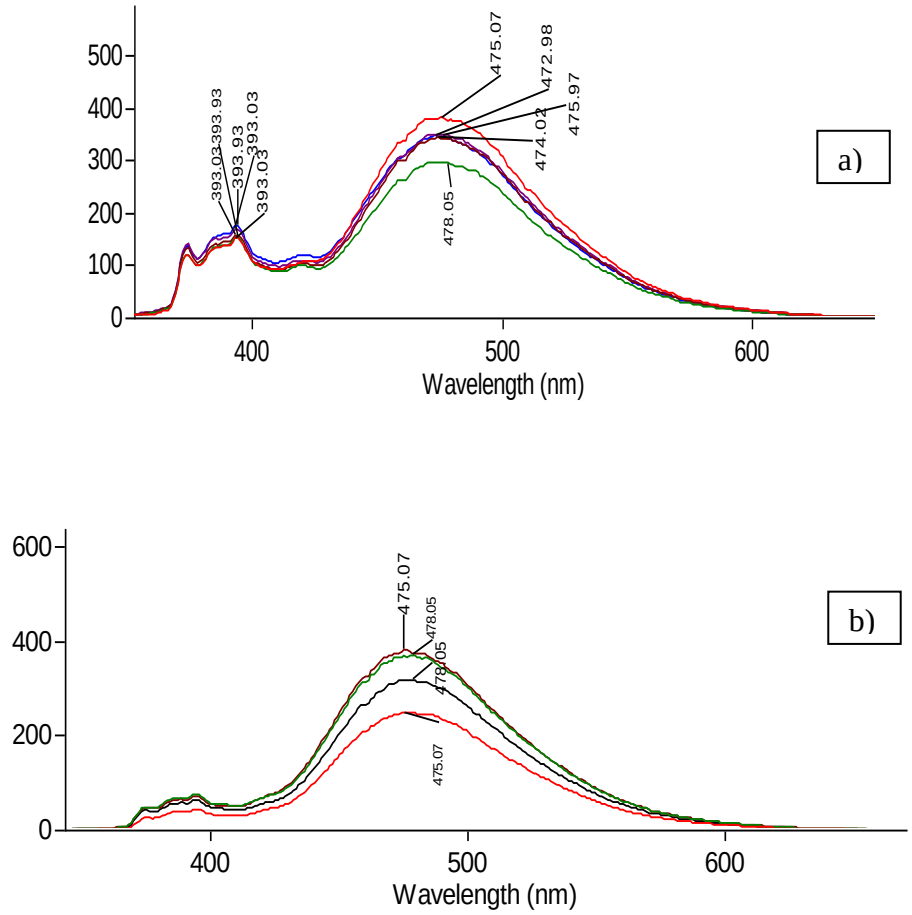
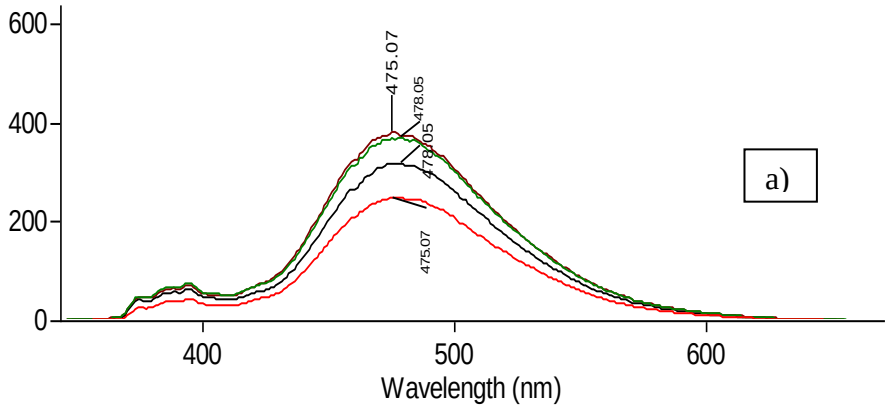


Fig.1. The dependence of microstructure of protein-lipid contact zone of plasmatic membrane in yeast cells on UV-B rays (excitation of pyrene at 275 nm). a) Control-red; $1.0.3 \cdot 10^4$ erq/mm^2-blue; $2.0.7 \cdot 10^4$ erq/mm^2- violet; $3.1.5 \cdot 10^4$ erq/mm^2-brown; $4.2.2 \cdot 10^4$ erq/mm^2-green. b) 1.Control-brown; 2. $3.0 \cdot 10^4$ erq/mm^2-blue; 3. $3.7 \cdot 10^4$ erq/mm^2-green; 4. $4.5 \cdot 10^4$ erq/mm^2-red



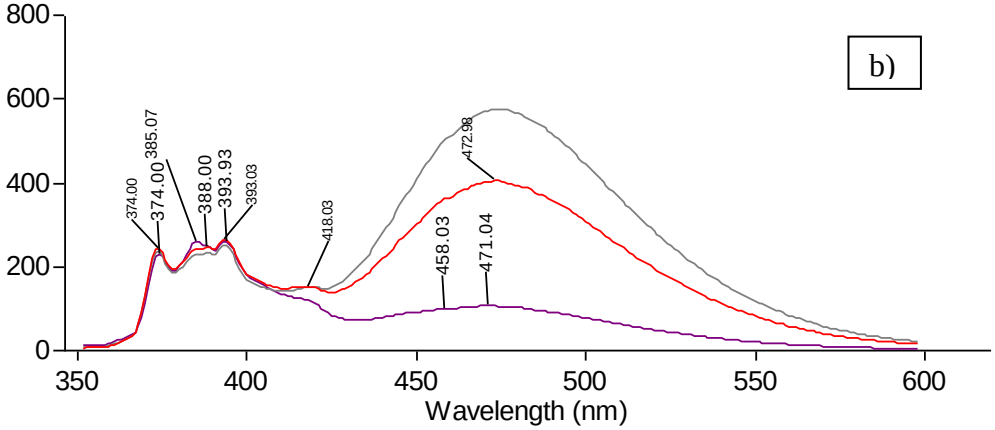
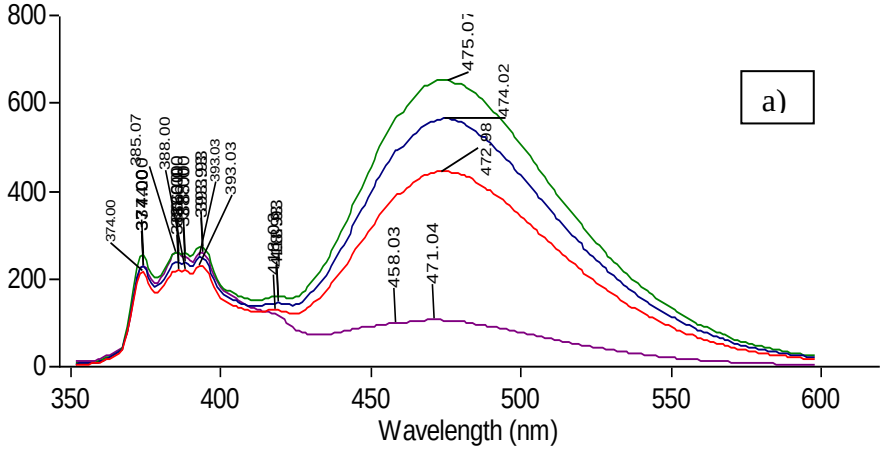


Fig.2. The dependence of the lipid bilayer microstructure of the plasmatic membrane of yeast cells on the dose of UV-B rays (excitation of pyrene at 334 nm) a) 1. Control- violet; 2. $0.7 \cdot 10^4$ erq/mm^2-green; 3. $1.5 \cdot 10^4$ erq/mm^2-blue; 4. $2.2 \cdot 10^4$ erq/mm^2-red; b)1. Control- violet; 2. $3.7 \cdot 10^4$ erq/mm^2-red; 3. $4.5 \cdot 10^4$ erq/mm^2-grey

The polarity of annular lipid and lipid density compared to control increases by 60% and 10%. Based on literature, in practice, the change in the value of the plasma membrane polarity of cells can be coordinated to changes in the surface load as a result of radiation exposure. Based on obtained results, it is assumed that the physiological changes in the cell membrane are associated with modification of lipid components.

It is known that an increase in the amount of free radicals (oxidative stress) in the living organisms and the acceleration of the process of lipid peroxidation is accompanied by certain changes in the properties and functions of biological membranes. First- the process of peroxidation of lipids causes oxidation of thiol (sulfhydryl) groups of membrane proteins (Pr). In fact, it can lead to the non-enzymatic reaction of SH-groups with the free radicals of the lipids.

At that time sulfhydryl radicals are formed. The second result of the LPO process-is having the ability to increase directly the ionic conductivity of the lipid bilayer in peroxidation products. LPO products increase the permeability of the membrane lipid phase for hydrogen and calcium ions. This also causes the mitochondrium to lose ATF synthesis, and the cell is exposed to energy hunger.



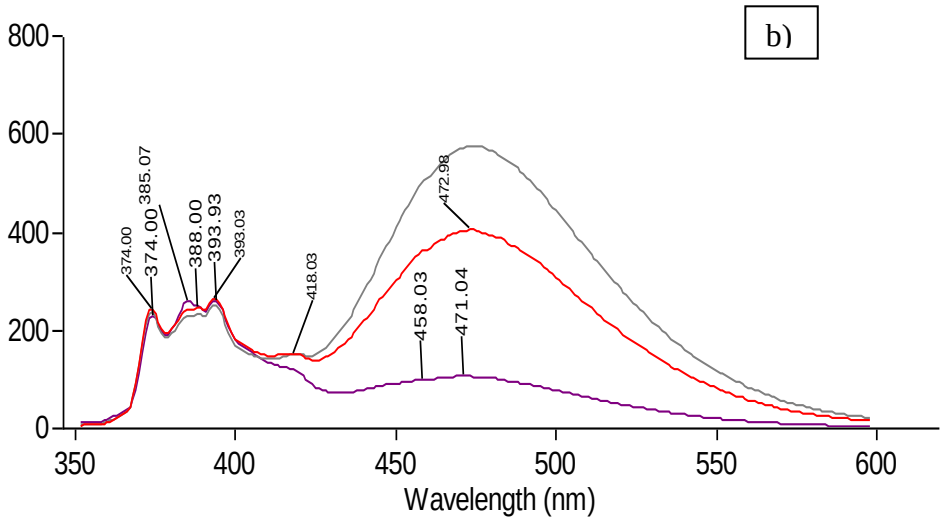


Fig.3. The dependence of lipid bilayer polarity of the plasma membrane in yeast cells on the dose of UV-B rays (excitation of pyrene at 334 nm)
a) 1. Control- violet; 2. $0.7 \cdot 10^4$ erq/mm^2-green; 3. $1.5 \cdot 10^4$ erq/mm^2-blue; 4. $2.2 \cdot 10^4$ erq/mm^2-red; b) 1. Control- violet; 2. $3.7 \cdot 10^4$ erq/mm^2-red; 3. $4.5 \cdot 10^4$ erq/mm^2-grey.

At the same time, calcium ions pass into the cytoplasm, which damages the cell structure. The third result of peroxidation - is the violation of lipid bilayer stability, which can lead to electrical breakdown of the membrane through its membrane potential, which is due to the influence of the electrical potential difference present in the living cell membrane. As a result of LPO the increase of viscosity in the cell membrane is due to the migration of the peroxidized chain of fatty acid bilayer to a higher surface area from deeper layer.

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