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MSEC-DELAYED EMISSION OF ACRIDINE ORANGE LIGHT IN YEAST CELLS UNDER THE DETERGENT INFLUENCE

Natella Kocharli, Samira Hummatova*

Department of Biophysics and Molecular Biology, Faculty of Biology, Baku State University, Baku, Azerbaijan

Abstract. At present work has been investigated msec- delayed light emission (msec-DLE) acridine orange (AO) in yeast cells under the influence of detergents. Gained data show that, under the influence of sodium dodecyl sulfate (SDS) on cells in the concentration less critical concentration micelle formation (CCM) intensity msec-DLE AO increases on comparison with control, or significantly doesn't change. SDS in concentration exceeding CCM significantly decreases and quenches msec-DLE AO in yeast cells. On base of literature and gained data, it can be supposed that, SDS in concentration higher CCM solubilizes cytoplasmic unicellular membranes. By treating yeast cells with triton X-100 at concentration CCM 10-4M within 10-60 min. msec-DLE AO is completely quenched, which is perhaps connected with the solubilization and destruction of membranes.

Keywords: *Delayed fluorescence, acridin orange, detergent, yeast cells.*

Corresponding Author: *Samira Hummatova, Department of Biophysics and Molecular Biology, Faculty of Biology, Baku State University, 23 Z. Khalilov Str., Baku, Azerbaijan, e-mail: sam_bio@mail.ru*

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1. Introduction

The investigation of influence of biogenic and abiogenic medium agents in the biological systems, is one of the actual problems of modern biology. One of such factors is detergent. The detergent binding with membranes depends on the number of linking site and on the degree of their affinities to the detergent moleculars. The binding site on the high affinity level is small, in its turn, slightly effects on the conformation of membrane proteins. By the increase of detergent concentration the binding site begins gradually to be saturated with high affinity to detergent (Borsukov 2004; Metola *et al.*, 2017). That can be explained by the "soft" influence of the non-ion SAS (KSAS) on membranes, so the value of their critical concentration micelle formation is low, than in the case of anionic SAS (ASAS). It was determined that, during the investigation of KSAS influence sequence on cytoplasmic membranes of streptococci membrane permeability changes firstly. That is due to a violation of membrane function, which depends on their native permeability-substance transporting and energy reformation. The influence of higher KSAS concentration followed by the solubilization of protein and lipid membrane component, with changing of secondary protein structure and inactivation their enzyme system. The violation of membrane streptococci permeability under the cation detergent influence is identified by the action of that unit on lipid component in membrane that leads to the change of structure organization of their hydrophobic sphere (Meddy & Dann, 1979; Metola *et al*., 2017; Seddon *et al*., 2014). During the simultaneously presence of detergent and lipid in composition of the same

unit arise conflict between the aspiration of detergents to get micelle configuration and lipid tendency to keep bilayer packing of moleculars in membrane. The knowledge on molecule self- assembly in membrane structure gives us on one hand, the opportunity to realize how the membrane is formed inside the cells, on the other hand-allows to apply these methods for constructingan artificial system, perspective for the opportunity of their practical applications (Boldyrev 1990; Boussambe *et al*., 2018).

In the work by authors have been used detergents: ionic (cetyltrimethyl ammonium bromide -CTAB; sodium dodecyl sulfate-SDS) and non-ionic (polyoxyethylenesorbitanmonolaurate-Tween-20; octylphenol polyethylene glycol-Triton X-100). It was identified that, Tween-20 and Triton X-100 solubilize fewer proteins from membranes, than SDS, CTAB, unlike SDS denature proteins, forming with them negatively charged complexes. Considerable difference of influence on ionic and non-ionic detergents has been identified (Lichtenberg *et al*., 2013; Lishko & Shevchenko, 1987; Markova *et al*., 2009; Metola *et al*., 2018).

2. Materials and methods

At present work has been studied the influence of detergents on the character and kinetic parameters of millisecond delayed light emission (msec-DLE), induced to interaction of fluorescent probe acridine orange (AO) with components of membrane systems in *Candida guilliermondii-916* yeast cells. In the work was used photometrical installation, allowing to register sec-DLE. In the installation was used phosphoroscope (Tarusov & Veselovsky, 1978). The experiences have been carried out by following methods: into the suspension of yeast cells with certain density (10^8 cell/ml was input aquous solution of acridine orange at concentration 10^{-5} M.

3. Results and discussion

Non- ionic detergent octylphenol polyethylene glycol (triton X-100) and ionic detergent sodium dodecyl sulfate were used as aquoussolution, which was added into the cell suspension within the limit of concentration SDS ($1\cdot10^{-2}$ M-$8\cdot10^{-3}$ M), triton X-100 ($5\cdot10^{-6}$ M-$1\cdot10^{-4}$ M). The concentration was treated by the continuous mixing. Then the cells were cooled by centrifugation, were washed two times and used in experiences.

The curves, characterizing changes of irradiation intensity in yeast cells, treated by detergents before, at the intermittent excitement by the visible light at the millisecond interval time have been determined. Analyzing the curves, it is possible to note that the intensity of msec-DLE AO in cells treated before by SDS changes depending on concentration and time of detergent influence. During the processing the cells of SDS concentration $1\cdot10^{-3}$ M during 5-6 minutes is watched the intensity increase of msec-DLE compared with control (Fig. 1).

Analogical results have been gained also in SDS $3\cdot10^{-3}$M concentration. The intensity of msec-DLE AO in yeast cells, treated by ionic detergent SDS (concentration $1\cdot10^{-3}$M, $2\cdot10^{-3}$M, $3\cdot10^{-3}$M) depending on the influence time increases to 2-14% compared with control. It also determined that the maximum increase of msec-DLE AO intensity was watched in concentration SDS $1\cdot10^{-3}$M. The high concentrations of SDS $5\cdot10^{-3}$M and $8\cdot10^{-3}$M (influence time 5-10 min.) induce to the decrease of emission output from 2 to 42 %, and the concentrations $8\cdot10^{-3}$M and 10^{-3}M during the 15-60 minutes influence on cells completely removes the msec-DLE AO excitement. At the

work was also studied the influence of non-ionic detergent triton X-100 on yeast cells. In experiences with triton X-100 was identified that, depending on the concentration and time of detergent influence the intensity of msec-DLE AO changes. After the treatment of cells by triton X-100 concentration 10^{-4}M within 5 min. was watched the intensity decrease of msec-DLE AO. It was not watched in higher concentrations of triton X-100 msec- DLE AO cells. The concentrations of triton X-100 $8 \cdot 10^{-6}$ M and $5 \cdot 10^{-6}$ M (influence time 5-6 min.) decrease msec-DLE to 6%-75 % respectively (Fig.2).

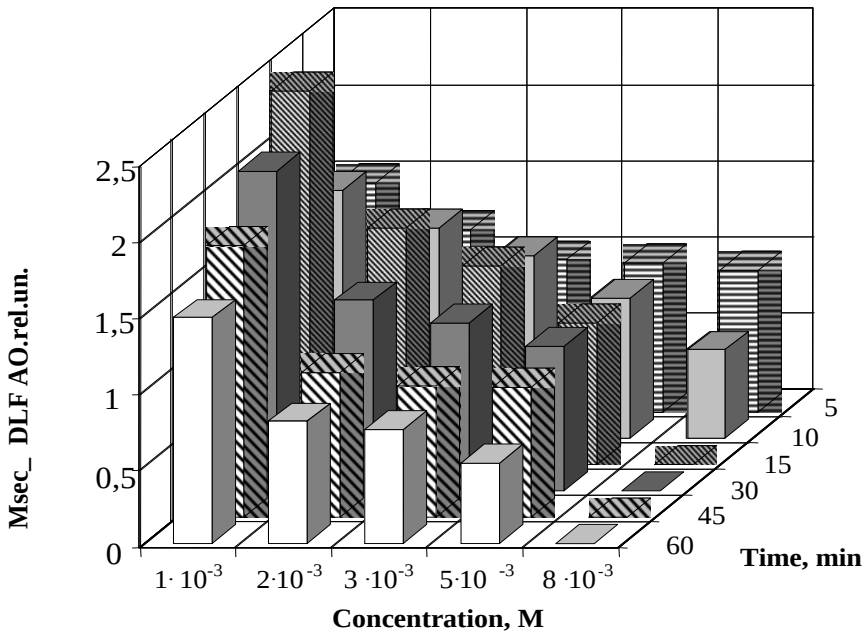

Fig.1. The independence of msec-DLE AO intensity in yeast cells on concentration and the time of SDS influence

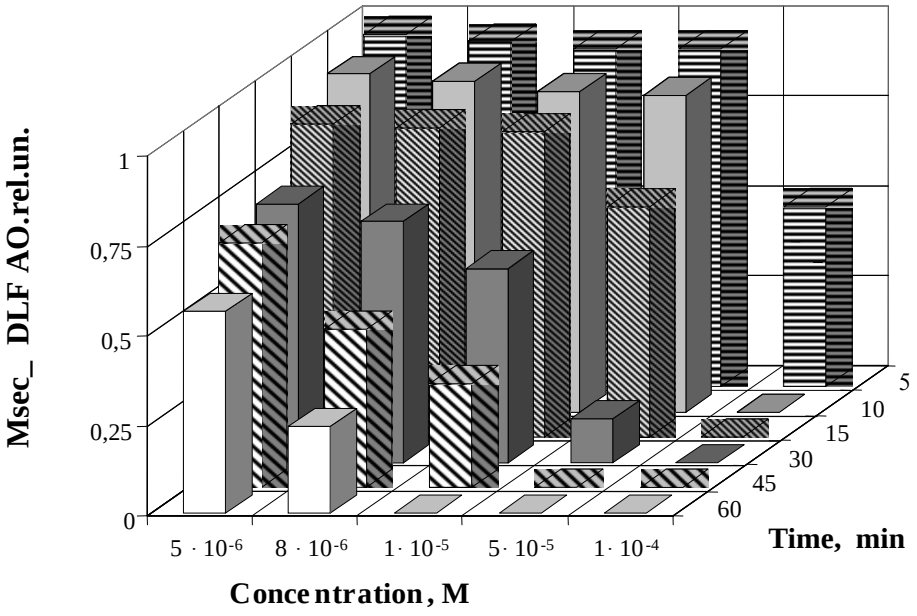

Fig.2. The independence of msec-DLE AO intensity in yeast cells on concentration and the time of influence of triton X-100

It is known that, the speed and solubilization type of biological membranes depend on the state and nature of detergents (Kragh-Hansen *et al.*,1998). During the non-micellar binding of detergent with the membrane, the saturation of membranes occurs in a cooperative manner in concentration, which is lower from CCM. This process followed by slowly binding and solubilization of membranes (Le Maire *et al.*, 2000). During the detergent influence, in concentration higher CCM was watched complete solubilization of membrane structure and the inclusion of membrane components in their own micelles detergent. If the used concentration does not reach CCM, the membrane modification happens without complete destruction of bilayer structure (Markova *et.al,* 2009). Based on reference data, for SDS CCM $8 \cdot 10^{-3}$M, but for triton X-100 $1 \cdot 10^{-4}$M.

Gained data show that, during the SDS influence on cells in the concentration less CCM, the intensity of msec DLE AO increases compared to control, or considerably does not change. SDS in concentration exceeding CCM considerably decreases and quenches msec-DLE AO in yeast cells. Based on reference and gained data we can consider that, SDS in concentration above CCM solubilizates cytoplasmic and intracellular membranes (Pakishina *et al.*, 1977; D'Auria *et al.*, 2001; Zhavodnic & Loshina, 2000). During the treatment of yeast cells by triton X-100 in concentration CCM 10^{-4}M within 10-60 minutes msec-DLE AO completely is quenched, apparently due to the solubilization and destruction of membranes.

Based on the gained data we can conclude that, msec-DLE AO in yeast cells allows to identify the influence character of ionic and non-ionic detergents on cells.

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