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ABSTRACT

of the dissertation for the degree of doctor of philosophy

STRUCTURAL LABİLİTY AND FUNCTİONAL ACTİVİTY OF PLASMALEMMA *NİTELLOPSİS OBTUSA* CELLS

Specialty:	2411.02 - Plant physiology
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GENERAL DESCRIPTION OF THE STUDY

The relevance of the study. Functional identification of the plasmatic membranes of plant cells was started after development of precise measurement of its electrophysiological metrics. Original and adequate samples of such methods were introduced into the research practice on 30s last century and successfully applied to identify several electrophysiological metrics (membrane potential, resistance, capacity, resistance, admittance, ionic fluxes, ion penetrability and etc.) without disrupting cells intactness. Membrane conductivity modifies were classified following the variable nature of such metrics against the backdrop of exogenous cells effect. However, little consideration was given to study lability of electric capacity as an indicator of its structure and polarization status. This is associated with a number of problematic aspects of its quantitative measurement in the first place. On the other hand, the classic overviews devoted to analysis of the research materials in this regard[1] presented such quantities as absolute and invariable nature of biological membranes.[2] Our studies were performed in vivo in intact cells. In this regard, application of standard modifies of the plasmatic membrane carriage features as an exogenous factor might be a successful step to identify the structural – polarization changes caused by them. It appears at the beginning phase of the researches that change in plasmatic membrane structural – polarization state is due to identification of the shares of lipid and protein phases.

Object and subject of research. *Chara, Nitella,* and *Nitellopsis* cells, which are model objects, are usually used to conduct research in the presented field. The large size of these cells,

[1]Алмерс, В. Воротные токи и движение зарядов в возбудимых мембранах // Мембраны: Ионные каналы, сборник статей, – Москва: Мир, – 1981, – с. 129-236.

[2]Мусаев, Н.А. Механизмы модификации транспортных свойств плазматической мембраны растительных клеток / Н.А.Мусаев, В.М. Юрин, А.И.Соколик [и др.] // Труды Беларусского Государственного Университета, – Минск, – 2012. Т. 7. ч. 1: Физиология растений, – с. 154-160

transparency, clear differentiation of structural phases provides regular recording of electrophysiological parameters with the application of microelectrode techniques[3] while maintaining their integrity. These features can seriously guarantee the successful completion of the dissertation and the objective presentation of the results. Thus, the objective of thesis work was to identify its possible structural lability on the eve of functional activeness of plasmatic membranes of plant cells and perform electrophysiological analysis of the mutually occurring events.

Purpose and tasks of the research. To achieve the set goal, the following research matters are scheduled to be addressed:

1. Differentiate protein and lipid phases of its electric capacity using the effect of protein type agents as modifiers of substance carriage in the plasmatic membrane;
2. Perform electrophysiological analysis of functional activeness and structural lability of plasmatic membrane using the effect of lipophile substances;
3. Identify structural lability and electrophysiologicallly analyze this effect on the eve of channel and pump differentiation of electrogenic activeness of plasmatic membrane;
4. Identify structural liability and electrophysiologically analyze of the plasmatic membrane when conductivity of its selective ion channels are modified;
5. To successfully address the above matter, generate method-logical solution comprising development of a new structure of plug device ensuring longterm operation of "glass microelectrode holder".

Main points presented to the defense of the dissertation:

- Electric capacity of the plasmatic membranes of plant cells is the indicator of structural – polarization of its lipid phase;

[3] Мусаев, Н.А., Воробьев, Л.Н. Электрогенная активность и структурная лабильность плазмалеммы клеток *Nitellopsis obtusa* при повышенных температурах // Физиология растений, – 1981. Т. 28. № 1, – с. 86-93; 48.

- Functional activeness of H^+- pumps of the plastic membrane is defined by the physical state of its lipid surrounding alongside its electric supply;
- Co^{2+} - importance of micromoloar concentration in most of the nutritious environments and blood for plants is designed to ensure functionally active state of proton pumps operating in plasmatic membrane.
- Electrogenous activeness of plasmatic membrane may be regulated by exerting force on its lipid phase;
- Enhancement of the electric capacity of plasmatic membrane under Ca^{2+} effect is the outcome of fermentative reactions bringing about oxidization of plasmatic membrane activated on account of increased intracell cation concentration.

Research methods. The theoretical basis of the research is the works of Azerbaijani, Russian and European researchers. The methodological basis of the research is formed from a set of methods that allow to solve the tasks arising from the purpose of the research. During the research, general, empirical methods were used to assess the objective situation: measurement, comparison, experiment and theoretical research methods: axiomatic, analysis, synthesis, induction, deduction, systematic approach and other methods.

The scientific novelty of the research. Following analysis of (R_m), φ_m ($[K^+]$) and $R_m([K^+])$ dependences of membrane potential (φ_m) and resistance in the wide diapason of K^+ ion concentration ($[K^+]$) in the environment, operation of two types of K^+ channels in the plasmatic membrane of cells *Nitellopsis obtusa* was identified and decision made regarding design of their conductivities and diapason of the active membrane potential. It was decided based on analysis of change in the reactions of carrying peculiarities of the plasmatic membrane under the influence of 2 types of proteic antibiotics having different molecules that insensitivity of the electric capacity in respect of the modifiers is due to the fact that share of membrane proteins in its electric capacity is unnoticeably low. To the contrary serious change in its electric capacity was

identified from effect of lipophil substances (dimetylsulfoxide, dicyclohexilcarbidiimid) on plasmatic membrane. It was decided from the analysis of these outcomes that electric capacity of the plasmatic membrane is the indicator of its lipid phase. "Surplus effect", hysteresis effect was identified reflecting rough interconnection with the surface of cation membrane when the plasmatic membrane is washed by the nutritious environment including the most effective concentration of resistance Co^{2+}. The impact effects of cation's membrane lipids have harmonized with its electrogenous activeness. It was established that the functional activeness and structural lability of plasmatic membrane align when Ca^{2+} cations have an effect on it. Role of the lipid phase in electrophysiological activeness of the plasmatic membrane was also confirmed when lipophil solving dimethylsulfoxide had an effect on the membrane. The methodological innovation ensuring multiple prolongation of the operation period of the microelectrode holder in constant mode in the thesis has been certified by Biophysics Institute, (ANAS).

The scientific and practical significance of the work. New plug device of the glass microelectrode holder suggested in this work may be widely used both for electrophyiological research purposes and on industrial scale. This project was also demonstrated at the exhibition held in connection startups set up at the higher educational institutions within the frame of "National Project for intellectual property policy at the University and scientific – research institutes" (March 07 2019) "Day of Science" event titled "Future prospects of molecular biophysics and molecular biochemistry" held at (ANAS) Biophysics Institute and was awarded a proper certificate. The work was also demonstrated at the International Educational "EduExpo" exhibition arranged by the Ministry of Education of the Republic of Azerbaijan (October 12 2019) and posted on the Ministry's official website. The provisions defined in the thesis may be used to build up the general theory of ion carraige in the plasmatic plant membrane. Almost all of the results obtained may be successfully used to identify electrophysiological effects of all xenobiotics. Functional activeness

of plasmatic membrane and alignment of its structural lability under the effect of Ca^{2+} cations may be used to trace cation concentration in tissues in medical practice. The results obtained may be used to address the issues of optimizing mineral nutrition of biophysical, biochemical, ecological, toxicological application and especially agricultural cultures. The dependences defined using mathematic statistics methods may be used at certain phases of the general theory of ion carriage in plasmatic membrane.

Approbation of the work. Key provisions of the thesis were demonstrated at VII International Scientific conference devoted to "Innovative Approaches in Modern Biology" of Young Scientists and Researchers (April 27-28 Baku -2017), International Scientific conference on "Protection of cultural heritage and biodiversity in the context of urbanized industrialization" (Ganja April 29-30 2017), VIII International Scientific Conference devoted to "Innovative Approaches in Modern Biology" of Scientists and Researchers (April 27-28, Baku -2018), General Republic conference of young researchers devoted to the 90th anniversary of J. Aliyev (Baku, October 31 2018), the exhibitions held in connection with startups and their commercialization setup at the higher educational institutsions within the frame of the "National Project for intellectual property policy at the University and scientific – research institutes" (March 07 2019) and was posted on the official website of the Ministry of Education of the Republic of Azerbaijan; It was further reported on and put to discussions at the "Day of Science" event titled " Future prospects of molecular biophysics and molecular biochemistry" held at the ANAS Biophysics Institute on March 14-15 2019, International symposium on "New untraditional plants and prospects of their usage" (Pushino, June 17-21, 2019), VI Assembly of Russian Biophysicists (Sochi, October 16-19 2019), Baku Expo Center of the Ministry of Education of the Republic of Azerbaijan XIII Azerbaijan International Educational "EduExspo" exhibition (Baku, October 10-12, 2019), International scientific conference titled "Urgent matters of biological physics and chemistry" (Sevastopol, September 21 -25 2020), and seminar of Cell Biophysics laboratory of Botanics Institute, (ANAS) has been printed

Publications. Key outcomes of the thesis have been commented on in 16 scientific works. In those publications, articles in 10 reviewed journals, 6 articles in the journals adopted by SAC as having the classification of international publication, 2 conference articles, and the thesis issued at publications of international conference belong to it.

The structure and volume of the dissertation work. The thesis encompasses introduction, list of acronyms, background, literary summary, methodoligical part, outcomes and their discussion (9 sections), conclusion, main scientific and experimental results, and bibliographic list. The work is composed of 154 pages, including illustrations, 32 figures, 2 tables, and the literature list covers 208 source titles (Russian 73 and English 133).

CHAPTER I. LITERATURE REVIEW

In the first chapter of the dissertation, the interpretation of the "Literature review" compiled in a proportionate volume is given. The review is mainly devoted to the analysis of the change reactions of the electrochemical characteristics of substance transport in different types of membranes under the influence of modifiers from different classes. As a result of this critical analysis, the research questions were defined by the author and the program for their solution was interpreted. More than fifty percent of the sources used in the review cover the publications of the last ten years.

CHAPTER II. OBJECTS AND RESEARCH METHODS

As one of the objects of electrophysiological researches, we have used interarticular cells of *Nitellopsis obtusa*, which is a green alga falling under Ch*ara* group. Cylindric shaped large sizes (dia up to 11 and length 10 cm), high electrogenous activeness (-110÷ -270 mV), transparency, clear differentiation of its structural phases enables us to assess their electric resistance, capacity, current density in those phases, and ion flux.

The environment where plants were raised and multiplied was an artificial pond water (APW) with ion content (mM/l): 1,0-$NaHCO_3$, 0,1- KH_2PO_4, 0,4-$CaCl_2$, 0,1-$MgSO_4$, 0,2-Mg $(NO_3)_2$, pH 6,9÷7,2, and temperature 20-22℃. Diameter of the cells used for experiemental purposes 0,4-0,6 mm and length not greater than 2 cm. This limitation was set to ensure measurement accuracy of the resistance of unified plazmalemma surface (R_m). Plants were raised and multiplied in aquaria sizes 0, 3x0,4x0,6 m lighted 12 hours 6 W/m^2. 2 cells were usually taken from the pical portion of the alga for electrophysiological measurements. Intensiveness of physiological processes in such cells are already decided upon on a stationary level, while measurement error of electrophysiological metrics don't exceed the threshold.

Regular recording of electrophysiological cell metrics has been implemented through electrophysiological device specifically developed for *Chara* cells. This method bases on regular application of sound frequency alternating and stable currents to (density 10^{-4} A/m^2) the object. The electrical connection between the internal phase of cells (vacuol or cytoplasm) and inlet of the measurement devices was made possible through application of glass microelectrodes.

R_m measurements was made possible by applying both currents, for which purpose, the additional voltage drop on account of the current released from plasmalemma (ΔU), i.e, electronic capacity was recorded. Calculation of plasmalemma R_m was made following Ohm law for electronic capacity and current rate $\dot{I}_0$ flowing through it. Electric capacity C_m of a uniform plasmalemma area was calculated as $C_m = 1/2\pi f X_c$ from the formula of capacitance at fixed alternating current frequency (usually 30 Hrz). Here X_c – plasmalemma capacitance.

To determine the electrochemical capacity gradient present in plasmalemma, the cytoplasmatic activeness a_k^+ of K^+ ions has been measured in practice with the help of sensitive microelectrodes K^+ in cytoplasm. This value was $a_k^+ = 104 \pm 6$ mM. Nuclear magnetic resonance spectrometers (Bruker -300, Germany) were used to check cleanliness of some modifiers. pH of the solvents used was

controlled via Metrohm-827 (Switzerland) device. The results obtained were processed using Microsoft Exel – 16 computer programs.

CHAPTER III. RESULTS AND DISCUSSION

3.1. Key electrophysiological metrics of N*itellopsis obtusa* cells under standard environmental condition

Key electrophysiological metrics of plasmatic membranes in plants are its resistance, capacitance and electric capacity. However accurate measurement the above values also requires assessment of the resistance and capacity of the cell membrane. Potentials difference in values is noted when the cell membrane is touched with the tip of the measurement microelectrode at the initial phase of measurement. The difference between these potentials is called the potential of the cell membrane. This obtained figure of the cell membrane coincides with the figure measured in *Triticum aestivum* cells. The currency rate released through the cell is I_0 and resistance R_o of its unified surface area was calculated for voltage drop $\Delta\varphi$ in the cell membrane. The average number of this figure for 64 cells was R_o=0,34±0,01 Om·m^2. Measurement of this figure is very important for accurately determining the plasmatic capacitance.

After both microelectrodes were included in the experimental cell, its capacity (φ_m), resistance (R_m) and impedance (Z) were regularly recorded and uninterrupted alternating currency 30-40 Hz was released from it for this purpose. Density of the alternating currency was one rate (0,1A/m^2) lower than the threshold value for Charas, thus the intactness of the cell under research was disrupted on the eve of experiment.

Positioning of the electrophysiological cell metrics on stationary level took place in the range of 50-80 min. Process kinetics was entirely determined by the initial level of the cell potential. φ_m – positioning on stationary level at relatively low (-120 mV) and higher (-230 mV) levels took place faster.

Regular recording of cells impedance has made it possible to regularly control the capacity of its plasmatic membrane.

It was also vital to regularly record the potential of cell membrane corresponding to solution of the posed research matters. Sowe could analyze the order of change in four electrophysiological metrics for the purpose of identifying its structural lability during functional activeness of the plasmatic membrane of the cells under research.

In measurements, currency microelectrode (CM) was included in experimental cell *Nitellopsis obtusa* in the first place and a shortterm (60-80 sec.) inhibition of protoplasm movement speed occurred. The second measurement microelectrode (MM) was included in the cell after the movement speed protoplasm recovered.

In standard environment, it ranged between φ_m - 100 ÷ -240 mV for 100 cells, R_m 2,3÷ 9 Om× m², C_m 0,44-1,4 mkFcm^{-2} for 30 cells and average value numbers made up: φ_m = -171 ± 0,4 mV, R_m = 3,8 ± 0,15 Om× m², C_m = 0,93 ± 0,12 mκf ⋆ m-^{2}.

3.2. K^+- characteristics of plasmatic membrane of *Nitellopsis obtusa* cells

The primary role in processes carried out on the level of cell membrane belongs to K^+ ions. These ions are the most important substrates of the substance carriage system (pump, diffusion exchange, simplified diffusion, cotransport systems and etc.). K^+ ions have "earned" the key potential generating ion status as a result of all these processes and consequently the analysis of the structural – functional modifications taking place in the cell membrane is not possible without considering K^+ characteirsitcs. Membrane's K^+ characteristics means the aggregate of the electrochemical effects of its electrophysiological metrics occurring when concentration of K^+ ions increase in the environment. In this regard, the cells with high membrane potential -180 ÷ -270 mV didn't react to increased cation concentration in the environment. In this case, plasmatic membrane is subject to zFφ=18 kC/mol electrochemical potensials gradient. Here our first observation was that 10,100 times increase in **K^+** ions concentration in *Nitellopsis obtusa* environment didn't change structural – polarization state of the plasmalemma. In this case,

change in φ_m, R_m cell metrics was quantitatively below the initial level of their membrane potential (Fig.1). 2 types of K^+ channels were found to be operating at various intervals of membrane potential in cell plasmalemmas following analysis of such dependenceies. In scientific literature, these channels are termed as calium channels rectifying internally (DDKK) and externally (XDKK). The active interval of the membrane potential at the active state of the channel is termed the activation diapazone of such channels and the value is measured by mVs.

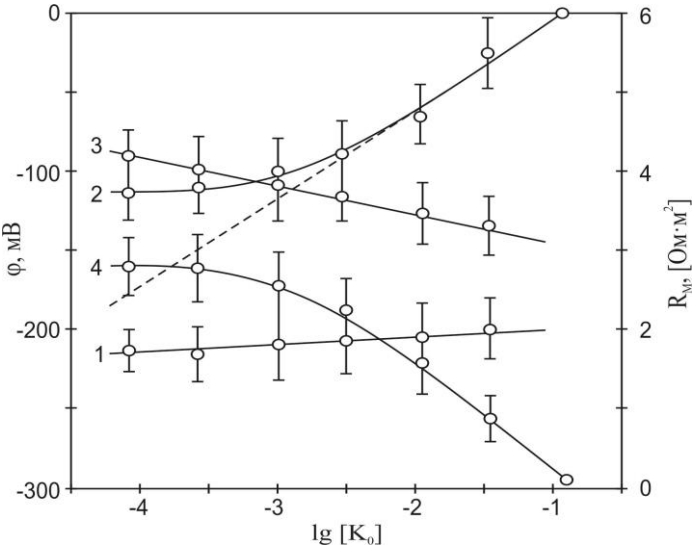

Figure.1. Plasmatic membrane potential of *Nitellopsis obtusa* cells φ_m and resistance is the R_m dependence of the decimal logarithm of calim ion concentration in the environment, 1,3 dependancies is the corresponding potential and resistance of the cells at active state of internally rectifying K^+ channels, while 2,4 dependancies are the same values of cells at active stage of externally rectifying K^+ channels. Nernst dependence is shown by dotted lines.

This we have made the following conclusion from analysis of the reaction of electrophysiological metrics of *Nitellopsis obtusa* cells to increased concentration of K^+ ions in outer environment. There are 2 types of operational K^+ channels rectifying internally

(DDKK) and externally (XDKK) respectively with activation range -270 ÷ -170 mV and -170 ÷ -120 mV in the plasmatic membranes of intact cells.

3.3. Differentiation of the electrogenous activeness of plasmatic membrane to the pump and channel

Analysis of the structural – functional properties of plasmalemma is not possible without the value of current flow force H^+ as one of its functional elements, internal resistance, density of the generated currency, determination of pump's operation mode (operation in currency or voltage modes), and adequate screening of the quantity and nature of bypass load. Solution of such matters may be achieved through inhibition analysis of H^+ pumps' operations.

For our research, inhibitor analysis was conducted by applying dicyclihexylcarbodiimin (DSKD) as a specific inhibitor of H^+-ATPaz-s (pumps). Membrane potential of *Nitellopsis obtusa* cells used for inhibitor analysis was in the range -247 ÷ -128 mV and membrane resistance 3,8. In this subsection, the value of current flow force of H^+ of plasmatic membranes of the cell, its internal resistance, generated current density, and pump operation mode (operation in current and voltage modes) are planned to be established. We have achieved solution of the posed matters via inhibitor analysis of H^+ pumps' operation.

Inhibitor analysis was conducted by applying dicyclihexylcarbodiimidin (DSKD) as a specific inhibitor of H^+-ATPaz-s. Membrane potential of *Nitellopsis obtusa* cells prepared for inhibitor analysis ranged between -247 ÷ -128 mV.

In this subsection, the value of current flow force of H^+ pump of the plasmatic membrane, its internal resistance, generated current density, pump's operation mode (operation in current or voltage mode) are planned to be established. We have achieved solution of posed matters via inhibitor analysis of H^+ pumps.

Inhibitor analysis was conducted applying dicyclihexylcarbodiimidin (DSKD) as a specific inhibitor of H^+-ATPaz-s (pumps). Under a standard condition, membrane potential

of the *Nitellopsis obtusa* cells prepared for inhibitor analysis was in the range -247 ÷ -128 mV and membrane resistance 2,9÷7 Om× m². Average numbers of such values were -170± 8 mV and 3,8± 0,5 Om·m^2. Average value of the membrane capacity was 0,96± 0,1 mkF·sm^{-2}. Differentiation of electrogenous activeness of experimental cells to K^+ channels and pump was made by applying 10^{-6} and $5 \cdot 10^{-6}$ M concentrations of the inhibitor. Kinetics of inhibitor's effect reactions is given in Fig. 2.

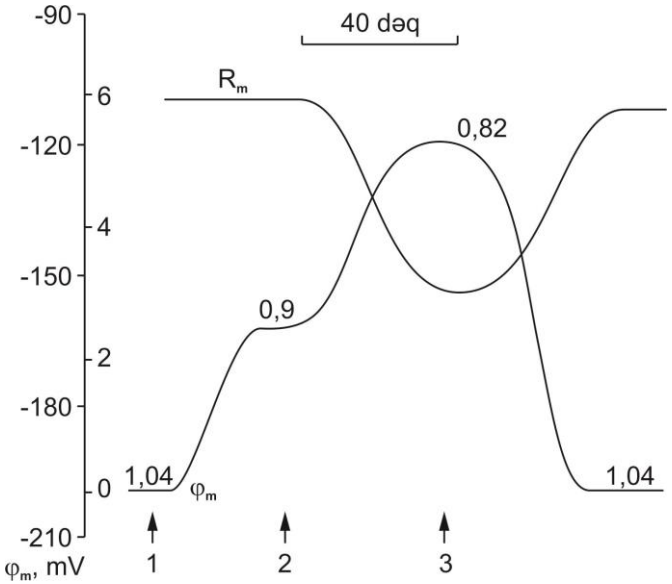

Figure 2. Change kinetics of φ_m, R_m, C_m metrics when 10^{-6} and $5 \cdot 10^{-6}$ M DSKD of *Nitellopsis* cell is placed in the environment with active DDKK. Arrows 1 and 2 in the figure show the moment of entry of inhibitor concentrations 10^{-6} and $5 \cdot 10^{-6}$ M into the environment, while arrow 3 shows the movement of its removal from the standard environment. Change in the electric capacity (mkF ·cm^{-2}) of the plasmatic membrane may be traced from the figures on its kinetic curve φ_m.

Following the comparison of V-A characteristics in the standard and inhibitor placed environment of *Nitellopsis obtusa*

cells, the internal resistance of H^+ pump was found to be R_p= 4 Om·m^2, -360 mV for EHQ, and 0,09 A/m^2 for short circuit current. How ever inhibition of H^+ pump was accompanied by 27% decrease in the electric capacity of plasmalemma. This fact may be an indicator of structural – functional relationship of plasmalemma.

3.4. Differentiation of electric capacity of plasmatic membrane into lipid and protein phases

For this purpose, we have made up our minds to conduct analysis of electrophysiological reactions taking place when both linearly and cyclically conformed proteins are included in the plasmalemma content of *Nitellopsis obtusa* cells.

Membrano – trop effect at the bilipid layer of cyclically conformed valinomycin used for our experiment was thoroughly studied and classified as an engine carrier of K^+ ions. More effective electrophysiological reactions of cell plasmalemma in our experiments were found in cells in the activation range of internally rectifying K^+ channels. Placement of 10^{-6} M valinomycin in the standard environment with presence of such cells dipolarized the plasmatic membrane up to 25-30mW within 30 min. period. The potential of plasmatic membrane resulting from dipolarization has dropped to the level of K^+ balance potential by absolute value. Dipolarization of plasm was accompanied by 25-30% reduction in membrane resistance. Changes in R_m and φ_m values proceeded against the backdrop of serious stability of the electric capacity of plasmatic membrane. Tenfold increase in the plasmatic membrane concentration hasn't caused significant changes in the electrophysiological metrics of the plasmatic membrane. Electric capacity of the plasmatic membrane remained at its previous level on the eve of such electrophysiological cell reactions. φ_m and R_m metrics of the plasmatic membrane recovered their initial levels after removal of antibiotics from the environment. In other words, effect of the abovementioned antibiotics concentrations on plasmatic membrane were fully reversible. At the stated levels of the membrane potential (-170 ÷ -270mV), plasmalemma is subject to electrochemical potential gradient $ZF\Delta\varphi$ = 9,65 kC/ mol of K^+

ions. In the given range of membrane potential, the calculated value of intracell activeness of K^+ ions is $a_k+ = 104$ mM. It may be seen clearly seen that the gradient is directed into the cell. Dipolarization of plasmatic membrane and considerable decrease in its resistant may be explained by this factor.

We have used A (10^{-8} M) antibiotic of gramicidin in linearly conformed ionophore quality. Hydrophobic nature of antibiotic molecule causes its transformation from linerar conformation into spiral by being twisted in the liquid environment, which ensures its easy inclusion into the membrane composition. Spiral length is as thick as membrane monolayer (half of bilayer thickness). Two monolayers of gramicidin channel are obtained when spirals combine and form a single unit ion channel.

Key electrophysiological effects of gramicidin also in *Nitellopsis obtusa* cells have been found in activation range of internally rectifying K^+ channels. It may be seen from careful look into concentration of Na^+ and K^+ ions in cytosol and standard environments that the plasmatic membrane is subject to electrochemical potential gradient directed to intracell environment. As this gradient is directed to intracell environment, emergence of "additional paths" for carriage of Na^+ and K^+ ions formed in plasmatic membrane under gammisidin influence makes it possible to realize the gradient. This has led to reduced membrane resistance and electrophysiological effect of cell dipolarization.

This antibiotic effect is certainly a proof of the development of ion carrying complexes in plasmatic membrane. Positioning of the electrophysiological metrics at the initial level when antibiotics were removed grom SGS took place at least within 45 minutes. This fact shows that gramicidin joins plasmatic membrane and develops an ion channel there. Ionophone wash process in the membrane doesn't exceed 20 minutes when it develops mobile carrier. The most interesting factor established as a result of the experiments are that the electrophysiological effects caused by gramicidin took place against stability of the electric capacity of plasmatic membrane as in valinomycin. It may be asserted that the effect of antibiotics, which has been studied, might not cause any changes in

the ultrastructure of the plasmatic membrane as long as the electric capacity is the indicator of polarization features of the plasmatic membrane. There is no escaping of one fact here. Emergence of "additional paths" for carriage of K^+ and Na^+ ions under antibiotic effect in plasmatic membrane took place against stability of membrane capacity. This fact shows that the K^+ and Na^+ ions entering the gramicidin channel are relieved from their hydrate layers. Otherwise there could have been a substantial increase in the electric capacity of plasmatic membrane.

3.5. Dimethylsulfoxidine effect on functional activeness and structural – polarization properties of the plasmatic membranes of *Nitellopsis obtisa* cells

As a polar solvent, dimethylsulfoxidine (DMSO) demonstrates high penetration ability in living system. In this regard, electrophysiologicala nalysis of the structural – functional changes taking place in plasmalemma under its effect may provide very valuable information to identify the relationships between such properties. There for electrophysiological analysis of the plasmalemma of solvent's *Nitellopsis obtusa* cells formed the next stage of our researches.

Reaction of the electrophysiological metrics of cells' plasmatic membrane to DMSO addition into the environment was both qualitatively and quantitatively dependent on the level of their membrane potential in the standard setting. Minimum DMSO concentration (threshold concentration) causing electrophysiological reaction in SGS was 1%. Plasmalemma reaction to 1% DSMO addition into standard environment at φ_m - 180 mV levels resulted in 20-25 mV hyperpolarization of membrane resistance at stable R_m=4,4 $Om \cdot m^2$ level and 22-26% increase in membrane capacity. Depolarization value of the plasmatic membrane made up 60-70 mV. Kinetic curves of the plasmalemma depolarization as effected by different solvent concentrations in general are presented in Fig. 3 This process was accompanied by considerable reduction in the reaction of plasmatic membrane and further increase in electric capacity.

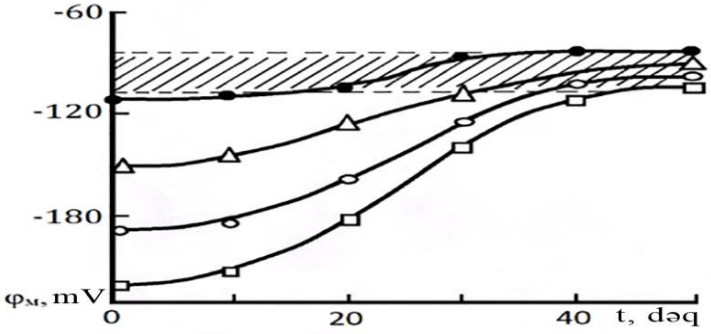

Figure 3. Dependences of the potential of plasmatic membrane cells *Nitellopsis obtusa* on DMSO concentration in SGS. The dots in charts are consistent with □-2% ○-3%, Δ–4% ●-5% concentrations of the solvent in the environment. Here the dots and average values of experimental numbers are presented. Mean quadratic deviation hasn't exceeded 9%.

The most attractive point of the above points from among the DMSO effect on the structural and functional properties of plasmatic membrane is the hyperpolarization against the backdrop of increase in its electric capacity. One may obtain information asto the physical state, polarization degree (amount of surface loads and nature of their distribution), linear sizes (membrane thickness in particular), dielectrical properties by measuring the electric capacity of cell membrane. According to the available literary data and the previously made conclusions in the thesis, electric capacity of biological membranes reflects the structural – polarization state of their lipid phases. The attempt to differentiate into the lipid – protein phases of the electric capacity of plasmatic membrane in *Nitellopsis obtusa* cells reaffirmed the above argument. Quantiative modification of protein phase in plasmatic membrane once again proves that its electric capacity is not the nature of the lipid phase. On the other hand, functional activeness of membrane proteins is regulated by its lipid phase from conformation point of view. It becomes appearant here that enhanced electrogenous activeness of plasmatic membrane observed in our experiments reflects the changes in structural polarization properties of its lipid phase as

affected by DMSO. When the standard environment is replaced by solution in the measurement chamber, membrane resistance could retain its value manifold above its value in its standard setting despite reduction to zero of the membrane potentsial. It shows that cell membrane retains its integrity even under the influence of the highest DMSO concentration. In other words, integrity of the cell membrane is assured by its lipid matrix and structural proteins.

By finalizing analysis of the MDSO electrophysiological effects one may see that at the initial phase of the solvent effect reconstruction processes take place manifesting itself as change in the electric capacity of structural – polarization properties of plasmatic membrane. Enhanced electrogenous activeness of cells takes place against the backdrop of such reconstruction processes. Inactivation of the carrying communication of plasmatic membrane takes place on account of conformation changes at the subsequent phase of the solvent effect.

Thus, based on the theoretical and practical analysis of the order of change in the potential, resistance, and electric capacity of the membrane potential of *Nitellopsis obtusa* cells as effected by DMSO, we have made the following final conclusions dimethylsulfoxide may be applied as an effective regulator of the carrying properties of biological membranes, the initial phase of dimethylsulfoxidine effect on cell membrane is manifest in the change of the electric capacity taking place in its lipid phase.

Aggregate of the established facts proves there is connection between functional activeness and structural lability of cell membranes.

3.6. Electrophysiological properties of the change in electrogenous activeness and structural – polarization states of the plasmatic membrane of *Nitellopsis obtusa* cells as affected by Co^{2+} ion

The nature of electrophysiological reactions to cation effect of the plasmalemma under research was dependent on its concentration and the level of membrane potential of researched cell

in standard setting. Electrophysiological effects of Co^{2+} ions were mainly found in cells with active externally rectifying K^+ channels.

Placement of micromolar cation concentration in the environment resulted in hyperpolarization of plasmatic membrane under stable membrane resistance within 30-40 mV and 15-20% reduction in electric capacity. As seen above, hyperpolarization of the plasmatic membrane as affected by cation is due to change in the physical state manifest in the form of reduction in the electric capacity of lipid phase rather than reduction in its bypass load. Cation interaction with the surface plasmalemma loads appears to be holding the key position here. Furthermore, as $Co2^+$ ion viscosity increased in the environment in our experiments, the reduction in the value of electric capacity against the backdrop of changes in the membrane resistance and membrane potential was as much as 32% of its initial value. This cation effect appears to be also relfecting reduction in bypass load of cation's H^+-pumps.

Big value changes in the electrophysiological plasmalemma metrics was observed under the effect from 10^{-3} M $CoC\ell_2$ at cells with active K^+ channels externally rectifying the membrane potential. In this case, increase in absolute value of the membrane potential was 80-90 mV, increase in membrane resistance 4-5 times, and reduction amount in electric capacity was 30-35%. It becomes clear from these values that both reduction in bypass load of H^+ pumps and change in the physical state of lipid phase of plasmatic membranes of Co^{2+} ions had certain role in such a big value hyperpolarization.

One of the attractive effects of 10^{-3} M cation concentration took place when it was removed from the nutritious environment. As noted above, after Co^{2+} was removed from the nutritious content electrophysiological plasmalemma metrics were not recovered to initial level within 80 – 90 min. This is caused by hard contract between cation and membrane surface. On the other hand, when the cation concentration was placed in the environment R_m membrane resistance increased manifold again. However, the R_m positioned level during secondary cation effect was 30-50% above its previous level. Put in other words, the value of increase in membrane

resistance exceeded the cation effect in the first interaction. This case was also true for the level R_m positioned itself when washed in SGS. R_m positioned level exceeded its level at previous interaction again when cation affected the plasmatic membrane for the second time. R_m positioned level exceeded its level after the previous interaction by 25-40% when cation affected the plasmatic membrane for the second time. Mean values of R_m levels are placed on a straight line with 0,33 $Om \cdot m^2$ /min. verticality to the absciss axis (Fig 4.).

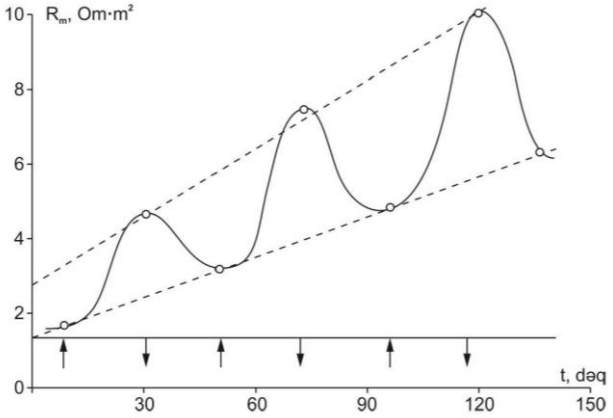

Figure 4. change kinetics curve of the plasmatic membrane resistance of *Nitellopsis obtusa* cells when they are placed in 10^{-3}M $CoC\ell_2$ for the second time. Placement of 10^{-3}M $CoC\ell_2$ into the nutritious environment is presented by upward axes and removal of 10^{-3}M $CoC\ell_2$ fgrom the environment is presented by downward axes.

Maximum R_m values in cation containing environment fall on the straight line with 0,58 $Om \cdot m^2$ /min verticality (Fig. 4).

The above described effect when intact cation cells interact with the plasmatic membrane was first discovered during our experiments. These cases primarily reflect the hysteresis case observed in inanimate system and they have been termed as

"surplus" effect. It is noteworthy that hysteresis was also discovered when the conduct of membrane was analyzed under the temperature effect in *Nitellopsis obtusa* cells and *Acetabularıa Meditterranea* as a kind of *Chara*.

3.7. Electrogenous activeness and structural lability of plasmatic membrane as effected by Ca^{2+} ions

Ca^{2+} ions have polyfunctional property in terms of effecting the structural – functional indices of cell membranes. There is always the possibility of cations ingress into cells as cation concentration in cell is mkM and mM in the environment. When this process is seen from isolated cell point of view it becomes clear that this assumption may be ensured by cation penetration into plasmalemma. How ever this process may not be assured by the gradient of highlevel electrochemical potential. The underlying reason is the dependence on potential of the active state of the paths where Ca^{2+} ions are carried through in the plasmalemma. In other words, conductivity of plasmatic membrane depends on potential. There fore analysis of the electrophysiological cell reactions to increase in Ca^{2+} concentration in the environment shall be performed individually for the potential intervals defining the conductivity statuses of the plasmatic membrane.

3.8. Effect of the Ca^{2+} ion concentration in environment to the electrophysiological properties of the plasmatic membrane with active externally rectifying K^+ channels

The cells with membrane potential in the activation range of externally rectifying K^+ channels reacted to 2-3 times increase in Ca^{2+} ions concentration, up to 15-20 mV hyperpolarization of plasmatic membrane, and 25% increase in membrane resistance. Membrane potential increased by 200 mV in absolute value and increase in membrane resistance was twofold on the eve of 10 times increase of cation concentration in the environment. 15-20% increase in electric capacity of plasmatic membrane was observed against the backdrop of cell hyperpolarization mentioned above. Up to 10^{-2} M cation increase in the environment resulted in cells

increase up to -220 ÷ -235 mV of plasmatic membrane, generally 3 times increase in the membrane resistance and 30% growth in electric capacity. Return of Ca^{2+} cations concentration in the environment to their initial level finalized with positioning of electrophysiological metrics of plasmatic membrane at their initial levels.

Following the laws of variation statistics, $\Delta\varphi = 74{,}16\text{-}0{,}607\varphi_m$ dependence (n=20) was found by r = 0,70 coefficient between the membrane potential of the cell φ_m and its hyperpolarization number $\Delta\varphi$. It may be easily concluded here that $\Delta\varphi$ value equaling zero corresponds to φ_m = -242, 34 mV value of the membrane potential. Even the most effective cation concentration hasn't changed plasmatic membrane potential where the value of membrane potential is φ_m = -230± -8 mV. Such hyperpolarization of plasmatic membrane (cell) was accombanied by 3 times increase in membrane resistance and 30% growth in membrane capacity. In other words, hyperpolarization of the plasmatic membrane took place against the backdrop of change in its structural – polarization state and decrease in its ion conductivity.

3.9. Electrophysiological reactions of *Nitellopsis* cells with active K^+ cells internally rectifying the plasmatic membrane to increase in Ca^{2+} ions concentration in the environment

The key feature of such cells in standard environment setting is that they may have high electrogenous activeness (potential membrane level may reach up to 270 mV) and have huge resistance (3-6 $Om \cdot m^2$). Its main reason is due to the fact that resistance of internally rectifying active K^+ channels in these cells is one point higher than resistance of externally rectifying active K^+ channels. Cation positioniong at standard concentration level in the environment resulted in recovery of electrophysiological cell metrics to their initial stationary levels.

Stepwise increase in environment of Ca^{2+} cations was accompanied by increase in electric capacity of plasmatic membrane. Naturally this cation effect is the data regarding change in the structural – polarization properties that took place in the

plasmatic membrane as effected by it. On the other hand, this fact is the expression of structural lability of the plasmatic membrane on the eve of functional activeness. So, this increase in membrane capacity in our experiments was accopmanied by change in its conductivity (resistance) and electrogenous activeness. During the experiments, the initial membrane potential coincided with 3 times increase (in the context of 10 time increase in cation concentration in the environment) of the resistance of plasmati cell membrane in the activation range of externally rectifying K^+ channels and increase in its phase potential (hyperpolarization). This means the hyperpolarization of plasmatic membrane is shut down by cation of its K^+ channels. Increase in cells membrane resistance as effected by cation was also specific to cells in the activation range of internally rectifying K^+ channels. It may be thus concluded that Ca^{2+} cations are blockers of both internally and externally rectifying K^+ channels of the plasmatic membrane of *Nitellopsis obtusa* cells.

Poor sensitivity of cells with electrophysiological metrics in the activation range of internally rectifying K^+ channels (-300 ÷ -160 mV) to relatively low Ca^{2+} concentrations (10^{-3} ÷ $3 \cdot 10^{-3}$ M) is due to the fact that in this range of membrane potential neither Ca^{2+} channels and nor H^+/Ca^{2+} transporters can be active. First ones as they are not in relevant φ_m activation range and second ones as there is no necessary level of concentration gradient for H^+. Further increase in Ca^{2+} ion concentration φ_m placed in extenrnally rectifying K^+ channels activation range resulting from inactivation of H^+ pumps and this was accompanied by multiple decrease in the membrane resistance due to mass activation of the channels.

Electric capacities of plasmatic cell membranes with membrane potential in the activeness range of internally and externally rectifying K^+ channels have qualitatively new, but quantitatively different reactions to increase in Ca^{2+} concentrations in the environment. Increase in environment of Ca^{2+-} concentration was accompanied by blockage of both types of K^+ channels, which case manifested itself as increase in membrane resistance and reflected itself in self – regulation of K^+ channels internally rectifying the potential. More vertical increase in membrane

capacity when intracell processes become activated is the indication of the fact that the functional activeness of plasmatic membrane is accompanied by its structural lability.

Thus, it may be seen from analysis of the reaction of structural – polarization state of *Nitellopsis obtusa* cells plasmatic membrane to the effect of various class of modifiers that the H^+ pumps identifying the functional state of cell membranes and functional activeness of 2 types of K^+ channels are identified by the physical state of lipid phase (temperature, existing electrochemical potential and concentration gradients, chemical modifier properties, electric supply to pumps and etc.) forming the base of structural membrane frame.

CONCLUSIONS

1. Stationary electrophysiological characteristics of the research object Nitellopsis obtusa cells were determined: cell envelope potential φ_o=20±0.3 mV Ro=0.34±0.01 Om.m^2, plasmatic membrane potential φ_m=171±2.4 mV, resistance Rm=3.8±0.15 Om.m^2. From the analysis of the scatter diagram of these quantities, a correlation dependence of Rm=0.032 φ_m -0.03 was determined between them; membrane capacity Cm=0.93±0.06 μF.cm^{-2}, activity of K+ ions in cytosol ak=104±6 mM. Based on the analysis of the dependence of the potential of the plasma membrane on the logarithm of the K+-channels in the environment, the activation ranges of the K^+-channels, which direct outward and inward, were determined: -170÷-120mV; -170÷-270 mV, and φk=-172±8 mV was obtained for K^+-equilibrium potential. [3]
2. Differentiation of electrogenic activity of plasmatic membrane into channel and H+-pump was carried out by application of dicyclohexylcarbodimid and analysis of volt-ampere characteristic: Na+-K+-potential φ_mNa-K=-120 mV, EHQ of H^+-pump -360 mV; short circuit current 0.09A/m^2; R=4 Om.m^2 for internal resistance of H+-pump; the structure-polarization index of the plasmatic membrane was observed to decrease by 27%

due to the effect of the inhibitor, an alternative mechanism of H+-pump inhibition was proposed based on the analysis of the obtained quantities. [9]

3. During the modulation of the protein phase with antibiotics of cyclic and linear molecular structure (valinimycin, gramicidin) for the differentiation of the electric capacity of the plasma membrane, which is an indicator of structure and polarization, the membrane capacity Cm remained stable against the background of changes in other electrophysiological parameters. This fact confirms that the electrical capacity of biological membranes is an indicator of their lipid phase. [2]
4. Under the influence of the polar solvent dimethylsulfoxide, the 2-phase increase in the electric capacity of the plasmatic membrane (40%), synchronously, the first decrease in the membrane resistance (25%), and then a multiple increase, the change in the membrane potential of the cells is an indicator of the functional activity of the solvent against the background of their structural lability, and this fact approves the property of the regulator of processes. [9]
5. High electrogenic activity accompanied by a multiple increase in plasma membrane resistance under the influence of Co^{2+}, a blocker of K^+ channels, reflects a decrease in the shunting load of H^+ pumps due to the blocking of K^+ channels, while hyperpolarization of the plasma membrane and membrane capacitance Cm= The decrease according to the law of $0.93-0.005 \cdot \lg[Co]$ indicates that the functional activity of H^+-pumps is strengthened due to the change in the physical state of membrane lipids. [4]
6. The increase in the concentration of Ca^{2+} ions in the environment occurred against the background of complex electrophysiological reactions reflecting the blocking of K+-channels of Nitellopsis obtusa plasma membranes, activation of Ca^{2+}-channels, changes in the functional activity of H+-pumps. It was decided that against this background, the change in the electrical capacity of the plasmatic membrane can be the result of both the interaction of Ca2+ ions with the

plasmatic membrane and the activation of some intracellular processes, especially enzymatic processes. [12]

7. During the inhibition of H^+-pumps of the plasma membrane (with DSKD), partial washing of the lipid phase (with DMSO), blocking of type 2 K^+-channels (with Co^{2+}, Ca^{2+}) other parameters of the electric capacity of the lipid phase (φ_m, R_m) electrophysiological analysis of the processes of change over time shows that the electrogenic activity of cells occurs against the background of its structural lability. [5]

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