Bioelectric characteristics of *Chara fragilis* (*Characeae*) cells

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Abstract. For the first time the data on the distribution of the potential (φ_m) and resistance (R_m) in the plasma membrane and cell wall (R_0) of *Chara fragilis* cells were obtained using the Hogg method by the number of *C. fragilis* cells in standard conditions. The Hogg method allows simultaneous measurement of electrophysiological parameters, such as φ_m and R_m. The stationary values of the membrane potential and resistance varied in a rather wide range: –90...–300 mV and 1.0–32.6 Ohm·m², respectively. The average values of φ_m were –183.0 ± 4.9 mV, R_m –9.0 ± 1.2 Ohm·m². Using standard modifiers of membrane transport, the electrogenic activity of the cells was differentiated into two types: K^+-channels and H^+-membrane pumps. The activation ranges of the K^+-inward rectifying channels and K^+-outward rectifying channels are –130...–50 and –300...–162 mV, respectively. The cytosolic activity of K^+-ions was 61.6 mmol/L. Since in our research, *C. fragilis* cells were studied for the first time, their electrogenic activity and ionic conductivity were tested using ammonium metavanadate (VO_3^-) – a proton pump inhibitor and tetraethylammonium (TEA^+) – a universal blocker of K^+-channels of plasma membranes. *Chara fragilis* cells can be recommended as a test object for establishing the mechanisms of changes in plasma membrane transport under exogenous stress factors influence.

Keywords: *Chara fragilis*, H^+-pumps, K^+-channels, membrane potential, membrane resistance, plasmalemma, TEA^+-iodide, vanadate

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Реферат. За допомогою методу Хогга вперше отримані дані з розподілу потенціалу (φ_m) та опору (R_m) плазматичної мембрани та клітинної стінки (R_0) клітин *Chara fragilis* у стандартних умовах за кількістю клітин *C. fragilis*. Метод Хогга дозволяє одночасно вимірювати електрофізіологічні параметри φ_m і R_m. Стаціонарні значення мембранного потенціалу та опору варіювали в досить широкому діапазоні: –90...–300 мВ та 1,0...32,6 Ом·м², відповідно. Середнє значення φ_m становило –183,0 ± 4,9 мВ, R_m –9,0 ± 1,2 Ом·м². Із застосуванням стандартних модифікаторів мембранного транспорту здійснена диференціація електрогенної активності клітин на два типи: K^+-каналів та H^+-мембранних насосів. Діапазони активації K^+-каналів внутрішнього та зовнішнього випрямлення становили –130...–50 та –300...–162 мВ відповідно, цитозольна активність K^+-іонів – 61,6 ммоль/л. Оскільки в наших дослідженнях клітини *C. fragilis* використовувалися вперше, перевірялася їхня електрогенна активність та іонна провідність з використанням метаванадату амонію (VO_3^-) – інгібітора протонної помпи та тетраетиламонію (TEA^+) – універсального блокатора K^+-каналів плазматичних мембран. Клітини *C. fragilis* можуть бути рекомендовані як тест-об'єкт для встановлення механізмів зміни мембранного транспорту в плазматичній мембрані під впливом екзогенних чинників та стресових факторів.

Ключові слова: *Chara fragilis*, H^+-насоси, K^+-канали, ванадат, мембранний опір, мембранний потенціал, плазмалема, TEA^+-йодид

Introduction

Experimental studies of *Chara* algae contributed to establishing the components and kinetic characteristics of primary active transport in the plasma membrane of plant cells (Yurin et al., 1991; Véry, Sentenac, 2003; Volkov, 2006), and to revealing the mechanisms of modification of membrane transport by a number of xenobiotics, pharmacological agents, etc. (Musayev et al., 2009; Musayev, 2012). The application of *Chara* algae as a test object is not restricted to the study of cellular mechanisms of the above listed exogenous stimuli action. Isolated *Chara* cells are a very promising tool for detecting adaptive reactions during the modification of membrane transport in the plasma membrane under stress (Demidchik, 2012). To this end, the involvement of new objects with different electrochemical and kinetic characteristics could stimulate identifying meaningful patterns of the response of isolated cells and plants in general to exogenous stimuli. This paper describes the bioelectric parameters of the cells of *Chara fragilis* Desvaux, which we first introduced into the electrophysiological practice. Using specific modifiers of the transport properties of the plasma membrane, the components of the primary active transport and electrogenic activity of *C. fragilis* cells were identified.

The use of vanadate, an inhibitor of H^+-pumps (Opritov, 2000; Larskaya, Zabotin, 2001), could provide valuable information about the possible interactions of the two components of the ion transport system in the plasma membrane of plant cells – H^+-pumps and K^+-channels. Such studies are especially important for cells of freshwater alga *C. fragilis*, since so far there is insufficient information about transport properties of the plasma membrane of these cells (Hasanova et al., 2017). Thereby this work is devoted to the study of the electrogenic and diffusion properties of the plasma membrane of *C. fragilis* under the influence of different concentrations of ammonium vanadate. The aim of the work is to monitor the state of K^+-channels at inhibition of the electrogenic transport implemented by the H^+-pumps of the plasmalemma.

The established facts indicate a close relationship between the two main components of the ion transport system of the plasma membrane, can be used in constructing a general theory of the interaction of xenobiotics with biological membranes. The high sensitivity of *C. fragilis* cells to low concentrations of the inhibitor can be used for express diagnostics of the ion transport system of plant cells.

Materials and methods

The object of the study was the alga *Chara fragilis* found in a small pond, a collector of sewage from a number of mountain springs, on the territory of "Katib Bulagy" in the Tovuz Region of the Azerbaijan Republic. *Chara fragilis* belongs to the family *Characeae* Ag. emend. Hollerb. of the *Charophyceae* class. Taxonomic position of the alga was identified by its anatomical and morphological characteristics using the *Guide to Fresh-Water Algae of the USSR* (Gollerbach, Krasavina, 1983), with the kind support of the algologists of the Institute of Botany, National Academy of Sciences of Azerbaijan.

The length of mature internodal cells of *C. fragilis* reached 6–11 cm with 0.3–1.0 mm diameter. Due to the large size, the internodal cells of *C. fragilis* may be used for several days in the microelectrode assays. The mineral composition of the "Katib Bulagy" water was determined by atomic absorption spectroscopy AAS-1 spectrophotometer company Bruker, Germany, corresponded to the mineral composition of artificial pond water (APW), containing 0.1 M K^+, 1 M Na^+, 0.4 M Ca^{2+}, 0.3 M Mg^{2+}-cations and Cl^-, HCO_3^-, NO_3^-, PO_4^{3-}, SO_4^{2-}-anions at pH 6.9–7.2 (Hasanova et al., 2017). Plants harvested from the "Katib Bulagy" pond were well adapted to laboratory conditions. Plants were grown on APW, in aquariums with $0.3 \times 0.4 \times 0.5$ m dimension under room light conditions (with intensity 6 W/m^2) 12–14 hours per day. The optimal temperature ranges for growing *C. fragilis* was 18–23 ºC. Increasing the temperature of growing medium to 30 ºC and higher was lethal for the plants.

The cells no longer than 20 mm in length were used in the experiments to ensure the high accuracy of membrane resistance measurement. This value corresponded to the length of the second cell from the top of the *C. fragilis* plants. The day before the experiments, an intranodal cell of *C. fragilis*, second from the top, was separated from neighbor internodes and placed in Petri dish with a standard nutrient medium. It was necessary for an accurate assessment of the direction of fluxes and gradients of electrochemical potentials. To measure the main electrophysiological parameters (the membrane resistance R_m, the potential φ_m of the plasma and vacuolar membranes) we used the two-electrode Hogg method (Hogg et al., 1968) developed for cells having a cylindrical shape. The Hogg method made it possible to simultaneously register the membrane potential φ_m and resistance R_m in algal cells for many hours. The direct current pulses with a density of 10^{-4}

A/m² in a duration of 2–3 seconds was transmitted through one microelectrode introduced into the center of the cell. Values φ_m and the electrotonic potential ΔU generated by passing a direct current through the cell was recorded via the second microelectrode. We also carried out a measurement of the current passing through the middle of the experimental cell. The numerical value of the current was determined by the voltage drop at the etalon resistance, connected in series with the experimental cell in the current circuit. R_m of cells is calculated by the formula

$$R_m = \frac{\Delta U}{I} \pi dl,$$

where I – current transmitted through the middle of the cell, l, d – length and diameter of the experimental cell.

APW was used as a solvent in the preparation of working solutions of inhibitors and blockers. Cells were treated with inhibitors or blockers in the measuring chamber during measurement, not in Petri dishes. The growth medium (APW) or working solution was regularly introduced into the corresponding part of the measuring chamber using thin (1.5 mm diameter) silicone tubes (inflow tube), and then removed using these tubes (outflow duct). To treat cells with APW modifiers in the measuring chamber, they were replaced with appropriate solutions.

Statistical processing of experimental results was carried out according to the laws of variation statistics (Lakin, 1990) using of computer programs Excel-2016 and SigmaPlot 12.0.

Results

Statistical analysis of the electrophysiological parameters of *Chara fragilis* cells under standard conditions

Since the *C. fragilis* cells were first introduced by us in the practice of electrophysiological research, we needed to conduct a statistical analysis of the distribution of electrophysiological parameters by the number of cells in standard conditions. It was found that the stationary values of φ_m and R_m varied in a rather wide range: –90... –300 mV and 1.0–32.6 Ohm·m², respectively. The average value of φ_m was –183.0 ± 4.9 mV. The sample size m of the experimental material was 106. The distribution of φ_m obeyed to the Gauss – Moivre – Laplace law of normal distribution. The value of the membrane

resistance, which is calculated from the resistance level of the cell membrane, was 9.0 ± 1.2 Ohm·m² (m = 45). The distribution of the set of values of R_m did not obey the law of normal distribution. The distribution curve compresses on the right. The majority of cells were characterized by less resistance (more conductivity).

Upon contact of the measuring microelectrode tip with the cell membrane under standard environmental conditions, a signal was recorded which value was $\varphi_0 = -30.0 \pm 2.5$ mV (m = 28). It represents the potential of the cell membrane relative to the environment, and this value is commonly called the potential of the cell wall. By passing current through the cell with a current microelectrode, on the cell wall a voltage drop was recorded ΔU_0, by which the value of its resistance $R_0 = 3.8 \pm 0.7$ Ohm·m² (m = 28) was calculated.

The number of classes was determined by the formula proposed by Brooks and Caruzer and amounted 10. The sample size m of the experimental material was 106, the class interval i = 21 mV. Therefore, we presented the variational series φ_m as a distribution of the total volume between 11 classes (Fig. 1). The variational series of φ_m, represented as a histogram, had a maximum at –174 mV.

The estimation of this value, according to our empirical data, yielded $\chi^2 = 16.75$. And the theoretical value of critical value for the 0.5% significance level was 16.75, i.e. $\chi^2 = \chi^2_{kr}$ This means that the distribution of φ_m in classes corresponds to the normal distribution.

The reached stationary levels of φ_m and R_m under standard conditions were stable for several hours. Spontaneous transitions of φ_m, R_m to other levels were not detected.

Similar calculations were made for the membrane resistance. The distributions of the empirical and theoretical frequencies sharply differed among themselves. The distribution of the set of values of R_m did not obey the law of normal distribution. There was no correlation between φ_m and R_m. The correlation coefficient between these values was r = –0.019878. In the total set of cells, proportion of cells with increased conductivity dominated.

Electrogenic activity and conductibility of plasma membrane in *Chara fragilis* cells under influence of vanadate

The next step in our research was testing the limits of the electrogenic activity of the H⁺-pumps and the conductivity of K⁺-channels of the plasma membrane using the appropriate modifiers. The electrogenic composing of the membrane potential was determined using vanadate,
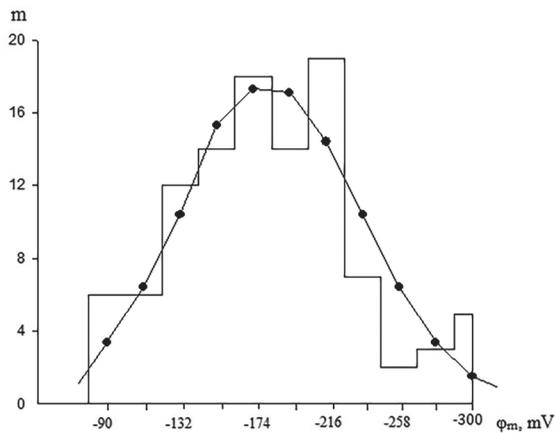
Fig. 1. Histogram of the membrane potential φ_m distribution of the *Chara fragilis* by the number of cells (m). The figure shows the theoretical frequencies calculated from the variation statistics of the dotted variants. A continuous line shows the calculated normal distribution

a common inhibitor of H^+-pumps of plasmalemma. The minimum concentration of the inhibitor, which caused a noticeable depolarization of the plasmalemma, was 10^{-5} M. An increase of its concentration in the medium up to 10^{-3} M, led to the depolarization of the plasmalemma to 80–100 mV within 30–60 min (Fig. 2).

In 89% of the cells of the total population, VO_3^--induced depolarization of the plasma membrane was accompanied by a monotonic decrease of the R_m. The relative decrease in R_m in these cells under the influence of a 10^{-4} M inhibitor for 33–30 minutes was 33%, but under the influence of the most effective concentration of the inhibitor 10^{-3} M was 14% (Fig. 2).

The removal of the inhibitor from the solution of artificial pond water was accompanied by the restoration of the φ_m, R_m values in the investigated cells. However, φ_m, as a rule, was restored to levels exceeding the initial level on 36 mV. On the contrary, under normal environmental conditions after inhibitory treatment, the final R_m levels of of the investigated cells processing were 7–8% lower than the initial values (Fig. 2).

Electrophysiological analysis of changes in the kinetics of φ_m and R_m in *Chara fragilis* cells under the influence of tetraethyl ammonium

To test the electrogenic activity of *C. fragilis* cells while reducing the shunt load of H^+-pumps of plasma membrane, we used in our measurements iodide salt of tetraethyl ammonium (TEA^+) – a blocker of all types of K^+-channels. The minimum concentration of the blocker that caused the primary bioelectric response of the cells
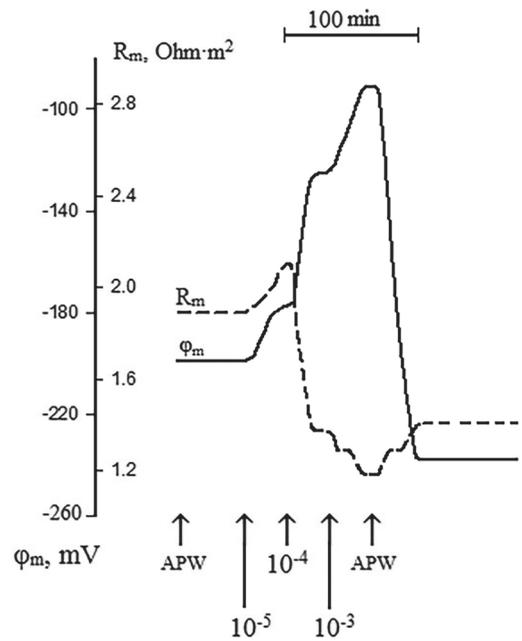

Fig. 2. The potential φ_m and resistance R_m of the plasma membrane of *Chara fragilis* cells in the presence of vanadate. The arrows indicate the moments of the appropriate concentrations (molar) addition in the nutrient medium (APW). Cell size: length 7.1 mm, diameter 0.325 mm

was 10^{-7} M in the medium. The electrophysiological reaction of the plasma membrane of *C. fragilis* was rather complicated in the presence of different blocker concentration in the medium (Fig. 3).

K$^+$-characteristics of *Chara fragilis* cells

The dependences of φ_m and R_m on the K^+-concentration in the medium were illustrated by three-phase curves (Fig. 4). The first phase of the dependence in the range of φ_m –300...–165 mV probably shows the activation of K^+-channels of inward rectification (KCİR), and the third phase in the range of φ_m –120...–50 mV – K^+-outward rectifying channels (KCOR). The second phase can probably be called a transition phase, because presumably the partial inactivation of the K^+-inward rectifying channels and activation of the K^+-outward rectifying channels occur in this phase.

For the other *Chara* species, the K^+-equilibrium potential in a medium with 10^{-4} M K^+ corresponded to the boundary range of the activation for two kinds of K^+-channels. According to it, φ_m for *C. fragilis* is –162 mV (Fig. 4). In view of this value, the intracellular activity of K^+-ions (a_{K^+}) can be calculated from the Nernst formula, which was 61.6 mmol/L.
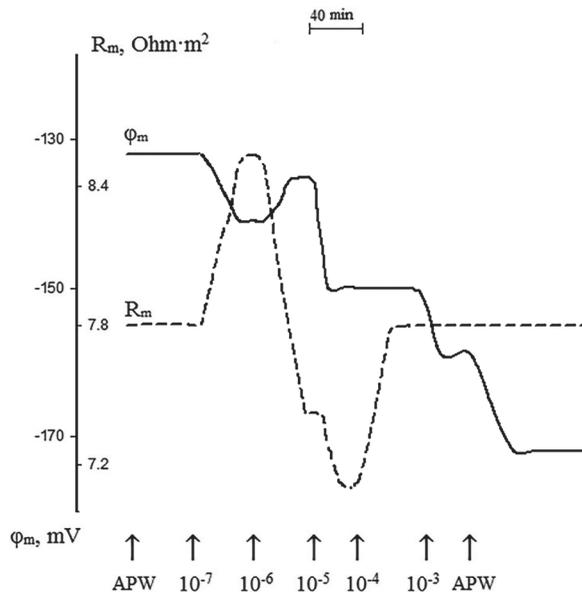
Fig. 3. Changes of the potential φ_m and resistance R_m of the plasma membrane of the *Chara fragilis* cells with a progressive increase in TEA^+-iodide concentration 10^{-7}–10^{-3} M in the nutrient medium. The arrows indicate the moments of the appropriate concentrations (molar) addition in the nutrient medium (APW). Cell size: length 10.6 mm, diameter 0.45 mm

Discussion

The established ranges of φ_m reflecting the conductivity states of the two types of K^+-channels are consistent with the analogous parameters of other species of *Characeae*: *Nitella flexilis* (Musayev et al., 2008; Musayev, 2012), *Chara gymnophylla* (Sokolik, Yurin, 1986; Musayev, Ismailov, 2007) and *Nitellopsis obtusa* (Musayev, 2012). For these cells, the measured K^+-equilibrium potential in a medium with 10^{-4} M K^+-corresponded to the boundary of the activation ranges for two kinds of K^+-channels. According to this position, φ_k for *Chara fragilis* is –162 mV (Fig. 4). In view of this value, the intracellular activity of K^+-ions (a_k) can be calculated from the Nernst formula, which was 61.6 mmol/L. The obtained value of a_k is much lower than in *Nitellopsis optusa* cells (Musayev, 2012). However, it is comparable to the value of a_k established for another species of *Chara* – *C. gymnophylla* (70 mmol/L) (Musayev, Ismailov, 2005).

The electrogenic composing of the membrane potential was determined using vanadate – a common inhibitor of H^+-pumps of plasmalemma. The inhibitor has been most effective in concentration 10^{-3} M. The addition of an inhibitor at this concentration to the standard medium caused depolarization of the plasma membrane by –170...–180 mV in cells with the initial
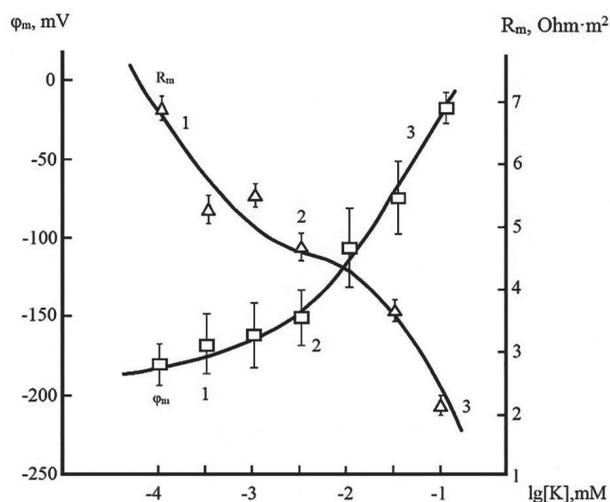

Fig. 4. Dependence of stationary values of φ_m and R_m of the PM of *Chara fragilis* cells on the concentration of KCl in the APW. The marks 1, 2, 3 on the curves indicate the particular phases of change of the corresponding values. Each point reflects the average of the measured values of φ_m and R_m for 9–10 cells. Vertical lines indicate the standard deviation of the mean of φ_m and R_m

φ_m = –250 mV (Fig. 2). This indicates a significant contribution of H^+-pumps to the electrogenic activity of the plasma membrane of *C. fragilis*. The decrease R_m on the background of progressive depolarization of the plasma membrane represents the massive activation of K^+-outward rectifying channels, that was also found in *C. gymnophylla* cells (Musayev et al., 2008).

For testing the electrogenic activity of *C. fragilis* with a decrease in the shunt load of H^+-pumps of plasma membrane, we used iodide salt of tetraethyl ammonium (TEA^+) – a blocker of all types of K^+-channels (Yurin et al., 1991). The minimum concentration of the blocker that caused the primary bioelectric response in the cells was 10^{-7} M. The electrophysiological reaction of *C. fragilis* plasma membrane was rather complicated in the presence of different blocker concentration in the medium (Fig. 3). When analyzing the kinetics of the electrophysiological reaction, it is necessary to consider the multifacetedness of the blocking action of TEA^+. In the cells of *Nitella flexilis* (Vysotskaya et al., 2005) and *C. gymnophylla* (Musayev, Vorobev, 1983), TEA^+-blocking of K^+-channels led to stimulation of the H^+-pump of the plasma membrane and reduction of its integral resistance. Earlier, we also observed changes in the structural-polarization state in the cells of *Nitellopsis obtuse* under the influence of the blocker (Musayev, Vorobev, 1983).

The distribution of the potential and resistance by the number of cells indicates that a significant part of the total number is composed of cells with high electrogenic activity.

Conclusion

Based on the results reported in this study, it can be proposed that the interstitial cells of *Chara fragilis* with stable electrophysiological parameters can serve as a new promising object for the investigation of the membranotropic actions of exogenous stimuli, including xenobiotics, nanoparticles, phytoprotectors, pharmacological agents, etc. Established dependencies of the resistance and potential of the plasma membrane on the concentration of K^+ in the medium made it possible to identify the ranges of the membrane potential within which the K^+-channels of the inward or outward rectification are in the conducting state. Inhibitory analysis allowed to determine the proportion of the electrogenic component in the total membrane potential. Accordingly, the differentiation of the membrane potential into K^+-channels and H^+-pumps was implemented. Significant values of the potential and resistance of the cell membrane allow the using of isolated *C. fragilis* cells for studying the ion-exchange properties of the plant cell membranes.

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