Baku State University Journal of Life Sciences & Biology ***2025****, v.2 (3), p. 13-18*

journal homepage: http://bsuj.bsu.edu.az/en

https://doi.org/10.30546/300045.2025.2.3.2026

MAIN ELECTROPHYSIOLOGICAL PROPERTIES OF CHAROPHYTE CELLS IDENTIFIED IN TWO WATER BODIES OF AZERBAIJAN

Shalala Najafaliyeva*, Nagi Musayev

Baku State University, 23 Z. Khalilov St, AZ-1073, Baku, Azerbaijan

Received 23 september 2025; accepted 30 september 2025

Abstract

The paper presents the results of the analysis of electrophysiological parameters of isolated cells of two species of algae taken from separate natural water bodies of Azerbaijan. *Chara gymnophylla* cells were collected from Guzgugol lake, which is part of the lake system of the Goygol lake Nature Reserve. *Chara fragilis* cells were collected from a small water body in the Katib Bulagy fount of Tovuz district. Isolated cells from both plants were subjected to detailed electrophysiological analysis and proved to be unique for the analysis of ion exchange patterns and electrogenic transport between the environment and the cell, using glass microelectrodes. A comparison of the electrophysiological properties of the noted species of charophytes is carried out for the first time.

Keywords: *Chara fragilis, Chara gymnophylla, isolated cells, membrane resistance, membrane potential, cellular resistance*

1. Introduction

Isolated cells of charophytes have generally proven to be successful subjects for classical and modern electrophysiological studies. The use of these cells as a research object using microelectrode technology allows us to monitor the progress of physiological and biochemical processes at the level of cell membranes without disturbing their integrity. In this regard, the data found in two water bodies of the Republic of Azerbaijan and their use as a research object made it possible to establish a number of their electrophysiological properties under standard conditions and under the influence of modifiers of the transport properties of cell membranes.

Chara fragilis algae were collected from a small water body in the Tovuz district, Katib Bulagy, and *Chara gymnophylla*, from Guzgugol lake, which is part of the lake system of the GoyGol Nature Reserve.

2. Materials and Methods

The taxonomy of the *Chara* algae we discovered was determined with the participation of researches of the relevant department of the Institute of Botany using Gollerbach's identification guide. The ionic composition of the water in the lakes from which the plants were selected was determined by atomic absorption spectroscopy using an AAS-1 N spectrophotometer (Germany): (mM) K^+ - 0.1; Na^+ - 1.0; Ca^{2+} -

*Corresponding author. Tel.: +994 10 324 18 86
E-mail address: sh.nacafaliyeva@mail.ru

0.4; pH = 6.8. Thus, the main mineral elements of the water of these lakes coincided in quantity with the mineral components of artificial pond water (APW) adopted as a standard, which contained: (mM) KH_2PO_4 - 0.1; $CaCl_2$ - 0.4; $NaHCO_3$ - 1.0; $Mg(NO_3)_2$ - 0.1; $MgSO_4$ - 0.1; pH = 7-7.5. Plants were grown in IPV with the addition of Shollar water (1:1) in aquariums measuring 0.3x0.4x0.5 m. The aquariums were illuminated with fluorescent lamps (8 W/m^2) for 14-16 hours per day. The growing environment temperature varied between 18-23°C. Increasing the temperature of the growing environment to 30^0C and above led to the complete destruction of plants.

Plants collected during the summer adapted poorly to laboratory conditions. Their growth and reproduction rates decreased to a minimum.

Internodal cells of *Chara gymnophylla* and *Chara fragilis* have proven to be very convenient objects for microelectrode studies according to the following parameters:

1. Large size, which allows the insertion of several glass microelectrodes into the cell without compromising its integrity;
2. The cylindrical shape makes it easy to accurately calculate the current density passing through the lateral surface, as well as the resistance per unit area of the lateral surface (membrane resistance) of the cells being studied;
3. Elasticity of the plasma membrane, which allows the insertion of microelectrodes into the cell with virtually no disruption to its integrity;
4. High electrogenic activity, allowing variation of the membrane potential (φ_m) under the influence of physicochemical factors over a wide range;
5. High sensitivity of membrane resistance (R_m), an indicator of the state of the ion-transporting system of the cell membrane, to the action of ion channel modifiers.

The cells of *Chara gymnophylla* and *Chara fragilis* are very similar in appearance. They differ only in that *Chara gymnophylla* cells have pinches on their surface, resembling processes of the epidermal cells.

Measurement of electrophysiological parameters of cells was carried out using the Hogg method developed for long cylindrical cells of the *Nitella, Nitellopsis* and *Chara* types [2]. Hogg's measurement method is based on standard microelectrode technology. To simultaneously and continuously record membrane resistance R_m and potential φ_m, direct current pulses of 1-2 s duration and a density of 10^{-5} A/m^2 were passed through the center of the cell being studied. Using a second intracellular microelectrode (the measuring microelectrode), inserted at a fixed distance from the current microelectrode, the electrotonic potential (shift φ_m) arising from the passage of a direct current ΔU) was measured. The resistance per unit area of the membrane was calculated using the formula:

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

Here d and l - are the diameter and length of the experimental cell, I - is the current strength passed through the middle of the experimental cell, which were measured on a scale installed in the eyepiece of a binocular magnifying glass.

3. Results and discussion

When the tip of the measuring microelectrode (MI) came into contact with the cell surface, the potential of the cell membrane was recorded, the value of which was φ_0=40.5±3.3 mV. When passing current through the cell, an additional voltage drop on the cell membrane ΔU_0 was recorded, from which the resistance value of the cell membrane R_0=0.9±1.14 $Om{\cdot}m^2$ was calculated. When the measuring electrode was insulated by a "sheath" formed at the tip and the membrane system of the cell was irreversibly damaged, φ_m fell to the level of φ_0, and R_m to R_0. When the tip of the measuring microelectrode was localized in the cytoplasm or in the vacuole, φ_m and ΔU were recorded, which were used to determine R_m of *Chara gymnophylla*. The values of φ_m and R_m were -167±2.5 *mV* and 4.51±1.55 Ohm·m^2, respectively. R_m was calculated from the R_0 level. R_0 accounted for up to 16% of the total resistance between the vacuole and the environment, reflecting the fact that *Chara gymnophylla* cells have a dense and rigid cell wall. After calcining the cell membrane, the tip of the IM, penetrating the cytoplasm (10 μm thick), ended up in the vacuole of the cells being studied. The movement of IM through the tonoplast into the cytoplasm was not reflected in the value of R_m, and a potential jump in the tonoplast of 10-15 *mV* was clearly recorded. Thus, the sum of the resistances of two series-connected membranes – the plasma membrane and the tonoplast – did not differ from the value of the plasma membrane resistance. Therefore, we consider that the φ_m and R_m values we measured reflect the

actual state of the *Chara gymnophylla* plasma membrane. Under normal conditions, φ_m and R_m had a wide range of values: 90÷240 *mV* and 1÷13 Om·m^2, respectively. When constructing the variation series, the set of values was divided into classes, the number of which was determined using the Brooks and Caruthers formula: K=5 lg*n*, where n is the sample size (number of measurements), which in our experiments was 100. The class interval was determined using the standard rule, which was 15 *mV*. To equalize the empirical distribution, the theoretical class frequency was calculated using the Gauss-Moivre-Laplace equation. Pearson's χ^2 test was used to compare the sets of empirical and theoretical frequencies φ_m. According to our empirical data, χ^2 was 14.87, which means that the distribution of φ_m, by classes at a significance level of 5%, follows a normal distribution (Fig. 1).

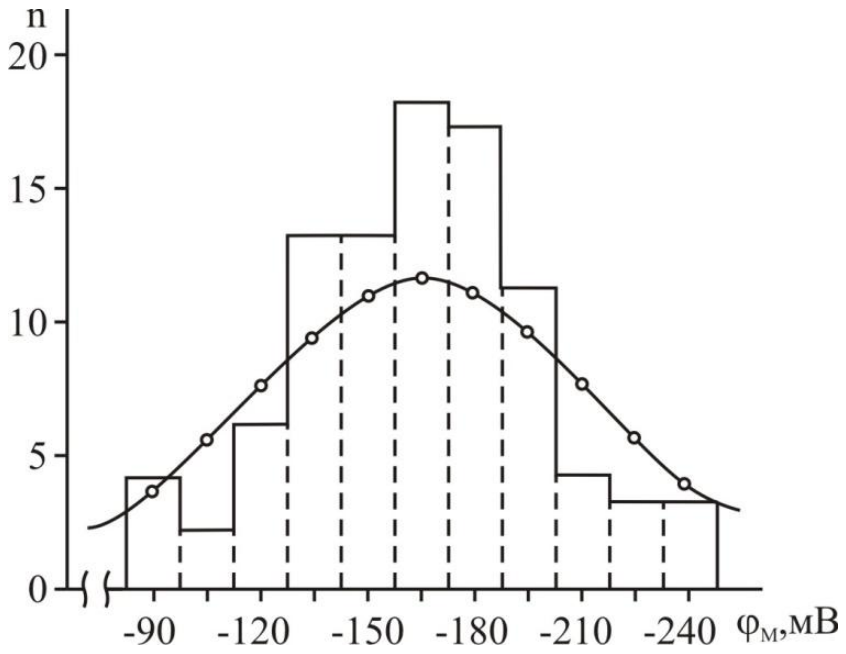

Fig. 1. Histogram of the distribution of the membrane potential φ_m of *Chara gymnophylla* by the number of cells n. The continuous line shows the theoretical dependence of the normal distribution.

However, the distribution of empirical and theoretical frequencies for R_m differed sharply. The peak of the distribution curve is shifted to the left of the center. The asymmetry is right-sided. Without using additional criteria, it could be seen that the empirical distribution of R_m by the number of cells does not obey the law of normal distribution. Analysis of the scatter plot of R_m and φ_m showed that there is no correlation between these values, as evidenced by the low correlation coefficient r=0.0025.

A 10-fold increase in the K^+ concentration in the medium (in a normal medium 0.1 mM K^+) in cells with an initial φ_m of -240÷-110 *mV* depolarized the plasma membrane by 10÷40 *mV* within 15 minutes. A 100-fold increase in the concentration of K^+ in the nutrient medium caused a sharp drop in φ_m by 80÷150 *mV*. Steady-state φ_m levels in a medium with 10 *mM KCl* were located in the amplitude range of 25 *mV*. Depolarization of the plasma membrane under the influence of 1 mM K^+ was accompanied by a decrease in R_m by 24%, and a sharp decrease in φ_m with a 100-fold increase in K^+ in the medium was accompanied by a decrease in R_m by 70%. The weak sensitivity of the parameters φ_m, R_m of cells to a 10-fold increase and the increased sensitivity to a 100-fold increase in the K^+ content in the medium were clearly revealed as a result of statistical processing of the kinetic patterns (Fig. 2). In the concentration range of 10÷100 *mM* in the medium, a linear dependence of the membrane potential on the cation concentration was revealed. The slope of the linear portion of the dependence with a 10-fold change in the K^+ concentration in the medium was 58 *mV*, which made it possible to use this portion of the "K^+ characteristic" of the membrane potential to determine the intracellular activity of K^+ using the Nernst equation (Fig. 1). Thus, the K^+ activity in the vacuole of *Chara gymnophylla* cells was 70 *mM*, the K^+ equilibrium potential φ_K, under normal environmental conditions was -160 *mV*. The bioelectrical responses of *Chara gymnophylla* cells to an increase in the K^+ content in the nutrient medium were completely reversible.

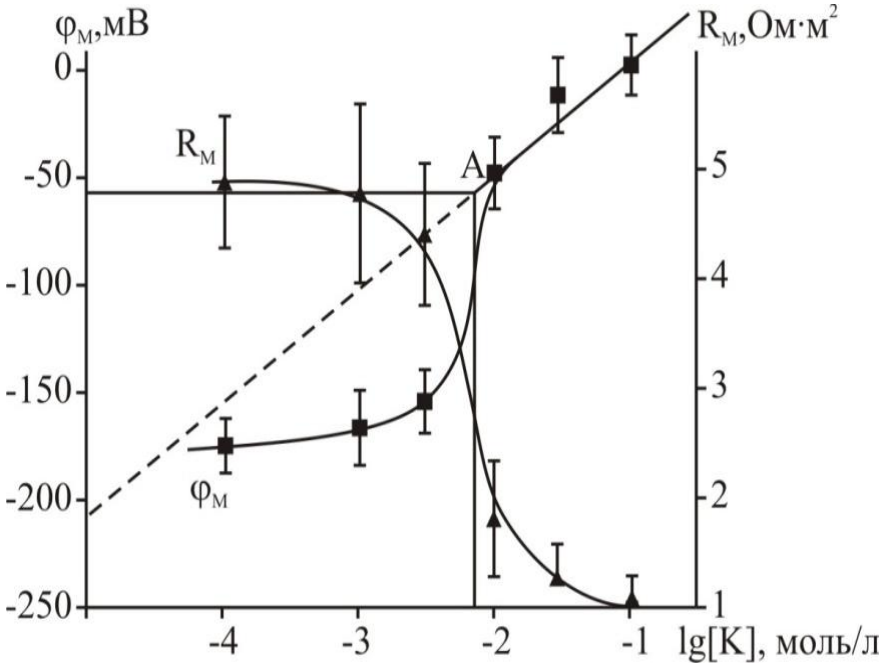

Fig. 2. Dependence of the steady-state properties and resistance of the Chara *Gymnophylla plasma* membrane on the logarithm of the KCl content in the medium. The dotted line indicates the K^+ equilibrium potential. Using the coordinates of any point on the dotted line, the intracellular K^+ activity (concentration) can be calculated using the Nernst formula. The coordinates of point A are x = 70 mmol, y = 58 mV.

To differentiate the functional states of the plasma membrane, a three-phase dependence of φ_m on the concentration of K^+ in the medium was used (Fig. 2). The first phase of plasma membrane depolarization to -160 *mV* possibly reflects the displacement of Ca^{2+} by an increased concentration of K^+ from the plasma membrane, which may cause the appearance of pathways for the passive entry of H^+ from the environment into the cell [3]. In the second, steeper phase of the dependence (φ_m~-160÷-50 *mV*), we believe that a massive increase in the number of outward rectifying K^+ channels occurs, which is evidenced by a 3-fold decrease in R_m (Fig. 1). This proposal is in good agreement with the range of K^+ channel activation established for other charophyte species [4, 5]. Therefore, it can be assumed that the plasma membrane conductivity at $|\varphi_m|$>160 *mV* is determined by the conductivity of H^+-pumps. On the other hand, $|\varphi_m|$=160 *mV* is apparently the lower limit of activation of inwardly rectifying K^+-channels. However, due to the high inward resistance, the rectifying K^+ channels can be bypassed by the low resistance of the H^+ pumps, and it can be assumed with great confidence that the current passing through the plasma membrane at $|\varphi_m|$>160 *mV* is generated by the H^+ pumps. Based on this assumption, the current density of H^+-pumps through the plasma membrane was estimated to be 5,5 *mkA⁄sm*^{2}. This is in good agreement with the data obtained on *Nitellopsis* cells [4, 6].

To establish the adequacy of the data obtained from *Chara gymnophylla* cells, a similar analysis was performed on *Chara fragilis*, a species of charophyte algae discovered in a small water body (Katib Bulagy) in the vicinity of Tovuz, Republic of Azerbaijan.

The taxonomy of the algae we discovered was determined as in the case of *Chara gymnophylla*

Chara fragilis cells are being used for the first time in electrophysiological research. This necessitates a detailed statistical analysis using strict mathematical criteria.

The measured values of φ_m and R_m were distributed within the ranges of -90 ÷ -300 mV and 1 ÷ 32.6 Ohm m^2, respectively. The number of φ_m and R_m variants was 106 and 45, respectively. The average values of φ_m and R_m were -183±4.9 mV and 9 ± 1.2 Om m^2. Taking into account the numerical values of the electrophysiological properties of *Chara fragilis* cells, as well as the Caruthers formula and the Pearson criterion, it was established that the distribution of φ_m by classes follows a normal distribution law, which was also characteristic of the distribution of the numerical values of φ_m of *Chara gymnophylla* cells. (Fig. 2).

When the tip of the measuring microelectrode came into contact with the cell surface, a small potential difference of -30 ± 2.5 mV (n=28) was recorded, which corresponded to the cell membrane potential.
In this position of the measuring electrode, when current was passed through the cell, a potential difference ΔU_0 was recorded, which corresponded to the cell membrane resistance R_0 = 3,8 ± 0,7 Om·m^2.

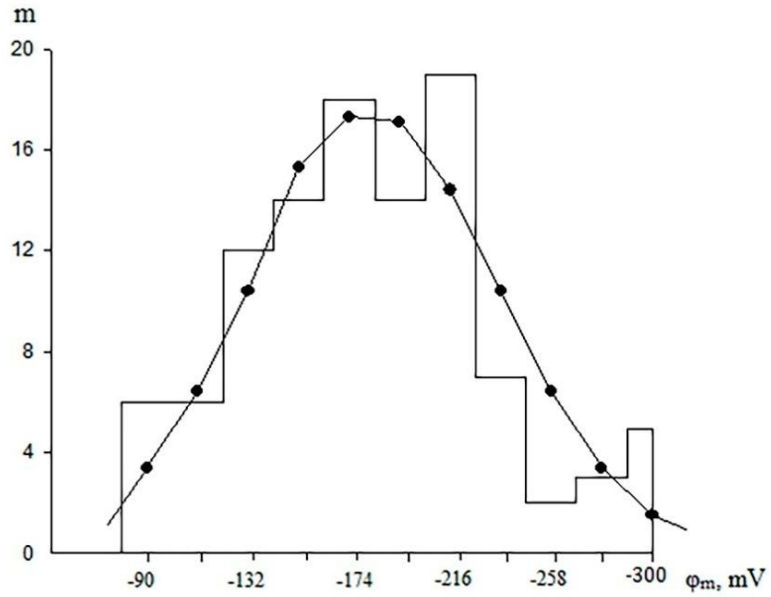

Fig. 3. Histogram of the distribution of the membrane potential φ_m of *Chara fragilis* by the number of cells n. The continuous line shows the theoretical dependence of the normal distribution.

The correlation between the values φ_m and R_m has not been established, as evidenced by the calculated coefficient r = -0.019878.

Analysis of the dependence of membrane potential on the logarithm of K^+ concentration has established the rectifying property of the plasma membrane of *Chara fragilis*, which indicates the presence of two types of K^+ channels – internal and external rectification. Ranges of membrane potential have also been established that reflect the active state of the corresponding channels. The values obtained in this regard are in close agreement with those obtained for other species of *Chara: Nitella flexilis* [7, 6], *Chara gymnophylla* [9, 8] and *Nitellopsis obtusa* [10]. It is particularly noteworthy that the boundaries of the activation ranges of the two types of K^+ channels and the values of K^+ equilibrium potentials are also in close agreement, which is 162 mV. Taking into account the value of the K^+ equilibrium potential, the activity of K^+ ions in the vacuole of *Chara fragilis* cells was calculated using the Nernst formula, which was 61.6 mmol/l.

To conclude this overview of *Chara fragilis* as a novel object of electrophysiological research, it should be emphasized that the internodal cells of these algae exhibit high electrogenic activity.The membrane potential of some cells we measured in these algae reached 300 mV (Fig. 3). The generator of a enormous amount of electrochemical activity is an H+- pump, operating on a plasmatic membrane of algae cells, which has been proven by inhibitory analysis with application of 10^{-3} M vanadate. A significant decrease in R_m against a background of depolarisation reflected massive activation of K^+ channels of external rectification [6].

4. Conclusions

Thus, two species of charophytes have been discovered for the first time in two natural bodies of water in the Republic of Azerbaijan. These are *Chara gymnophylla*, found in Guzgugol lake, part of the lake system of the Goygol Nature Reserve, and *Chara fragilis*, found in the small Katib Bulagy water body in the Tovuz District. Isolated algae cells have long been used as a research object in the practice of electrophysiology and in studies of the biophysical basis of cellular processes. The internodal cells of both algae proved to be very convenient objects for identifying ion channels based on their functional state, selectivity, and conductivity. Using ion channel modifiers, the possibility of selectively altering their permeability was established.

In the future, using the above-mentioned types of algae, it is possible to obtain protoplasmic droplets for further observation of the possible assembly and disassembly of selective ion channels..

References

[1] Gollerbach VI, Krasavina L.K. Identification guide to freshwater algae of the USSR. Leningrad: Nauka, 1983, 185 p.

[2] Hogg ., Williams EJ, Jhonston RI. A simplited method for measuring membrane resistances Nitella translucens // Biochim. et Biophys. Acta, 1968, v.150, p.518

[3] Beilby MJ. Potassium channels at *Chara* plasmalemma // *J. Exp. Botany*, 1985, v.36, №163, p. 228-239

[4] Magerramov MG. Study of the influence of auxin and fusicoccin on the electrochemical regulation of H^+-pumps in plant cells. PhD thesis in biological sciences. Baku, 1989, 159 p.

[5] Yurin VM, Sokolik AI., Kudryashov AP. Regulation of ion transport through plant cell membranes. Minsk: Nauka i Tekhnika, 1991, 272 p.

[6] Musayev,NA, Yurin,VM, Sokolik AI, Ali-zade VM. Mechanisms of Modification of Transport Properties of the Plasma Membrane in Plant Cells. Proceedings of the Belarusian State University, 2012, Vol. 7, Part 1: Plant Physiology, pp. 154–162.

[7] Musayev NA., Yurin VM, Sokolik AI, Ismailov E. Electrogenic Activity of the plasma membrane in *Chara gymnophylla* cells under blockage of K^+ channels by tetraethylammonium. Bulletin of the Belarusian State University, Series 2, No. 2, Minsk: BSU, 2008, pp. 46–49.

[8] Musaev NA, Ismailov ER. Membrane potential and resistance of *Chara gymnophylla* plasmamembrane during interaction wish cobalt (Co^{2+}). Prosiding of the ninth Baku international congress "Energy, ecology, economy", Baku, 7-9 June, 2007, p.336-340.

[9] Sokolik AI, Yurin VM. Potassium channels in plasmalemma of *Nitella* cells at rest // *J. Membr. Biol.*, 1986, v.89, № 1, p. 9-22.

[10] Musayev NA. Biophysical mechanisms of modification of transport properties of the plasma membrane in plant cells. Dissertation submitted for the degree of Doctor of biological sciences. Baku, 2012, 336 p.