ISSN 1990-7478, Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology, 2010, Vol. 4, No. 2, pp. 232–239. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © A.A. Bulychev, N.A. Krupenina, 2010, published in Biologicheskie Membrany, 2010, Vol. 27, No. 2, pp. 209–217.

ARTICLES

Inactivation of Plasmalemma Conductance in Alkaline Zones of *Chara corallina* after Generation of Action Potential[1]

A. A. Bulychev and N. A. Krupenina

Department of Biophysics, Faculty of Biology, Moscow State University, Moscow, 119991 Russia; fax: 7 (495) 939-1115; e-mail: bulychev@biophys.msu.ru

Received April 28, 2009

Abstract—A microelectrode study with *Chara corallina* cells has shown that post-excitation changes of membrane potential and plasmalemma resistance, induced by the action potential (AP) generation, differ substantially for cell areas producing zones of high and low external pH. In cell regions producing alkaline zones, the AP generation was followed by post-excitation hyperpolarization by about 50 mV, concomitant with four- to eightfold increase in plasmalemma resistance and a considerable drop of pericellular pH. In the acidic areas the post-excitation hyperpolarization was weak or absent, and the membrane resistance showed no significant increase within 1–2 min after AP. The membrane excitation in the acidic zones was accompanied by a noticeable pH increase near the cell surface, indicative of the inhibition of plasma membrane H^+ pump. The results suggest that the high local conductance of the plasmalemma is closely related to alkaline zone formation and the depolarized state of illuminated cell under resting conditions. Excitation-induced changes of membrane potential and pH in the cell vicinity were fully reversible, with the recovery period of ~15 min at a photon flux density of ~100 μE/(m^2 s). At shorter intervals between excitatory stimuli, differential membrane properties of nonuniform regions turned smoothed and could be overlooked. It is concluded that the origin of alkaline zones in illuminated *Chara* cells cannot be ascribed to hypothetical operation of H^+/HCO_3^- symport or OH^-/HCO_3^- antiport.

Key words: Characean algae, plasmalemma, H^+ transport, membrane conductance, intracellular patterns, action potential

DOI: 10.1134/S1990747810020169

Proton transport across the plasmalemma of plant cells plays a key role in the maintenance of cytoplasmic pH and generation of membrane potential [1]. The electrochemical proton gradient ($\Delta\mu_{H^+}$) created by the H^+ pump at the plasma membrane is the driving force for accumulation of nutrient elements, e.g., K^+ and NO_3^-, and participates in regulation of cell turgor and intracellular osmotic pressure. In aquatic plants inhabiting slightly alkaline waters, the active H^+ extrusion from the cytoplasm and acidification of the apoplast might stimulate photosynthetic activity, because a slightly acidic pH near the cell surface elevates the content of carbon dioxide (CO_2), a species that readily passes through the membranes, unlike poorly permeable charged forms HCO_3^- and CO_3^{2-} [2].

Depending on the function performed by the cell, the distribution of $\Delta\mu_{H^+}$ generators and $\Delta\mu_{H^+}$ consumers over the cell can be comparatively uniform or nonuniform. Heterogeneous spatial distribution of H^+ extrusion and passive H^+ inflow is spectacularly evident in some aquatic plants, characean algae in particular. Illuminated *Chara* cells produce alternating zones of low and high pH (6.3–6.7 and 9.5–10.0) that are separated by a periodic distance of 7–10 mm along the internode length [3, 4]. In this case the area of H^+ circulation apparently expands from the micrometer and submicrometer level to macroscopic dimensions.

Application of vibrating extracellular microelectrodes revealed the existence of circular electric currents between acid and alkaline zones of illuminated cells [5, 6]. In the acidic regions the electric current is directed outward, in consistency with the direction of active H^+ transport, and in alkaline regions the current flows from the medium into the cytoplasm. Based on these data, it is commonly assumed that the transmembrane charge transfer in the acidic zones is performed by the plasma membrane H^+ pump [7]. The origin of ion flows in alkaline areas is not yet elucidated. Phenomenologically, the alkaline zones can arise as a result of H^+ influx into the cell or OH^- efflux from the cytoplasm to the outer medium. The OH^-

[1] The article was translated by the authors.
Abbreviations: AP, action potential; MP, membrane potential; pH_o, pH of the outer medium near the cell surface; R_m, plasma membrane resistance.

efflux seems even more probable [8], because the local external H^+ concentration is so low at pH ~10 that the appreciable H^+ flux could be only attained at very high H^+ permeability of the membrane (orders of magnitude higher than OH^- permeability). Nevertheless, by analogy with the H^+ cycle concept validated for energy-coupling membranes of mitochondria and chloroplasts, it is common to implicate the inward H^+ flux [1, 9] via channels whose conductance increases enormously at high pH [10]. Such an assumption seems justified because the concentration of protons is strictly related to the OH^- concentration via the ion product constant for water.

The currently dominant hypothesis presumes that the inward H^+ influx along the gradient created by the H^+ pump in characean internodes is coupled to the influx of HCO_3^-; i.e., the transport is electrically neutral [6]. A variant of this hypothesis considers the OH^- efflux in exchange for HCO_3^- influx [11]. The question of whether the transmembrane H^+ flow in the alkaline areas is electrogenic or electroneutral can be resolved by measurements of membrane resistance upon the abrupt cessation of H^+ transport. The arrest of H^+ flow should not affect the plasma membrane resistance, R_m (or an inverse quantity, conductance) in the case of electrically neutral transport (H^+/HCO_3^- symport or OH^-/HCO_3^- antiport) but should be manifested in R_m changes in the case of noncoupled electrogenic transport. It is known that specific plasma membrane resistance in alkaline regions is substantially lower than in acid areas [12]. These differences provide indirect evidence for considerable H^+ or OH^- conductivity of the so-called "high pH channels" [8, 10, 13, 14].

A convenient way for simultaneous interruption of H^+ flows in alkaline and acidic zones of characean cells is triggering the action potential (AP) by stimulation with a short pulse of electric current [15]. Following the AP propagation, pH in alkaline zones decreases considerably, reflecting the stoppage of steady-state H^+ influx to the cytoplasm, while the pH in the acidic zones increased slightly. The AP-induced changes are reversible: the recovery of pH zones takes 10 to 30 min depending on light intensity and other factors [16].

The aim of this work was to examine the changes of plasma membrane resistance R_m in various regions of *Chara corralina* cell after the AP generation and cessation of transmembrane H^+ flows. We show that the arrest of H^+ flows is accompanied by four- to eightfold R_m increase in the alkaline areas, with insignificant R_m increase in the acidic zones. The results indicate that the H^+ entry into the cytoplasm in alkaline cell regions is electrogenic, i.e., not coupled with the cotransport of HCO_3^- anion.

EXPERIMENTAL

Chara corallina algae were grown in an aquarium at room temperature under scattered daylight. Individual internodal cells used in experiments measured about 6 cm in length and 0.9–1 mm in diameter. Isolated cells were placed into artificial pond water containing 0.1 mM KCl, 1.0 mM NaCl, and 0.1 mM $CaCl_2$ and were allowed to stay in this solution for at least one day prior to measurements. Young cells without calcium incrustations, as well as mature cells with crystal depositions on the cell wall were sampled. The visually distinguishable calcification zones served as a preliminary indicator of alkaline zone locations. The cell position in the measuring chamber was adjusted in such a way as to ensure the placement of the target area in the middle compartment between insulation gaps. In the absence of calcium incrustations, local pH differences were visualized by means of the indicator dye phenol red that brought yellow color to acidic zones and red color to alkaline zones.

Individual internodal cells were mounted in a plexiglass three-compartment transparent chamber, which was placed on a specimen stage of an Axiovert 25 CFL inverted microscope (Zeiss, Germany). Partitions between the chamber compartments ensured electrical insulation of different cell parts, while narrow slits in partitions served to fix cell position. The gaps between the cell and partitions were filled with insulating silicone grease (Baysilone, Germany). The distance between partitions in the central compartment was 3 mm, which is lower than the effective cable lengths for alkaline and acidic cell areas (3–5 and 10–15 mm, respectively) [12]. Silver–silver chloride electrodes were used for passing the pulses of electrical current through the transcellular route. Two interconnected electrodes for passing current were fixed in broad side compartments of the chamber. The narrow central compartment contacted with the second current electrode, which also served as reference electrode in the measuring circuit.

In order to measure membrane resistance of the selected cell region, square pulses of electrical current were passed through the current circuit (0.5 μA, pulse duration 180 ms) at a frequency of 2.5 Hz. These pulses produced hyperpolarizing shifts of the cell membrane potential in the central compartment. Periodic current pulses were obtained from an ESL-2 electronic stimulator connected to current electrodes via a stabilizing load resistance of 10^7 Ω. The plasmalemma membrane potential (MP) and its shifts induced by transcellular current pulses were measured by means of a microelectrode amplifier EPC-5 (List-Medical, Germany) and Pyrex capillary microelectrodes filled with 2 M KCl. Prior to microelectrode insertion and in the end of the experiment, the voltage drop on a series resistance (resistance of external medium, salt bridge, and the reference electrode common to current and measuring circuits) was deter-

mined. The small voltage drops on a series resistance were taken into account in calculations of membrane resistance R_m. The AP generation was induced by single depolarizing pulses of current (4–6 μA, 100–200 ms).

A microelectrode pH sensor with a tip diameter of about 20 μm was fabricated from a Pyrex glass capillary filled with molten antimony; it was positioned near the cell surface in the middle compartment of experimental chamber. The potential difference between the pH electrode and the reference electrode was measured with a VAJ-51 electrometric amplifier (Germany) having an input impedance of 10^{15} Ω. The output signals of EPC-5 and VAJ-51 were digitized by means of AD/DC converter (PCI-6024E, National Instruments, USA) and recorded with WinWCP computer program (Strathclyde Electrophysiology Software).

During experiments the cell was continuously illuminated from the upper light source of the microscope through a SZS-22 blue glass filter (photon flux density at the cell level ~100 μE/(m^2 s)). The method of chlorophyll fluorescence measurements on small cell regions was described previously [17]. Neutral glass filters were used for attenuation of light intensity during fluorescence measurements.

In the beginning of each experiment, the external pH near the cell surface (pH_o) was measured to verify the existence of alkaline or acidic zone around the selected cell region. The transport activity of the region of interest was additionally tested by measurements of the pH_o shifts induced by AP generation. Next, the membrane potential (MP) and plasma membrane resistance were measured under resting conditions and upon a single excitation of cell membranes. Following the AP generation, the cell was allowed to stay at rest for 15–20 min for the recovery of initial pH and MP levels. After the completion of measurements in 2–3 cycles of stimulation and relaxation, the cell was repositioned in the chamber for examining the cell region with oppositely directed H^+ transport, and a similar series of measurements was carried out. Figures display the results of representative experiments made in five replicates. Data on membrane conductances are presented as mean values ± standard error.

RESULTS

Under natural conditions, the emergence of alkaline and acidic zones on the surface of illuminated characean cells is related to circulation of extracellular currents between the areas with different pH_o [5, 6]. However, the task of differential measurements of membrane resistance in alkaline and acid zones necessitates the electrical insulation of the selected cell region from the remaining cell parts. Therefore, our first task was to find out if the cell is able to produce and maintain zones of different pH when it is placed in the chamber with insulating partitions. Considering the established relation between the nonuniform pH profile and circulation of electric currents [6], we attempted to promote zone formation in the central compartment by placing salt bridges between the central and side compartments of the chamber. The placement of conducting salt bridges (U-shaped glass tubes filled with agar and KCl) slightly facilitated the generation of alkaline or acidic zone in the central compartment. At the same time, the zone formation continued also after the removal of salt bridges between the compartments. Irrespective of existence or absence of salt bridges, the zone formation proceeded slower and the alkaline shift was smaller than in spatially unconstrained cells. The formation of alkaline zone upon the disruption of natural pathways for circulating electric currents in the outer medium might be reasonably explained from the assumption that insulating partitions reduced extracellular currents but did not eliminate them completely. In the narrow (3 mm) central compartment, variations of local pH_o along the cell length were often observed, which indicates the possibility of weak current circulation within the limits of the central compartment. It is also possible that weak extracellular currents might spread along the water-filled free space of the cell wall.

Figure 1a shows pH changes near the surface of alkaline and acidic regions of the same cell in a chamber with insulating partitions, which were caused by the AP generation upon stimulation with a suprathreshold pulse of electric current. It is seen that the AP generation was followed by a large pH decrease in the alkaline zone and a much smaller pH increase in the acid zone. The changes were reversible and developed synchronously, thus indicating the concurrent cessation of active H^+ extrusion in the zones of H^+ pump operation (acid zones) and the blockage of passive H^+ inflow in alkaline zones. Thus, the influence of AP on H^+ transport in different areas of the plasmalemma can be observed not only in free-lying cells but also upon separation of external medium into isolated compartments. The suprathreshold stimulation of the space-clamped cell caused also the decrease and oscillations of the maximum fluorescence F_m' in chloroplasts of alkaline cell regions (Fig. 1 b). These changes were similar to AP-induced fluorescence quenching in free-lying cells [17], which provides additional evidence for suitability of the model system examined.

Figure 2a shows AP-induced changes of membrane resistance in the acid cell region. Periodic pulses of inward electric current, passed through the cell region in the central compartment, produced short hyperpolarizing excursions of MP with the amplitude of about 20 mV. When a pulse sequence was applied continuously for a long time (≥ 120 s), the frequent MP shifts were displayed on records as a wavy band whose width is proportional to the membrane resistance R_m. The plasmalemma conductance in acid areas of resting cell

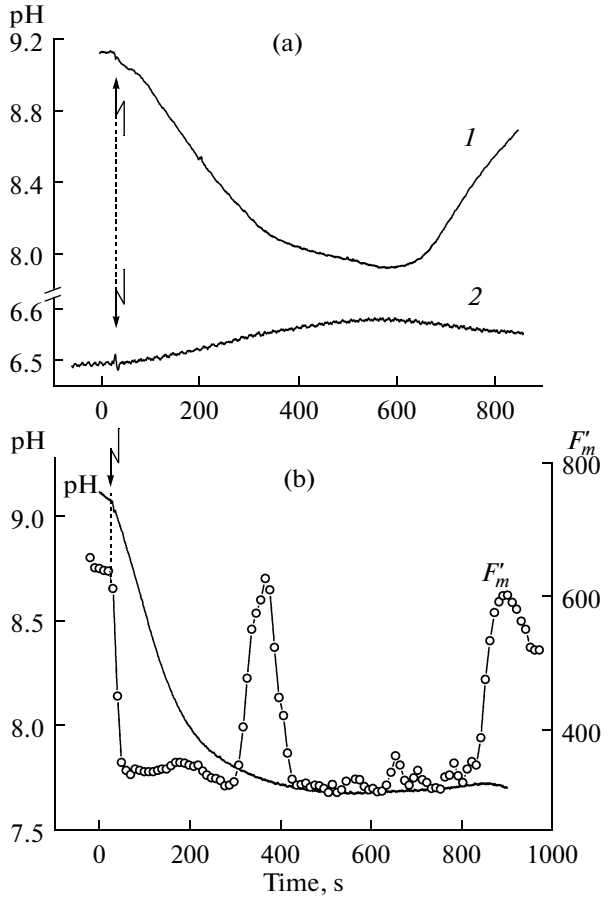

Fig. 1. Action potential-induced changes in pericellular pH and maximum chlorophyll fluorescence on microscopic areas of *Chara corallina* cell parts clamped in the central compartment between insulating partitions. (a) Oppositely directed changes of pH in the alkaline (*1*) and acid (*2*) zones of the same cell after AP generation at the moment marked with arrows. (b) Excitation-induced changes in apoplastic pH and chlorophyll fluorescence F'_m in the electrically insulated alkaline region of the cell.

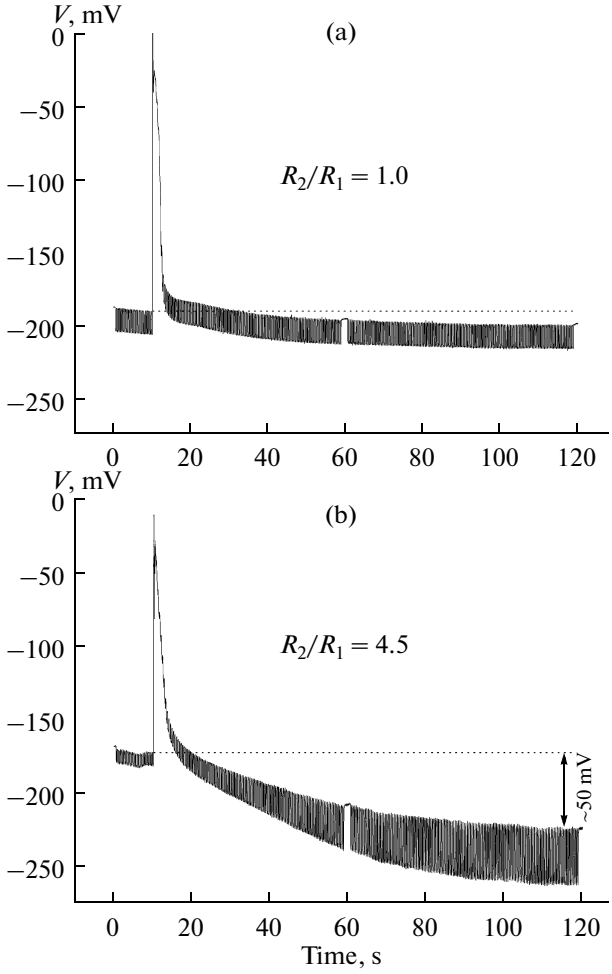

Fig. 2. Membrane potential (V) and plasmalemma resistance (R_m) changes during and after AP generation in space-clamped cell parts producing acid zone (a) and alkaline zone (b). The R_m values were judged from V shifts induced by passing periodic hyperpolarizing pulses of electric current (0.5 μA, 2.5 Hz) through a selected cell region with a surface area of 0.1 cm^2. The R_2/R_1 ratio characterizes the maximal increase in R_m after AP with respect to the R_m value at resting state.

was 4.2 ± 0.3 S/m^2 ($n = 7$). The AP generation after application of a single stimulus was followed by a short-term decrease in R_m with a subsequent restoration of the initial (before excitation) level. The ratio of R_m values measured in the end and the beginning of the recording (R_{m2}/R_{m1}) for the experiment shown in Fig. 2a was 1.0. In other cells this ratio for the membrane in acid regions varied in the range 1.0–2.0.

Figure 2b displays the results of R_m and MP measurements in the alkaline zone of the same cell at rest and after excitation. Under resting conditions the R_m in alkaline area was lower than in acid region, which is in line with published data [12]. The membrane conductance of resting membrane was 7.2 ± 0.5 S/m^2, which falls in the range of 5–8 S/m^2 reported in an earlier study [12]. The R_m decreased shortly during the spike generation but underwent a fourfold to eightfold increase (compared to R_m in the resting state) within 1–2 min after AP generation. The increase in R_m was accompanied by the hyperpolarizing shift of MP (marked with arrows in Fig. 2b). The R_m and MP changes were fully reversible: the recovery of both parameters at a photon flux density of 100 μE/(m^2 s) took a period of at least 15 min, required also for restoration of alkaline and acidic zones.

It is known that the positions of alkaline areas and calcified zones are not always coincident [18]. Figure 3a illustrates the case when the cell part in the central compartment contained calcium salt crystals (mainly $CaCO_3$ [19]) but did not form the high pH zone. In this case the AP generation did not lead to R_m increase and to post-excitation hyperpolarization of the plasmalemma. Furthermore, the initial R_m value under resting conditions was much higher than in the cell

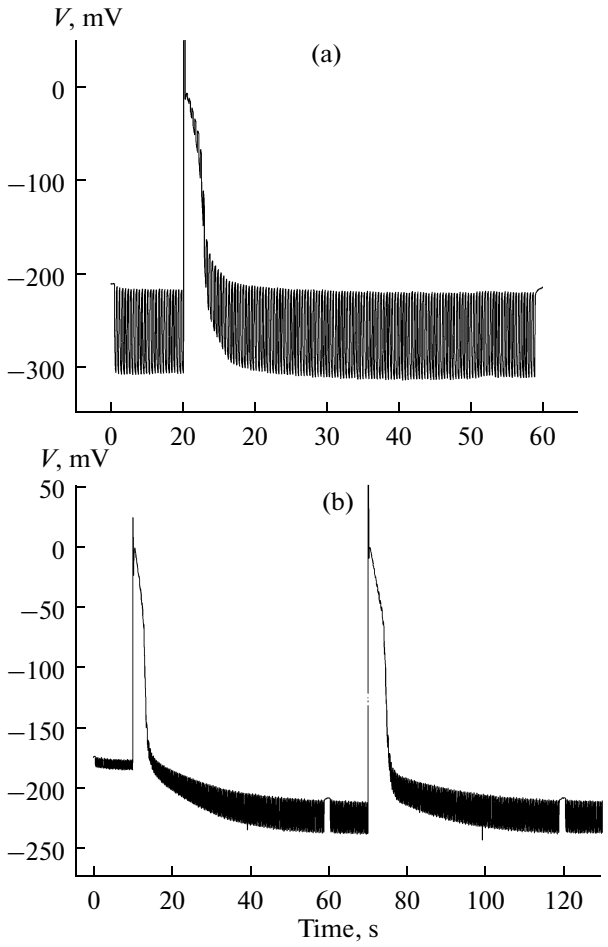

Fig. 3. Dependence of AP-induced increase in R_m on the cell condition prior to membrane excitation. (a) Changes in R_m during and after AP generation in the calcified cell region that lost the capacity of producing alkaline zone. The MP shifts were caused by passing the hyperpolarizing current pulses (1 μA through the cell region with a surface area of 0.1 cm^2. (b) Different patterns of R_m changes and different spike amplitudes as a function of the rest period preceding cell excitation: the first AP was triggered after 15-min cell rest period in the light; the second AP was triggered in 60 s after the first pulse. The strength of periodic current pulses was 0.5 μA.

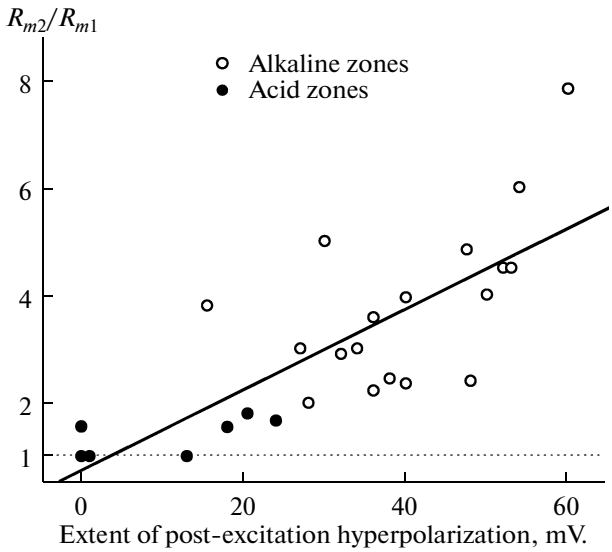

Fig. 4. Relation between the amplitudes of AP-induced hyperpolarization and the concurrent increase in plasmalemma resistance (R_{m2}/R_{m1}) for different cell regions of *Chara corallina*. Data points for cell regions producing acid and alkaline zones are marked with solid and open symbols, respectively. The R_{m2}/R_{m1} coefficient is the ratio of maximal membrane resistance observed after AP to the resistance measured prior to AP generation. Straight line is the linear approximation of data.

regions capable of producing high pH_o. A similar pattern of R_m changes was observed upon application of excitatory stimulus in 1–3 min after the previous excitation, when the cell was already hyperpolarized and the H^+ transport inhibited. In this case the short-term decrease in R_m during the spike was not followed by the subsequent R_m increase (Fig. 3b).

Figure 4 shows relations between the R_m shifts and the amplitude of hyperpolarization after AP generation in alkaline and acidic regions of various cells. The data points in the lower part of the plot (*solid symbols*) were obtained for cell areas classified as acid zones according to pH measurements. These cell regions were characterized by low coefficients of excitation-induced increase in R_m (R_{m2}/R_{m1} ratios) and low extents of post-excitation hyperpolarization. The data points in the upper right part of the plot were obtained for cell areas producing alkaline zones; these points correspond to high coefficients of R_m increase and large amplitudes of hyperpolarization (*open symbols*). The results show that the post-excitation hyperpolarization is clearly manifested in the alkaline regions and is weak in the acid regions. Despite some exceptions, the results reveal a clear correlation between the AP-induced R_m increase and the plasmalemma hyperpolarization.

DISCUSSION

Cells of characean algae represent a widely used model in studies of membrane transport in plants. This model is often further simplified by experimental conditions ensuring spatially uniform distribution of plasmalemma properties. For example, the ionic currents arising during cell excitation were only investigated for the uniform membrane state, which is stable in darkness or under weak light insufficient for generation of periodic pH profile [8, 20, 21]. By contrast, in bright light, the spatial distribution of transmembrane H^+ flows and membrane conductances is strikingly nonuniform [12, 22]. However, the inhomogeneity of membrane transport in illuminated cells remains often unnoticed and omitted from consideration.

The photosynthetically active light is known to affect the amplitude and shape of AP in *Chara* [23]. However, a possible relation of this phenomenon to generation of inhomogeneous distribution of pH_o and plasma membrane conductance has not been considered. In experiments with *Chara* cells, Smith and Beilby [24] observed the decrease in plasma membrane conductance by 30–40% within tens of seconds after AP generation (presumably, owing to inhibition of plasma membrane H^+ pump). However, the authors did not examine this phenomenon for the cell parts differing in H^+ pump activity. In light-exposed cells of *Chara globularis*, the AP generation was followed by a hyperpolarizing shift of MP [25], but this shift was ascribed to the unique property of the given species. In general, experimental data concerning membrane excitation in photosynthesizing cells are still fragmentary, and the processes involved remain incompletely recognized. The results of this study emphasize local differences in responses of illuminated cell to AP generation.

The main finding is that the AP generation induces considerable decrease of pH_o in alkaline cell regions in parallel with the plasma membrane hyperpolarization (MP shift ~50 mV) and up to eightfold increase in membrane resistance. Thus, the stoppage of passive H^+ flux from the medium into the cytoplasm was accompanied by a sharp decrease in membrane conductance and by the negative shift of MP. In contrast, in cell regions producing acid zones, the AP generation induced a small increase in pH_o in parallel with a slight increase in membrane resistance and insignificant hyperpolarization of the plasmalemma. The opposite directions of pH shifts indicate that the AP generation abolishes temporarily the active H^+ extrusion from the cytoplasm in acidic zones and the counter-directed passive H^+ influx (OH^- efflux) in the alkaline zones.

Local pH measurements provide direct indications of the H^+ pump activity; therefore, the pH_o increase after AP in the acidic zones presents a clear evidence for the H^+ pump inhibition. Remarkably, the suppression of active H^+ extrusion under the influence of AP was not accompanied by cell depolarization. Several factors could account for the lack of depolarization. The suppression of H^+ pump current might be compensated partly by the increase in R_m, which was observed in acid zones of some cells (Fig. 4). Furthermore, the H^+pump current was probably rather small even in the resting state because of the disruption of extracellular current pathways by insulating partitions. This is indicated by slow (tens of minutes) establishing of steady-state pH value in the acid zone of insulated (space-clamped) cell part.

This work is the first report of a manyfold R_m increase in the alkaline cell area of *Chara* after AP generation. The R_m changes in the areas of passive H^+ leak from the medium into the cytoplasm are almost an order of magnitude higher than the R_m increase by 40–50% observed previously after AP generation [24]. Nevertheless, the R_m changes in our experiments and in the earlier study [24] showed similar temporal patterns, which indicates their common origin and suggests that the methods used previously revealed these changes in strongly attenuated form.

The AP-induced decrease in pH_o occurring in parallel with the R_m increase and plasma membrane hyperpolarization imply the high membrane conductance for protons and its influence on the MP level. Since the electrochemical H^+ gradient across the plasmalemma did not change immediately after the potential spike, it is obvious that the arrest of H^+ flow is caused by an abrupt decrease in H^+ conductance of the membrane. It is possible that the diffusion component of MP in alkaline cell regions is largely determined by H^+ conductance, as was suggested by Kitasato with regard to the whole cell [9, 26].

The inactivation of H^+ conductance after AP generation shifts the MP values from the range near the equilibrium H^+ potential toward the hyperpolarized state, at which the diffusion MP component is presumably determined by K^+ ions [27]. Furthermore, the large increase in R_m might elevate the electrogenic component of MP despite the concurrent decline of the pump current. Our results indicate that the plasmalemma hyperpolarization after AP is not an exceptional property of particular characean species. The main requirement for its generation in *Chara corallina* is the creation of the cell state with inhomogeneous distribution of pH_o and R_m. The restoration of such state after a single AP would need a rest period of at least 15 min.

The exposure of higher plants to injurious treatments induces transient hyperpolarization of leaf cells [28], which is somewhat similar to the AP-induced hyperpolarization in *Chara*. The hyperpolarization of leaf cells after excitation was ascribed to activation of electrogenic H^+ pump, whereas in *Chara* cells the hyperpolarization is related, by contrast, with the H^+ pump inactivation. The development of hyperpolarization in *Chara* undoubtedly depends on the H^+ pump, which should be active prior to excitation for establishing the heterogeneous distribution of H^+-transporting systems over the plasmalemma and for creating high membrane conductance in the alkaline areas.

Our results allow the conclusion that high pH_o values in alkaline zones cannot result from operation of H^+/HCO_3^- symport or OH^-/HCO_3^- antiport. In the case of coupled electroneutral ion transport, the cessation of H^+ (or OH^-) transfer should have induced the drop in pH_o without the accompanying increase in R_m. Thus, further support is given to the working notion that active H^+ extrusion and counter-directed passive H^+ influx are spatially separated but coordinated by extracellular current flows carried in the medium by dominant ions [29].

The functional role of spatial separation of zones with active H^+ transport and passive H^+ conductance in characean algae is a matter of discussion. On the one hand, the passive H^+ uniport, unlike symport or antiport serving to accumulation of nutrient elements, might seem wasteful. However, one should consider that the long-distance separation between zones where protons are expelled and where they leak passively into the cytoplasm allows the cell to reduce pH on a large area of the cell surface (broad acidic zones) to the level comparable with pK ~6.3 for the CO_2–HCO_3^- equilibrium. Such a shift in pH enriches the boundary layers of the medium with dissolved CO_2, thus promoting the entry into the cytoplasm of this photosynthetic substrate. If generators and consumers of H^+ electrochemical gradient were distributed homogeneously over the cell surface, such a local decrease in pH would be impossible or hampered because of the H^+ leakage into the cytoplasm.

The well-known equivalent electric circuit of the plasmalemma consists of two parallel pathways with two electromotive forces (EMF), designating the H^+ pump EMF and the diffusion potential, connected in series with the respective resistances (inverse of conductances) [1, 25]. The H^+ pump conductance is thought to be comparable with the conductance of diffusional pathway [1]. In this case the pump current depends not only on the parameters of the H^+ pump but also on the conductance of ion channels. It is not excluded that changes of plasmalemma conductance in alkaline zones are involved in regulation of the H^+ pump activity. Based on this viewpoint, the minimal size of alkaline zones is determined by the required pump current that would suffice for the sustained photosynthetic activity. In this connection it is interesting to note that the pump current in acid zones ~10 μA/cm^2 [6] corresponds to the outward H^+ flux of about 100 pmol/(cm^2 s). If the protons expelled from the cell interact with HCO_3^-, converting it to CO_2, which is a consumable substrate in photosynthesis, the CO_2 influx in acid zones should correspond to the rate of H^+ extrusion. The H^+ flux ~100 pmol/(cm^2 s) is somewhat higher than the average rate of CO_2 fixation in *Chara* internodes under saturating light (40 pmol/(cm^2 s)) [30]). However, about one-third of the cell area belongs to regions with low photosynthetic activity; therefore, the rate of photosynthetic CO_2 fixation in acid zones should be approximately 1.5 times higher than the average rate (~60 pmol/(cm^2 s)). The latter value is comparable to estimates for the outward H^+ flux and the rates of CO_2 uptake.

Thus, after completion of cell growth, the function of the H^+ pump, related to accumulation of mineral nutrients (K^+, NO_3^-), apparently weakens for the sake of improvement of cell supply with a permeable photosynthetic substrate, whose content is depleted in a weakly alkaline aquatic environment.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project no. 07-04-00132).

REFERENCES

1. Tazawa, M., Cell Physiological Aspects of the Plasma Membrane Electrogenic H^+ Pump, *J. Plant Res.*, 2003, vol. 116, pp. 419–442.
2. Plieth, C., Tabrizi, H., and Hansen, U.-P., Relationship between Banding and Photosynthetic Activity in *Chara corallina* as Studied by the Spatially Different Induction Curves of Chlorophyll Fluorescence Observed by an Image Analysis System, *Physiol. Plant.*, 1994, vol. 91, pp. 205–211.
3. Bulychev, A.A., Zykov, S.V., Rubin, A.B., and Müller, S.C., Transitions from Alkaline Spots to Regular Bands during pH Pattern Formation at the Plasmalemma of *Chara* Cells, *Eur. Biophys. J.*, 2003, vol. 32, pp. 144–153.
4. Lucas, W.J., The Influence of Light Intensity on the Activation and Operation of the Hydroxyl Efflux System of *Chara corallina*, *J. Exp. Bot.*, 1975, vol. 26, pp. 347–360.
5. Lucas, W.J., Keifer, D.W., and Sanders, D., Bicarbonate Transport in *Chara corallina*: Evidence for Cotransport of HCO_3^- with H^+, *J. Membrane Biol.*, 1983, vol. 73, pp. 263–274.
6. Lucas, W.J. and Nuccitelli, R., HCO_3^- and OH^- Transport across the Plasmalemma of *Chara*: Spatial Resolution Obtained using Extracellular Vibrating Probe, *Planta*, 1980, vol. 150, pp. 120–131.
7. Fisahn, J.M., Hansen, U.-P., and Lucas, W.J., Reaction Kinetic Model of a Proposed Plasma Membrane Two-Cycle H^+-Transport system of *Chara corallina*, *Proc. Natl. Acad. Sci. USA*, 1992, vol. 89, pp. 3261–3265.
8. Beilby, M.J. and Bisson, M.A., *Chara* Plasmalemma at High pH: Voltage Dependence of the Conductance at Rest and during Excitation, *J. Membrane Biol.*, 1992, vol. 125, pp. 25–39.
9. Kitasato, H., Membrane Potential Genesis in *Nitella* Cells, Mitochondria, and Thylakoids, *J. Plant Res.*, 2003, vol. 116, pp. 401–418.
10. Beilby, M.J., Mimura, I.T., and Shimmen, T., The Proton Pump, High pH Channels, and Excitation: Voltage Clamp Studies of Intact and Perfused Cells of *Nitellopsis obtusa, Protoplasma*, 1993, vol. 175, pp. 144–152.
11. Shimmen, T. and Wakabayashi, A., Involvement of Membrane Potential in Alkaline Band Formation by Internodal Cells of *Chara corallina, Plant Cell Physiol.*, 2008, vol. 49, pp. 1614–1620.
12. Smith, J.R. and Walker, N.A., Membrane Conductance of *Chara* Measured in the Acid and Basic Zones, *J. Membrane Biol.*, 1983, vol. 73, pp. 193–202.
13. Bisson, M.A. and Walker, N.A., The *Chara* Plasmalemma at High pH. Electrical Measurements Show

Rapid Specific Passive Uniport of H^+ or OH^-, *J. Membrane Biol.*, 1980, vol. 56, pp. 1–7.

14. Yao, X. and Bisson, M.A., Passive Proton Conductance is the Major Reason for Membrane Depolarization and Conductance Increase in *Chara buckellii* in High-Salt Conditions, *Plant Physiol.*, 1993, vol. 103, pp. 197–203.
15. Bulychev, A.A., Kamzolkina, N.A., Luengviriya, J., Rubin, A.B., and Müller, S.C., Effect of a Single Excitation Stimulus on Photosynthetic Activity and Light-Dependent pH Banding in *Chara* Cells, *J. Membrane Biol.*, 2004, vol. 202, pp. 11–19.
16. Eremin, A., Bulychev, A., Krupenina, N., Mair, T., Hauser, M., Stannarius, R., Müller, S., and Rubin, A. Excitation-Induced Dynamics of External pH Pattern in *Chara corallina* Cells and Its Dependence on External Calcium Concentration, *Photochem. Photobiol. Sci.*, 2007, vol. 6, pp. 103–109.
17. Krupenina, N.A. and Bulychev, A.A., Action Potential in a Plant Cell Lowers the Light Requirement for Nonphotochemical Energy-Dependent Quenching of Chlorophyll Fluorescence, *Biochim. Biophys. Acta*, 2007, vol. 1767, pp. 781–788.
18. Serikawa, K.A., Porterfield, D.M., Smith, P.J.S., and Mandoli, D.F., Calcification and Measurement of Net Proton and Oxygen Flux Reveal Subcellular Domains in *Acetabularia acetabulum*, *Planta*, 2000, vol. 211, pp. 474–483.
19. Kiyosawa, K., Ca^{2+} and Phosphate Releases from Calcified *Chara* Cell Walls in Concentrated KCl Solution, *J. Exp. Bot.*, 2001, vol. 52, pp. 223–229.
20. Berestovsky, G.N. and Kataev, A.A., Voltage-Gated Calcium and Ca^{2+}-Activated Chloride Channels and Ca^{2+} Transients: Voltage-Clamp Studies of Perfused and Intact Cells of *Chara, Eur. Biophys. J.*, 2005, vol. 34, pp. 973–986.
21. Lunevsky, V.S., Zherelova, O.M., Vostrikov, I.Y., and Berestovsky, G.N., Excitation of Characeae Cell Membranes as a Result of Activation of Calcium and Chloride Channels, *J. Membrane Biol.*, 1983, vol. 72, pp. 43–58.
22. Smith, J.R., Effect of a Spatially Inhomogeneous Membrane upon the Measured Electrical Properties of *Chara, J. Membrane Biol.*, 1983, vol. 73, pp. 185–192.
23. Baudenbacher, F., Fong, L.E., Thiel, G., Wacke, M., Jazbinsek, V., Holzer, J.R., Stampfl, A., and Trontelj, Z., Intracellular Axial Current in *Chara corallina* Reflects the Altered Kinetics of Ions in Cytoplasm under the Influence of Light, *Biophys. J.*, 2005, vol. 88, pp. 690–697.
24. Smith, J.R. and Beilby, M.J., Inhibition of Electrogenic Transport Associated with the Action Potential in *Chara, J. Membrane Biol.*, 1983, vol. 71, pp. 131–140.
25. Shimmen, T., Unique After-hyperpolarization Accompanying Action Potential in *Chara globularis*, *J. Plant Res.*, 1994, vol. 107, pp. 371–375.
26. Kitasato, H., The Influence of H^+ on the Membrane Potential and Ion Fluxes of *Nitella*, *J. Gen. Physiol.*, 1968, vol. 52, pp. 60–87.
27. Bulychev, A.A. and Krupenina, N.A., Facilitated Permeation of Methyl Viologen into Chloroplasts In Situ during Electric Pulse Generation in Excitable Plant Cell Membranes, *Biol. Membrany* (Rus.), 2008, vol. 25, pp. 343–351 [Transl. version in *Biochemistry (Moscow) Suppl. Series A*, 2008, vol. 2, pp. 387–394].
28. Zimmermann, M.R., Maischak, H., Mithöfer, A., Boland, W., and Felle, H., System Potentials, a Novel Electrical Long-Distance Apoplastic Signal in Plants, Induced by Wounding, *Plant Physiol.*, 2009, vol. 149, pp. 1593–1600.
29. Bulychev, A.A. and Krupenina, N.A., Effects of Plasma Membrane Excitation on Spatially Distributed H^+ Fluxes, Photosynthetic Electron Transport and Nonphotochemical Quenching in the Plant Cell, *Bioelectrochemistry Research Developments*, Bernstein, E.M., Ed., New York: Nova Science Publishers, 2008, pp. 159–188.
30. Lucas, W.J., Photosynthetic Fixation of ^{14}Carbon by Internodal Cells of *Chara corallina, J. Exp. Bot.*, 1975, vol. 26, pp. 331–346.