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Differential effects of plasma membrane electric excitation on H^+ fluxes and photosynthesis in characean cells

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Abstract

Cells of characean algae exposed to illumination arrange plasma-membrane H^+ fluxes and photosynthesis in coordinated spatial patterns (bands). This study reveals that H^+ transport and photosynthesis patterns in these excitable cells are affected not only by light conditions but also by electric excitation of the plasma membrane. It is shown that generation of action potential (AP) temporally eliminates alkaline bands, suppresses O_2 evolution, and differentially affects primary reactions of photosystem II (PSII) in different cell regions. The quantum yield of PSII electron transport decreased after AP in the alkaline but not in acidic cell regions. The effects of electric excitation on fluorescence and the PSII electron flow were most pronounced at light-limiting conditions. Evidence was obtained that the shift in chlorophyll fluorescence after AP is due to the increase in ΔpH at thylakoid membranes. It is concluded that the AP-triggered pathways affecting ion transport and photosynthetic energy conversion are linked but not identical.

Keywords: *Chara corallina*; Action potential; Proton transport; Acidic and alkaline zones; Chloroplasts; Nonphotochemical quenching; Spatial heterogeneity

1. Introduction

Electrical excitation occurs in many plant cells but its physiological consequences are not yet fully recognized. The well-known examples are leaf movements triggered by action potentials (AP) in *Mimosa* and insectivorous plants [1,2]. Cells of some liverworts, algae, and vascular plants generate AP in response to light, chilling, burning, as well as chemical, mechanical, and electric stimuli [3–8]. These signals are thought to initiate responses of cells and organisms to injury or environmental changes [9]. Like in animals, AP in plants are caused by a regenerative voltage-dependent process of ion channel opening–closing and exhibit threshold phenomena [10,11]. Recent findings showed that propagating electric signals in mimosa leaves result in transient suppression of photosynthesis [12].

Internodal cells of characean algae represent a convenient model for studying plant cell excitability. This model allowed researchers to identify Ca^{2+}, Cl^-, and K^+ fluxes through ionic channels during AP generation [10,11,13,14] and to characterize the electrogenic H^+ pump of the plasma membrane (PM) [15]. Owing to large cell dimensions and fixed single-layer alignment of chloroplasts, the internodes of Characeae are convenient for exploring interactions between electric excitation, photosynthesis, and ion transport. Characean cells are known to form under light alternating subcellular domains with H^+ extrusion and H^+ sink activities that account for pH banding [16–19] and photosynthetic patterns [20–22]. Although the pH banding phenomenon is known for years, its regulation and associated metabolic patterns are poorly characterized. In addition to photosynthesis pattern, inhomogeneous distribution of mitochondria was observed in illuminated cells [23]. Cell regions with active photosynthesis accumulate mitochondria, whereas regions with low photosynthetic activity were depleted in these.

The pH pattern is strikingly influenced by AP generation [24]. A single AP produces a long-lasting suppression of the banding profile, which implies either involvement of a signaling cascade or a powerful side effect of cell excitation.

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Analysis of the chlorophyll fluorescence of photosystem II (PSII) in different cell domains revealed excitation-induced changes that were dissimilar for alkaline and acid regions. The AP-induced phenomena were tentatively assigned to the rise in cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) during excitation. The excitation-induced events at PM (suppression of counter-directed H^+ flows) and in chloroplasts (changes in PSII quantum yield of electron flow) remain largely unexplored to date. It is not known whether these processes have similar light requirements and if they are strictly linked or can proceed separately.

The aim of this work was to examine effects of PM excitation on transmembrane H^+ fluxes, effective quantum yield of PSII electron flow, and fluorescence quenching in subcellular domains of *Chara* internodes at various light intensities. This was achieved by monitoring external pH near the cell surface (pH_o) with a microprobe and by measuring photosynthetic activity of small cell regions with microfluorometry, saturation pulse method, and amperometric pO_2 sensor. These methods were previously employed to study light-dependent formation of photosynthetic and pH bands [21,22].

2. Materials and methods

Chara corallina internodes about 6 cm in length and 0.9 −1 mm in diameter without apparent calcium depositions were excised and placed in the medium containing 0.1 mM KCl, 1.0 mM NaCl, and 0.5 mM $CaCl_2$ (pH 6.8−7.2). A transparent chamber with the cell was mounted on a stage of Axiovert-25 CFL inverted microscope (Carl Zeiss, Germany) equipped with a Microscopy PAM fluorometer (Walz, Effeltrich, Germany).

Cell regions of H^+ extrusion and H^+ influx were detected with pH microelectrodes having tip diameter of 10–20 μm [22]. The terms “alkaline and acid cell regions” denote cell parts producing zones of high and low pH_o, respectively. The potential difference across PM was measured with capillary microelectrodes filled with 1 M KCl.

Chlorophyll fluorescence was measured on small (diameter 100 μm) portions of a chloroplast layer with a Microscopy-PAM fluorometer [25] and WinControl program (Walz). The pH probe was positioned within the fluorometric window. Low-intensity measuring light had no actinic effect, as evidenced by high ratios of variable to maximum fluorescence ($\Delta F/F_m$). Actinic light was directed from the upper light source of an Axiovert microscope. The highest fluence rates (PAR) were 150 and 30 μmol m^{-2} s^{-1} for white light and blue light (a cut-off glass filter, $\lambda < 580$ nm), respectively. Neutral glass filters were used for attenuating photon flux density. A black screen with a small ($d = 2.5$ mm) opening was placed 3 mm above the cell to ensure local actinic illumination; it shielded the whole cell except for a small region coaxial with the microscopic field of view.

The effective quantum yield of PSII electron flow ($\Delta F/F_m'$) and the coefficient of nonphotochemical quenching (NPQ) were estimated from equations $\Delta F/F_m' = (F_m' - F)/F_m'$ and $NPQ = (F_m - F_m')/F_m'$. Here F_m and F_m' designate maximal yields of fluorescence induced with a saturation pulse in a dark-adapted cell and in a cell exposed to actinic light, respectively, and F is actual fluorescence yield in the actinic light.

Changes in local O_2 content were measured with glass-insulated open Pt electrode having a tip diameter of 100 μm [21]. With the potential of Pt cathode of 0.65 V with respect to Ag/AgCl electrode, the stationary electrode current proportional to O_2 content was fed into a current-voltage converter with a transfer coefficient of $3 \cdot 10^7$ V/A.

The cell excitation was elicited by a rectangular pulse of transcellular electric current (~ 10 μA, 150 ms) passing through external electrodes [24]. Experiments were performed at least in triplicates. Figures display results of representative experiments. Bars in Fig. 3 show mean values and standard errors.

3. Results

3.1. Kinetics of pH and membrane potential changes induced by excitation and darkness

It was previously established that the light-dependent pH pattern along *Chara* cell transiently disappears after cell excitation [24]. The smoothing of pH_o profile was manifested as a slight pH rise in acidic zones and a large pH decrease in the alkaline zones. Large pH shifts in the alkaline zones are particularly convenient for exploring interactions between excitation and light-controlled pH banding.

Fig. 1a compares local pH changes induced in the alkaline zone by AP and darkening. The cell was first exposed to darkness at time $t = 0$, which led to the vanishing of the alkaline band after a lag period (solid line 1). The kinetics of pH decay was fit to a sigmoid curve shown with symbols. When the alkaline band recovered after returning continuous light, AP was triggered (curve 2). The AP-induced decrease in pH followed an exponential kinetics with one or (sometimes) two exponential terms. The dark- and excitation-induced pH changes had comparable amplitudes and decay rates; however, the AP-induced pH drop started without a lag period. Remarkably, the AP-induced decrease in pH_o was also observed if the electric stimulus was applied 30 s after darkening, i.e., during the lag phase of the sigmoid decay curve (results not shown). Even in this case, the AP-triggered pH decline started earlier than the response to darkness. The above similarities in AP- and dark-induced pH changes point to the involvement of a common factor in excitation-induced and dark-induced permeability changes of PM.

Fig. 1b displays the membrane potential (MP) records, made from the cytoplasm of alkaline cell region, upon dark–light transitions and after AP generation. Turning off the actinic light caused a large hyperpolarization of PM, while the transfer of cell to light repolarized the membrane after a transient hyperpolarization (curve 1). This temporal pattern is similar to light-induced MP changes in *C. corallina* observed previously [26]. Our experiment was extended by triggering AP in the light. In Fig. 1b, the dark- and AP-induced responses are overlaid. The cell excitation induced a large after-potential (curve 2) comparable in extent and time range to the dark-induced hyperpolarization. It is seen that the hyperpolarization