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Triggered Permeation of Methyl Viologen into *In Situ* Chloroplasts upon Electrical Excitation of a Plant Cell

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ABSTRACT

Action potential (AP) generation at the plasmalemma of *Chara corallina* has a strong influence on photoprocesses in chloroplasts. Under physiological conditions the AP generation transiently suppresses photosynthetic electron transport and reversibly increases thermal losses of chlorophyll excitations. However, these changes provoked by membrane excitation became irreversible in the presence of artificial electron acceptor methyl viologen (MV) in the external medium. Incubation of *Chara* cells under resting conditions in the presence of this herbicidal agent had no effect on kinetics of chlorophyll P700 photooxidation, indicating that permeation of MV divalent cations across the cell and chloroplast membranes is a limiting factor and makes MV inaccessible at the sites of its interactions with photosystem I. On the other hand, the AP generation in the presence of MV irreversibly modified the P700 photooxidation signals, as measured from the difference of absorbance changes at 810 and 870 nm (ΔA_{810}). The results suggest that permeation of MV into *in situ* chloroplasts is triggered during or after AP generation. Photoinduced changes of cell membrane potential, similarly to ΔA_{810} signals, were insensitive to the presence of MV in the external medium until the application of the first excitatory stimulus. However, the AP generation in the presence of MV irreversibly modified cell electrical photoresponses, indicating that photosynthetic electron flow was redirected to MV reduction. In the herbicide-treated plants, the effect of AP on photosynthesis seems to be complex and includes permeability changes to MV in the system of membrane barriers comprising the plasmalemma and the chloroplast envelope.

Keywords: *Chara corallina*, membrane potential, paraquat, P700 absorbance changes
Abbreviations: AP, action potential; **MP**, cell membrane potential; **PSI** and **PSII**, photosystems I and II; **P700**, reaction center chlorophyll of photosystem I

INTRODUCTION

Plants with their sessile habit of life cannot move in the search for a favorable environment. Therefore, rapid mobilization of adaptive systems in response to environmental changes is particularly important for plant survival. Action potential (AP) is an important part incorporated into plant signaling systems. The AP is generated and propagated over the plant upon the action of chemical, mechanical, and thermal stimuli. These signals disturb cellular processes causing, in particular, temporal inhibition of photosynthesis (Koziolek *et al.* 2003; Bulychev *et al.* 2004; Lautner *et al.* 2005) and increased protection against excess light (Krupenina and Bulychev 2007).

Effects of the plasma membrane electrical impulses on organelle functions are primarily interesting in the context of intracellular regulation. Charophyte algae are considered close relatives of higher plants (Lewis and McCourt 2004). Unlike leaves of vascular plants, they combine the properties of electrical excitability and photosynthesis at the single cell level, thus representing a suitable model for studying the role of excitable membranes in chloroplast functioning. The mechanisms of AP generation in characean algae have been studied in detail (Lunevsky *et al.* 1983; Plieth *et al.* 1998). The potential spike is due to the rapid influx of external Ca^{2+} into the cytoplasm through voltage-gated channels (Berestovsky and Kataev 2005) combined with the Ca^{2+} release from intracellular stores (Plieth *et al.* 1998); it also involves the Ca^{2+}-activated efflux of cytosolic Cl^- to the outer medium and a subsequent K^+ efflux. The Ca^{2+} level in the cytoplasm increases dramatically, from 0.1 to about 10 μM (Berestovsky and Kataev 2005), whereas relative changes in concentrations of other ion species are comparatively small. The AP generation is followed by a long-term cessation of H^+ fluxes across the plasma membrane (Bulychev *et al.* 2004), which shifts the apoplastic pH in different cell regions from fractions of a unit up to 2.5 units. The areas of active H^+ extrusion and passive H^+ influx in illuminated characean internodes are spatially separated; therefore, the cessation of H^+ flows after AP might cause opposite shifts of cytoplasmic pH in various cell regions.

The AP generation in an intact cell diminishes the effective quantum yield of photosystem II (PSII) reactions and increases nonphotochemical losses of chlorophyll excitations (dissipation of light energy as heat), which is manifested as a reversible decrease in chlorophyll maximal fluorescence F_m' (Bulychev and Kamzolkina 2006; Krupenina and Bulychev 2007). The AP-induced F_m' quenching results from the increase in thylakoid ΔpH caused by the supposed entry of excess cytosolic Ca^{2+} to the stroma of illuminated chloroplasts and by consequent inhibition of the Calvin–Benson cycle reactions (Krupenina and Bulychev 2007). The largest decrease in F_m' after electric pulse generation at the cell membrane was observed on cells treated with a dicationic artificial electron acceptor methyl viologen (MV) (Krupenina and Bulychev 2008). In the presence of MV, unlike physiological conditions, the decrease in F_m' was irreversible, i.e., it developed only once, in response to triggering the first AP. The lack of discernible MV effect on functioning of chloroplasts *in situ* indicates that the resting cells possess effective barriers for MV permeation from the external medium into the chloroplasts. On the other hand, the photosynthetic electron transport pathways appeared strikingly modified after a single generation of AP in the presence of MV. The chlorophyll fluorescence data indi-

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cated that MV becomes accessible acceptor within the chloroplast stroma immediately after the electric pulse generation at the cell membrane (Krupenina and Bulychev 2008).

The influence of AP on chloroplast functioning might be complex and include changes in membrane permeability of the plasmalemma–chloroplast envelope system. In model membrane systems, the conductance for MV cations was shown to depend on pH (Létant *et al.* 2006) and membrane potential (Majumder *et al.* 2007). However, it is not yet known if biological cell membranes can increase their permeability to MV in response to short-term physiological depolarization. The recognition of a possible impact of AP on permeation of substances into the organelles is not only important for understanding the intracellular interactions but has also a practical aspect, because methyl viologen (1,1'-dimethyl-4,4' bipyridinium chloride, known also as paraquat) is an efficient herbicide acting on photosystem I (PSI) in chloroplasts. Methyl viologen accepts electrons from iron-sulfur centers on the acceptor side of PSI competing with the natural acceptor ferredoxin; it diverts electron flow from the assimilating pathway to the reactions associated with the production of reactive oxygen species and membrane destruction.

In this study we checked the assumption that MV does not permeate into the chloroplasts of resting *Chara corallina* cells within at least half-hour incubation but enters immediately into the plastids and modifies photosynthetic electron transport after the generation of a single AP at the electrically excitable cell membrane. The onset of MV-mediated photoreactions in the *in situ* chloroplasts can be judged from chlorophyll fluorescence measurements and from the induction changes in the redox state of chlorophyll P700 in PSI reaction centers. It is known that MV effectively removes electrons from PSI, thereby causing fast oxidation of P700 in the light. The accumulation of oxidized P700 form ($P700^+$) can be measured from the increase in absorbance at 810–830 nm (Baker *et al.* 2007). Therefore, we studied photoinduced absorbance changes of $P700^+$ in the resting cell before and after the addition of MV into the medium, as well as after AP generation in the absence and presence of this electron acceptor. Furthermore, the appearance of MV inside chloroplasts and shunting of CO_2-dependent electron flow to MV reduction was deduced from irreversible alteration of membrane potential changes during dark–light transitions.

MATERIALS AND METHODS

Plant material

Internode cells of *Chara corallina* Klein ex Willd. about 6 cm in length and 0.9–1 mm in diameter were excised from the strand and placed into artificial pond water containing 0.1 mM KCl, 1.0 mM NaCl, and 0.1 mM $CaCl_2$. The medium was supplemented with about 0.2 mM $NaHCO_3$ for adjusting pH to 7.0–7.1. In order to reduce the substrate limitation for photosynthesis, we used also the medium with 5 mM Mes–NaOH buffer (pH 6.2), where the equilibrium CO_2 concentration is elevated.

$P700^+$ measurements

Measurements of $P700^+$ absorbance changes on individual cells are exceptionally rare or lacking, because the content of P700 is quite small compared to the total chlorophyll content. The micromethods for such measurements are not yet invented, while the application of standard instruments is hindered by a small projective area of a single cell in the measuring beam cross-section. In our experiments we extended the sample area by placing four isolated internodes close to each other. The fixed position of cells in the experimental chamber was secured by narrow slits in the partition between two compartments of the chamber.

Oxidoreductions of chlorophyll P700 in the reaction centers of PSI were monitored from the difference of absorbance changes at 810 and 870 nm (ΔA_{810}). The absorption at 810 nm is attributed to oxidized P700 form ($P700^+$). The kinetics of ΔA_{810} was monitored using a Walz modulated detection system consisting of a PAM-101 control unit operating at modulation frequency of 100 kHz and ED-P700DW dual-wavelength emitter–detector unit (Walz, Germany). The dual-wavelength measuring system enables detection of $P700^+$ absorbance changes with the minimal distortion from other processes. A branched fiber-optic cable was used to deliver modulated measuring light and white or far-red actinic light to the sample and to direct reflected modulated light to the detector. The cells were placed between the mirror support and the end of the light guide cable. The measuring light passed the sample in forward and reverse directions, which extends the pathway length and increases the ΔA_{810} amplitude.

The sample was illuminated with white light obtained from a KL-1500 light source (Schott, Germany) and by far-red (FR) light obtained from an OI-28 source (a 70-W halogen lamp) fitted with a 717 nm interference filter. Far-red light is absorbed by PSI only and induces almost complete oxidation of P700. The photon flux density for white light equaled to 150 μE m^{-2} s^{-1}. The duration of light pulses provided with a KL-1500 source was controlled with a PAM-103 flash trigger control unit (Walz). The ΔA_{810} signals were recorded on a computer using a CED 1401 analog-to-digital converter (Cambridge Electronic Design, United Kingdom) and WinWCP software (Strathclyde Electrophysiology Software).

The induction changes of ΔA_{810} were measured under intermittent illumination with light and dark periods of 2.5 s and 5–6 min, respectively. In each experiment, a series of reproducible measurements was obtained at different stages during sequential replacement of cell conditions: (1) cell at rest in the absence of MV, (2) cell subjected to a single excitation in the absence of MV, (3) resting state in the presence of MV, and (4) post-excitation state imposed by AP generation in the presence of MV. The graphs show ΔA_{810} signals obtained in a representative experiment with a four-cell sample after averaging records for three consecutive measurements at each set of experimental conditions. Experiments were performed in four replicates.

Electric stimulation of the cell was accomplished by passing rectangular pulses of electric current with duration of 150–200 ms through extracellular electrodes placed in electrically insulated compartments of the chamber. The generation of AP was detected from temporary cessation of cytoplasmic streaming.

Electric potential and local pH measurements

The cell membrane potential (MP) was measured by means of Pyrex capillary microelectrodes filled with 1 M KCl. The microelectrodes were positioned with a KM-2 micromanipulator (Russia) under observation with an Axiovert-25 CFL microscope (Zeiss, Germany). The potential difference between the vacuole and external medium was measured with a VAJ-51 electrometric amplifier (RFT, Germany) and recorded on a computer by means of a 1401 Plus analog-to-digital converter (Cambridge Electronic Design). The cell was illuminated with saturating white light provided from the upper light source of an Axiovert microscope.

Local changes in extracellular pH upon light–dark transitions were measured with glass-insulated antimony pH microelectrodes having a tip diameter of about 20 μm. The potential difference between the pH microelectrode and a silver/silver chloride reference electrode was measured with VAJ-51 electrometer and 1401 Plus analog-to-digital converter similarly to electric potential measurements.

Figures show changes in MP and extracellular pH from representative experiments made in three replicates. Methyl viologen was obtained from Acros Organics (Belgium).

RESULTS

Induction changes of P700 redox state indicate different accessibility of MV for chloroplasts *in situ* under resting conditions and after AP

Fig. 1 shows changes in P700 redox state induced by the pulse of white light upon sequential changes of experimental conditions. Illumination of resting cell adapted to darkness for 5 min in the absence of MV (**Fig. 1A**) induced a rapid peak of P700 oxidation followed by the reduction

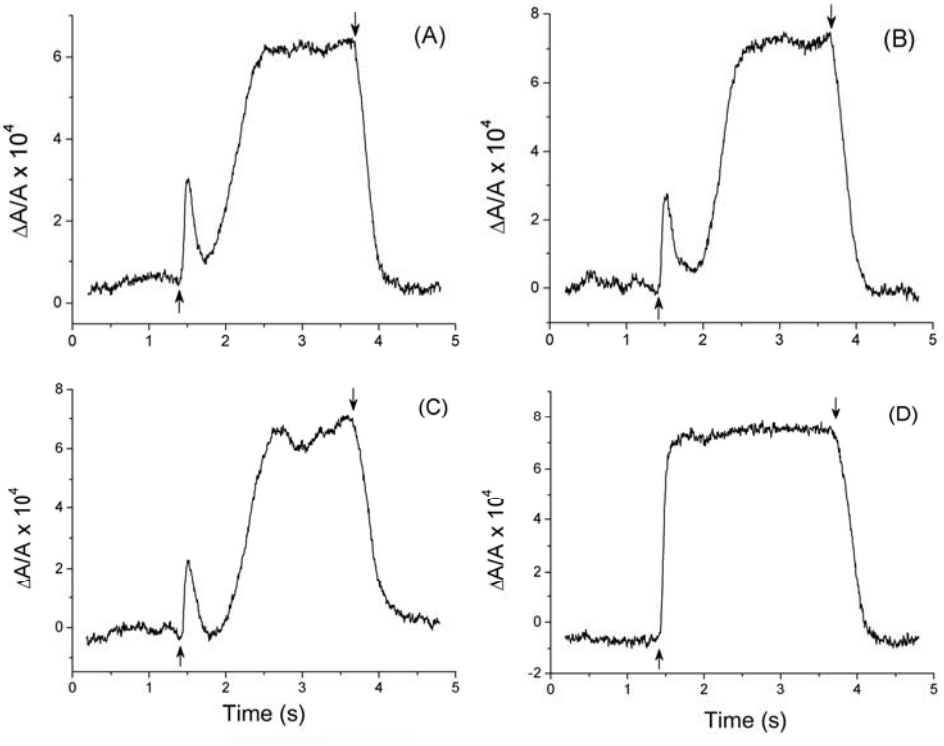

Fig. 1 Kinetics of chlorophyll P700 oxidation in *Chara corallina* cells induced by a white light pulse (150 μE m^{-2} s^{-1}) after 5-min dark adaptation under different experimental conditions at pH 6.2. (**A**) Resting state in the absence of MV; (**B**) after generation of a single AP in the absence of MV; (**C**) during 30-min incubation of resting cells in the presence of 0.2 mM MV; (**D**) after generation of a single AP in the presence of 0.2 mM MV. The increase in absorbance at 810 nm with respect to that at 870 nm (ΔA_{810}) corresponds to accumulation of P700 oxidized form, $P700^+$. Upward and downward arrows designate the moments when light was switched on and off.

stage and subsequent second wave of P700 oxidation. Such ΔA_{810} kinetics is also characteristic of leaves for the majority of higher plant species. The intermediary $P700^+$ reduction is related to the arrival of electrons from PSII after plastoquinone reduction, while the second wave of P700 oxidation reflects the release of restrictions for electron transport on the acceptor side of PSI (Schansker *et al.* 2003). By the end of the light period, the major part of P700 was in the oxidized state, as evidenced from equal amplitudes of ΔA_{810} signals induced by white light and far-red light. Similar changes of ΔA_{810} were also observed after AP generation in the absence of MV in the external solution (**Fig**. **1B**). The kinetic curve of ΔA_{810} comprised the fast peak of oxidation and the second, delayed wave of P700 oxidation. The addition of MV to the medium (in 30–40 min after triggering a single AP) and incubation of cells for 30 min in the presence of this acceptor had no influence on ΔA_{810} reflecting the dynamics of P700 redox state (**Fig. 1C**).

By contrast, the shape of the ΔA_{810} induction curve was modified rapidly and irreversibly after generation of a single AP provoked by excitatory electric pulse (**Fig. 1D**). In this case switching the light on was accompanied by rapid oxidation of P700 within about 100 ms without the stage of intermediary P700 reduction. Similar rapid photooxidation of P700 in the presence of MV was also observed on isolated pea chloroplasts (thylakoid suspensions) when permeation of this charged acceptor to thylakoids was not restricted by cell membrane barriers. Rapid oxidation of P700 reflects the capacity of MV as a very effective electron acceptor at the PSI level.

The essential similarity of ΔA_{810} signals in the absence and presence of MV in the medium (**Figs. 1A, 1C**) suggests that permeation of MV into chloroplasts *in situ* is prevented by effective membrane barriers, such as the plasmalemma and the chloroplast envelope. Since AP generation did not influence the shape of ΔA_{810} signals in the absence of MV but immediately modified ΔA_{810} in its presence, it is reasonable to suppose that the passage of MV towards the chloroplast thylakoids is facilitated abruptly during or after AP. The generation of a single AP was sufficient for the delivery of MV in effective concentrations. We did not observe any signs of MV incorporation into the electron transport chain under the action of subthreshold electrical stimuli, but these signs were manifested in full after the application of suprathreshold stimulus, in consistency with the all-or-none rule for AP generation.

Photoinduced changes of cell membrane potential under normal conditions and after triggered permeation of MV into the chloroplasts

Fig. 2 shows reversible changes of MP and pericellular pH in different cell regions upon light–dark transitions. All records started at continuous light, when alternating alkaline and acidic bands were formed in the unstirred layer along the cell length. Irrespective of microelectrode insertion in the alkaline or acidic regions, the transfer of cell to darkness resulted in a slow hyperpolarization of the cell. Upon subsequent return to light, the MP reversed to its initial depolarized state after a transient hyperpolarization. The essential similarity of MP kinetics recorded with microelectrode inserted in different cell regions is notable. The reasonable explanation is that the cable length constant for characean internodes estimated as 20–30 mm (Smith 1983) is much larger than the widths of alkaline and acidic zones (~2 and 5–7 mm, respectively). The light-induced changes of MP in *Chara* cells can be considered as an integral characteristic of electrically connected cell regions with different properties.

The origin of separate stages of light-induced MP changes in *Chara* cells was discussed elsewhere (Frost-Shartzer *et al.* 1992; Bulychev and Kamzolkina 2006). The cell hyperpolarization on darkening is thought to reflect the transition of the plasma membrane from the state with high H^+ conductance to the state with the dominant K^+ conductance on the background of residual activity of the electrogenic plasma membrane H^+ pump. The hyperpolarizing shift of MP after the transfer of cell to darkness was concurrent with disappearance of acidic and alkaline zones; i.e., it paralleled the pH increase in the acidic zone (**Fig. 2**, top) and the pH decrease in the alkaline zone (**Fig. 2**, bottom). In turn, the transition to depolarized state after switching the light on correlated with the pH lowering in the acidic zones (indicative of H^+ pump activation in the light) and with the pH increase in the alkaline cell regions (the increase in passive H^+ conductance). Thus, the MP changes in *Chara corallina* cells during intermittent illumination under physiological conditions are related to light-dependent formation of spatial pH pattern in the apoplast and to smoothing of the pH pattern in darkness. It is known that the heterogeneous spatial pH profile is coordinated with the distribution of photosynthetic activity in different cell regions and that such coordination is based on elevated CO_2 content in the

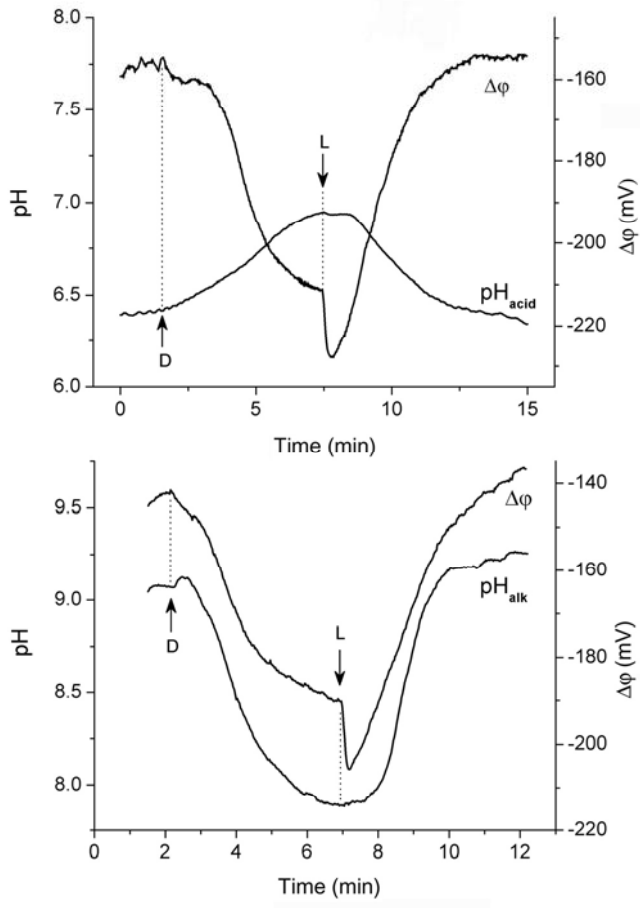

Fig. 2 Photoinduced changes of cell membrane potential (Δφ) and apoplastic pH in the acidic (top panel) and alkaline (bottom panel) regions of *Chara corallina* internodes. Arrows with symbols **D** and **L** in this figure and **Fig. 3** mark the moments when light (600 μE m^{-2} s^{-1}) was switched off and on, respectively.

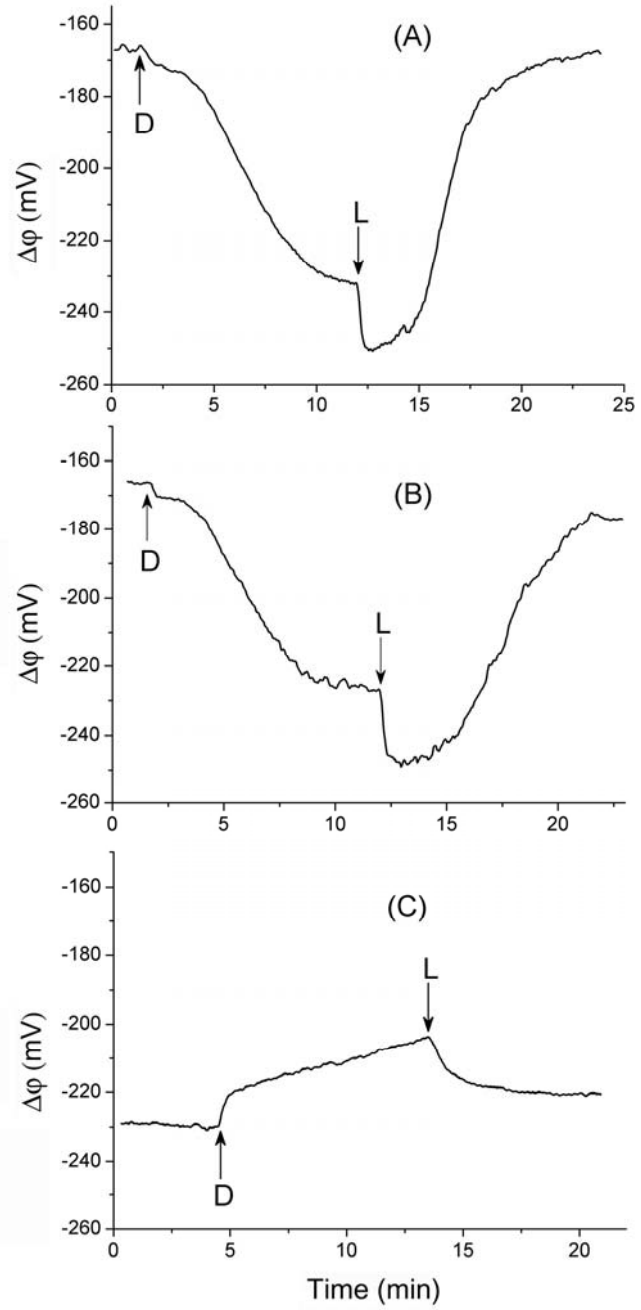

Fig. 3 Photoinduced changes of membrane potential (Δφ) in *Chara corallina* cells under sequential modification of experimental conditions. (**A**) Resting cell in the absence of MV in the medium; (**B**) resting cell incubated in the presence of 0.2 mM MV; (**C**) after generation of a single AP in the presence of 0.2 mM MV.

acidic regions and on deficiency of this membrane-permeable substrate at high pH (Bulychev and Vredenberg 2003; Bulychev *et al.* 2005). Hence, the light-induced MP transients reflect changes in membrane properties associated with natural photosynthetic electron transport.

Fig. 3 shows MP changes that were measured under control conditions (**A**), in the presence of MV at rest (**B**), and in the presence of MV after triggering a single AP (**C**). It is seen that the presence of MV in the medium had no appreciable influence on light-induced MP changes in the resting cell. On the other hand, the shape of MP changes was drastically altered after the generation of a single AP in the presence of MV. Following the electrical excitation of cell membranes, the light–dark transition did not lead to hyperpolarization, while the reverse transition did not cause cell depolarization. The MP responses to light and darkness contained only comparatively fast changes, which were apparently related to MV-mediated electron transport.

DISCUSSION

The results suggest that a single electrical pulse (AP) occurring at the plasma membranes can serve as a trigger that redirects electron transport in chloroplasts from a natural pathway to photoreduction of exogenous acceptor MV. It appears that the passage of MV to the sites of its action in chloroplasts *in situ* and eventual effectiveness of this herbicide are strongly affected by electric pulses propagated over the plant. The effects of membrane excitation on chloroplast functions remained unveiled for a long time because of methodical difficulties. The application of a dedicated microfluorometer based on the saturation pulse method discovered several fundamental effects of AP on photosynthetic activity and spatial organization of photosynthesis in characean cells as a plant model (Bulychev and Kamzolkina 2006; Krupenina and Bulychev 2007; Bulychev and Krupenina 2008). The depolarization spike in the presence of MV activated the electron flow to this exogenous acceptor. Strong energization of thylakoid membranes during MV photoreduction is known to occur even in very weak light (Salvucci *et al.* 1987; Neuhaus and Stitt 1989), which is particularly evident from nonphotochemical quenching of chlorophyll fluorescence at low light intensities.

Unlike the approach based on chlorophyll fluorescence measurements (Bulychev and Krupenina 2008; Krupenina and Bulychev 2008), this study focused on the kinetics of ΔA_{810} that reflect redox transitions of P700. The signal shape was used as a criterion of MV penetration into the chloroplasts. It is known that photooxidation of P700 in dark-adapted leaves is accelerated after effective treatment with MV, because MV circumvents the acceptor side limita-

tion for electron transport in PSI normally existing after dark adaptation (Schansker *et al.* 2005). The comparison of ΔA_{810} induction curves in **Fig. 1** shows clearly that MV added to the external medium remains chemically inactive until the moment of application of the first excitatory (supra-threshold) stimulus. On the other hand, it is also obvious that AP generation in the absence of MV had no substantial influence of photoinduced ΔA_{810} signals. Hence, rapid photooxidation of P700 after AP generation in the presence of MV was due to the increased accessibility of this acceptor in the chloroplast stroma, rather than to retardation of PSII activity after AP, which is normally evident both in the absence and presence of MV.

Fig. 2 contains evidence that the MP changes caused by intermittent light coincide in time with smoothing of non-uniform pH pattern upon darkening and with its restoration upon illumination. Since the pH distribution in the apoplast is concerted with the spatial pattern of photosynthetic activity and with relative content of membrane-permeable (CO_2) and impermeable (HCO_3^-, and CO_3^{2-}) forms of inorganic carbon, we suppose that CO_2-dependent electron flow plays the principal role in the formation of pH pattern and MP changes during illumination. Characteristic kinetics of MP changes during light–dark transitions can be a suitable indicator of operating the natural CO_2-dependent pathway of electron transport.

As can be seen in **Fig. 3**, the incubation of a resting cell in the presence of MV did not disturb photoinduced MP changes. Apparently, the electron transport pathways and associated processes remained unimpaired. This observation confirms the assumption that MV passage through the "plasmalemma–chloroplast envelope" membrane system is effectively blocked in the resting cell. Low permeability of cell membranes to MV is determined by its electrical charge (oxidized MV form is a divalent cation). In order to promote the permeation of MV into the cells, various procedures are applied, such as long-term (overnight) incubation, the treatment of plant material with detergents, intense stirring of leaf segments, and vacuum infiltration of leaves (Salvucci *et al.* 1987; Neuhaus and Stitt 1989; Schansker *et al.* 2005). Once the AP was triggered in a *Chara* cell by a short pulse of electric current in the presence of MV, the light-induced MP changes were irreversibly modified. Slow hyperpolarization and depolarization stages disappeared, and only faster changes of the opposite sign were retained. These results prove that MV becomes an available acceptor immediately after AP and switches photosynthetic electron transport from the CO_2-dependent pathway to MV reduction.

The question of how AP promotes the penetration of a divalent cation MV into the chloroplasts is presently unresolved. Experiments with artificial membrane systems revealed that permeability to MV can be affected by changes in membrane potential and pH of the medium (Létant *et al.* 2006; Majumder *et al.* 2007). However, the short-term displacement of MP by stimulating current (in the absence of AP generation) could not provide effective concentrations of MV in the cytoplasm and chloroplasts. Indeed, the functional activity of MV appeared in a stepwise manner under the action of supra-threshold stimulus but was not manifested in any form upon application of subthreshold pulses. Obviously, the activation of ion channels involved in cell excitation is significant for permeation of MV. These ion channels of the plasmalemma might provide a pathway for nonspecific permeation of MV during the passage of the principal ion species. Alternatively, they might modulate permeability of the chloroplast envelope to MV by changing the cytosolic concentrations of essential natural ions (e.g., Ca^{2+}). It should be pointed out that even a small influx during AP might be sufficient, because MV is effective at very low concentrations (~40 nM) (Neuhaus and Stitt 1989). Furthermore, the oxidized MV is not depleted during prolonged illumination because MV acts as a catalyst. Its reduced form is oxidized by atmospheric oxygen with the production of superoxide and hydrogen peroxide (Schreiber *et. al.* 1995), which may react together and produce hydroxyl radicals detrimental for membrane integrity. Therefore, even a passage of MV single portion might be sufficient for striking rearrangement of electron transport pathways in the chloroplast.

In the absence of MV, the chloroplast functional changes appear after AP only in the light. Furthermore, the chloroplast responses in alkaline and acidic zones are clearly different (Bulychev and Kamzolkina 2006). By contrast, the permeation of MV into the chloroplasts after AP generation was independent of the formation of such zones. During ΔA_{810} measurements, the light conditions were insufficient for spatial differentiation of cell regions differing in photosynthetic and transport activities. It is not excluded that the influx of MV into the cytoplasm during AP occurs irrespective of light conditions, while the uptake of MV from the cytoplasm by chloroplasts is light-dependent, by analogy with the light-driven Ca^{2+} uptake into the chloroplast stroma.

We conclude that the generation of a single electrical impulse at the plasma membrane accelerates considerably the permeation of a physiologically active agent, methyl viologen from the external medium into the chloroplasts of intact *Chara corallina* cells. If the herbicidal effect of MV on whole plant leaves depends on permeation through cell membrane barriers, the plants would show differential susceptibility to this herbicide depending on whether or not the electric signals were propagated over the plant during the treatment. The electric pulses were shown to arise in response to local injuries, heating, mechanical agitation, rapid cooling, application of KCl solutions, and other treatments. Based on experiments with a model plant system (characean internodes), it is not excluded that, in a group of plants equally treated with this herbicide, some plants could be selectively damaged by supplementary treatments known to induce the AP propagation. Taking into consideration the impact of AP on MV permeation in *Chara* might be helpful for improving weed control measures.

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