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Action potential in a plant cell lowers the light requirement for non-photochemical energy-dependent quenching of chlorophyll fluorescence

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Abstract

This study deals with effects of membrane excitation on photosynthesis and cell protection against excessive light, manifested in non-photochemical quenching (NPQ). In *Chara corallina* cells, NPQ and pericellular pH displayed coordinated spatial patterns along the length of the cell. The NPQ values were lower in H^+-extruding cell regions (external pH ~6.5) than in high pH regions (pH ~9.5). Generation of an action potential by applying a pulse of electric current caused NPQ to increase within 30–60 s. This effect, manifested as a long-lived drop of maximum chlorophyll fluorescence (F_m'), occurred at lower photosynthetic flux densities (PFD) in the alkaline as compared to acidic cell regions. The light response curve of NPQ shifted, after generation of an action potential, towards lower PFD. The release of NPQ by nigericin and the rapid reversal of action potential-triggered NPQ in darkness indicate its relation to thylakoid ΔpH. Generation of an action potential shortly after darkening converted the chloroplasts into a latent state with the F_m identical to that of unexcited cells. This state transformed to the quenched state after turning on weak light that was insufficient for NPQ prior to membrane excitation of the cells. The ionophore, A23187, shifted NPQ plots similarly to the action potential effect, consistent with a likely role of a rise in the cytosolic Ca^{2+} level in the action potential-induced quenching. The results suggest that a rapid electric signal, across the plasma membrane, might exert long-lived effects on photosynthesis and chlorophyll fluorescence through ion flux-mediated pathways.

Keywords: Chlorophyll fluorescence; Action potential; Energy-dependent quenching; Thylakoid membrane; Ionophores; *Chara corallina*

1. Introduction

Electric excitation in plant cells is a multifunctional regulatory signal whose possible influence on the use of light energy in photosynthesis remains largely unexplored. Plants and algae are subject to photooxidative damage under excessive light [1]. Photodamage is minimized by the plant's ability to regulate the balance between absorption and utilization of light energy [2–5]. Energy-dependent non-photochemical quenching (NPQ), termed q_E, is a harmless way for dissipation of excess light energy as heat, which provides rapid regulatory adjustments in the time range from seconds to a few minutes. If the absorption of light exceeds the capacity for CO_2 fixation, the thylakoid ΔpH is built up by photosynthetic electron transport. The acidification of the lumen activates enzymatic conversion of a carotenoid, violaxanthin to zeaxanthin, which is a quencher of chlorophyll excited states [6]. It also promotes protonation of carboxyl groups in photosystem II (PSII) polypeptides, which facilitates binding of zeaxanthin to the effector site in the antenna [7]. Furthermore, the increase in proton concentration displaces Ca^{2+} from its functional site in the O_2-evolving complex, which inhibits electron flow to P680 and induces quenching in the PSII reaction center because oxidized P680 ($P680^+$) accumulates [4,6,8,9].

A great deal of attention has been given to analysis of NPQ as a function of light intensity in plants exposed to fluctuating light or other environmental stimuli [10–12]. Imaging of NPQ and other chlorophyll fluorescence parameters during the induction period of photosynthesis revealed the transient appearance of periodic spatial patterns in leaves whose potential

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Abbreviations: AP, action potential; NPQ, non-photochemical quenching; PFD, photosynthetic flux density; PSII, photosystem II

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photosynthetic capacity was basically uniform [13–16]. Reversible transitions between homogeneous and heterogeneous patterns of photosynthesis are especially evident at the single cell level in characean algae [17,18]. When exposed to light, these cells produce alternating zones of low and high external pH and corresponding cell regions with elevated and suppressed activity of PSII. The origin of spatial heterogeneity of photosynthesis and H^+ fluxes across the plasma membrane are of particular interest in view of the fact that the extracellular pH pattern is highly sensitive to the action potential generated by a single electric excitation of the cell [19].

Electric excitation in plants is recognized as a signal involved in cell elongation, respiration, and osmotic regulation [20]. The primary event, when an action potential (AP) is induced, is a manifold increase in cytosolic Ca^{2+} level [21]. Cells of characean algae generate an AP upon electrical, chemical or mechanical stimulation. After induction of a single AP with a typical duration of about 1 s, the external pH pattern is damped for 20–40 min indicating temporal cessation of circulating H^+ fluxes across the plasmalemma [19]. There is evidence that an AP transiently suppresses primary photochemical processes of PSII in characean algae [19,22] and in excitable higher plants [23]. However, the origin of this phenomenon and its relation to photochemical and non-photochemical quenching components and to ion fluxes engaged in membrane excitation and post-excitation events remain largely unknown.

The aim of this study was to examine spatial patterns of NPQ in isolated cells of *Chara corallina* in relation to H^+ fluxes across the plasma membrane and to investigate effects of membrane excitation and addition of a Ca^{2+} ionophore on the light response curve of NPQ. The results suggest that induction of an AP inhibits PSII activity by promoting thermal losses of chlorophyll excitation at lower PFD compared to those sufficient for NPQ in unexcited resting cells.

2. Materials and methods

Chara corallina internode cells about 6 cm in length and 0.9−1 mm in diameter were excised and placed in a 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 an isolated single *Chara* 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 a tip diameter of 10–20 μm [18]. The terms “alkaline and acid cell regions” denote cell parts producing zones of high and low external pH, respectively.

Chlorophyll fluorescence was measured on small (diameter 100 μm) portions of a chloroplast layer with a Microscopy-PAM fluorometer [24,25] and WinControl program (Walz). The pH probe was positioned within the fluorometric window at a distance of about 20 μm from the cell surface. 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 and passed through a blue cut-off glass filter (SZS-22, $\lambda < 580$ nm). Irradiance at the cell level was determined with an 818-UV optical power detector (Newport, CA, United States) and recalculated to photosynthetic flux density (PFD). The highest PFD was 100 $\mu mol\ m^{-2}\ s^{-1}$. Neutral glass filters were used for attenuating the PFD.

The effective quantum yield of PSII electron flow ($\Delta F'/F_m'$), coefficient of non-photochemical quenching (NPQ), and coefficient of photochemical quenching qP were estimated from equations $\Delta F'/F_m' = (F_m' - F')/F_m'$, $NPQ = (F_m - F_m')/F_m'$, and $qP = (F_m' - F')/(F_m' - F_o')$. Here F_m and F_m' designate maximal fluorescence induced with a saturating pulse in a dark-adapted cell and in a cell exposed to actinic light, respectively, F' is the actual fluorescence in the actinic light, and F_o' is the minimal fluorescence observed after removal of actinic light [26,27]. During measurements of light intensity curves, fluorescence parameters F_m' and F' were taken after they reached the steady state values. Minimal fluorescence under weak measuring light (F_o) was recorded with accuracy (standard deviation) of about 3% of the mean value. In the literature alternative approaches have been described for estimating fluorescence quenching via the analysis of the relative values of photochemical and non-photochemical rate constants of energy dissipation in PSII [28].

The electrical excitation of the cell membrane was done with one single rectangular pulse of a transcellular electric current (~10 μA, 150 ms) passing through external electrodes [19]. Effects of ionophores were assessed after 1- to 3-h incubation. All experiments were performed at least in triplicate. Figures display results of representative experiments. Bars in figures show mean values and standard deviations of the means.

3. Results

3.1. Non-photochemical quenching in different cell regions: relation of NPQ pattern to pH bands and dependence on light intensity

In darkened *Chara* cells the external pH in boundary layers and the quantum yield of PSII are homogeneously distributed along the internode cell. After transfer to light, *Chara* cells develop spatial profiles of external pH [29,30] and effective quantum yield of electron flow in PSII [17,18,31]. Photosynthesis is more active in the regions of proton extrusion (acid regions, external pH 6.2–6.7) compared to cell regions adjacent to alkaline zones (pH 9.5–10) [17,18]. Fig. 1A shows longitudinal profiles of NPQ and external pH in an illuminated internode cell of *C. corallina*. Spatial profiles of NPQ and external pH (Fig. 1A) were clearly correlated, with a slope of 0.6±0.1 for the regression line of NPQ as a function of pH. Under given light conditions, the NPQ values were comparatively high (0.4–1.6) for chloroplasts in cell regions producing alkaline (inward current) zones and were low (0–0.2) in cell regions producing acidic (outward current, H^+ extrusion) zones.

Local properties of alkaline and acidic cell regions were further characterized by measuring NPQ at various fluence rates. The plots of NPQ as a function of PFD (Fig. 1B) fit well to sigmoid curves, both in alkaline and acidic regions of more than 30 cells. The light response curves of NPQ in alkaline cell regions were shifted to lower PFD compared to those in the acidic regions. The plots for alkaline and acidic regions coincided at low light intensities, diverged in the intermediate PFD range (it is this range where NPQ spatial profiles were evident), and differed slightly at the high light intensity range.

The extent of quenching and the steepness of the transition between non-quenched and quenched states varied in various cells, depending on cell age and transparency. In young non-calcified cells, the transitions were particularly steep. In mature and senescent cells, which were less transparent owing in part to light screening by calcium deposition on cell walls, the ascending segment of the plot was less steep, and the inflection point of the fitting curve was observed at higher PFD. It is known that NPQ is more sensitive to PFD at low CO_2 levels

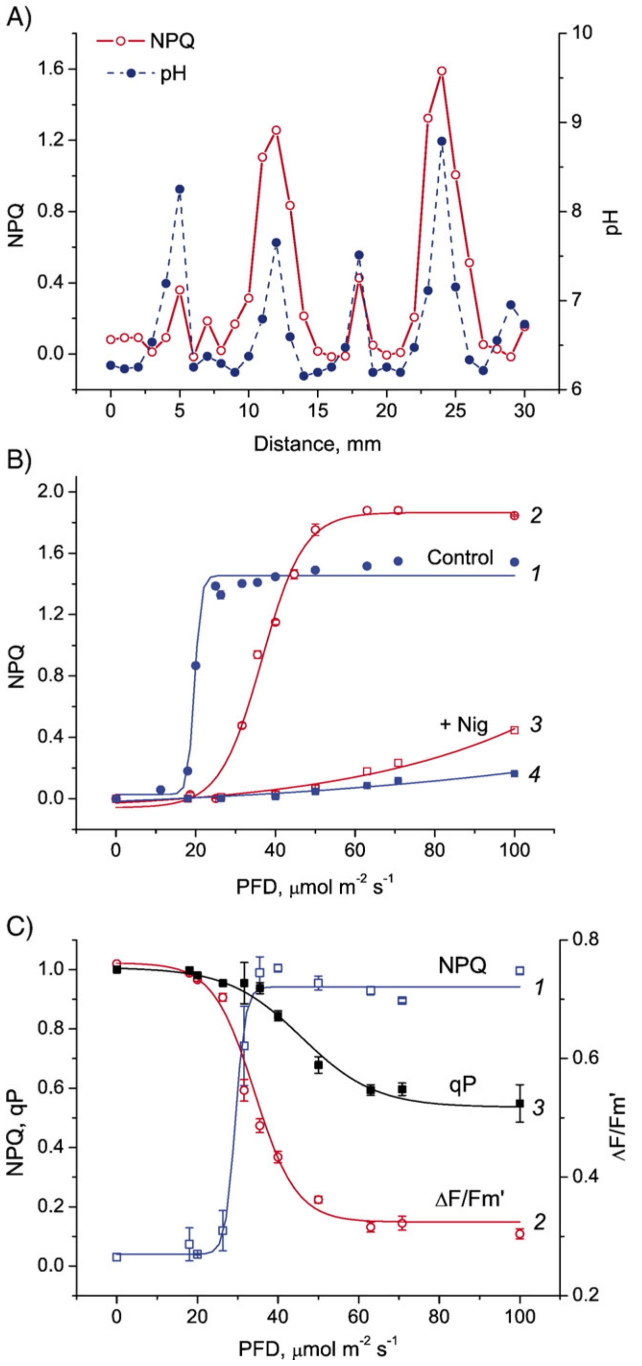

Fig. 1. Characteristics of non-photochemical quenching (NPQ) revealed by local measurements of chlorophyll fluorescence in *Chara* cells. (A) Profiles of NPQ (solid line) and external pH (dashed line) along the cell length, measured at a photosynthetic flux density (PFD) of 28 μmol m^{-2} s^{-1}. (B) NPQ in the alkaline (*1, 4*) and acidic (*2, 3*) cell regions as a function of PFD under control conditions (*1, 2*) and in the presence of 4 μM nigericin (*3, 4*). (C) Comparison of light response curves for NPQ (*1*), quantum efficiency of PSII, $\Delta F'/F_m'$ (2), and photochemical quenching, qP (*3*) in the alkaline cell region. Data points and error bars are time-averaged values ± SD with *n* ranging from 4 to 15.

[12]. Consistent with this notion and with the supposedly higher CO_2 level in the acidic zones [17,31], the slope of NPQ light curve was usually somewhat lower in the acidic regions compared to alkaline regions (Fig. 1B).

Fig. 1B shows also that NPQ was effectively released, both at high and low PFD, after the treatment of the cell with nigericin, a K^+/H^+ antiporter known to eliminate the thylakoid pH gradient [32] and ΔpH-dependent quenching of chlorophyll fluorescence [5]. The effect of nigericin suggests that NPQ in *Chara* cells is related to ΔpH and represents energy-dependent quenching, qE [5,6,33].

Fig. 1C compares the light intensity plot for NPQ in alkaline region with similar plots for the effective quantum yield of PSII electron flow, $\Delta F'/F_m'$ and photochemical quenching, qP. It is seen that $\Delta F'/F_m'$ changes occurred over a wider PFD range compared to NPQ changes. As PFD increased over the range where NPQ attained saturation, the decline in $\Delta F'/F_m'$ was approximately paralleled by the decrease in qP.

3.2. Effect of action potential on F_m': fluorescence properties of illuminated and darkened cells during the post-excitation period

The maximal fluorescence F_m' and NPQ in the alkaline cell regions were highly sensitive to AP generation (Fig. 2) at intermediate PFD range. The effect of an AP on NPQ was manifested as a large long-lived drop of maximum fluorescence, F_m' (Fig. 2A). The F_m' decrease was usually concurrent with a large pH decrease in the alkaline regions. Nevertheless, the AP-induced pH shifts (i.e., changes in H^+ transport across the plasmalemma) and the F_m' changes in chloroplasts were not strictly linked, differing in light requirement. Once the pH pattern had been pre-formed by light treatment, the effect of AP on external pH in the alkaline region was observed irrespective of whether the cell was illuminated or darkened at the moment of stimulation (Fig. 2B). In contrast, the F_m' response to AP generation was only observed under illumination and fully disappeared if light was turned off as short as 10 s prior to cell excitation. The increase in F_m' in Fig. 2B represents the usual F_m' rise observed upon darkening; there was no sign of AP-induced quenching in darkened cells.

In resting cells at low PFD (28 μmol m^{-2} s^{-1}), the F_m' fluorescence was close to F_m level of the dark-adapted cell and increased insignificantly upon the transfer of cell to darkness (data not shown). However, when the same cell was electrically stimulated and then darkened at the peak of the AP-induced F_m' drop (Fig. 2C), the light–dark transition caused a rapid recovery of F_m' to a high level with a halftime of about 20 s. A subsequent return of the cell to light caused rapid quenching of F_m'. These results suggest that NPQ developed at lower PFD after cell excitation than in the resting state, prior to excitation. The resting and excited cells did not differ in the maximal fluorescence (F_m) measured in darkness but showed strikingly different F_m' responses to dark–light transitions. One may suppose that AP converted the chloroplast–cytoplasm system into a "latent" state, which persisted in darkness and was rapidly transformed into the quenched state upon the onset of weak light. As shown in Fig. 2A and C, the AP-induced quenched state lasted for about 10 min or longer.

Fig. 2D shows F_m' changes in the cell exposed to AP-triggering stimulus in darkness and then transferred to light.

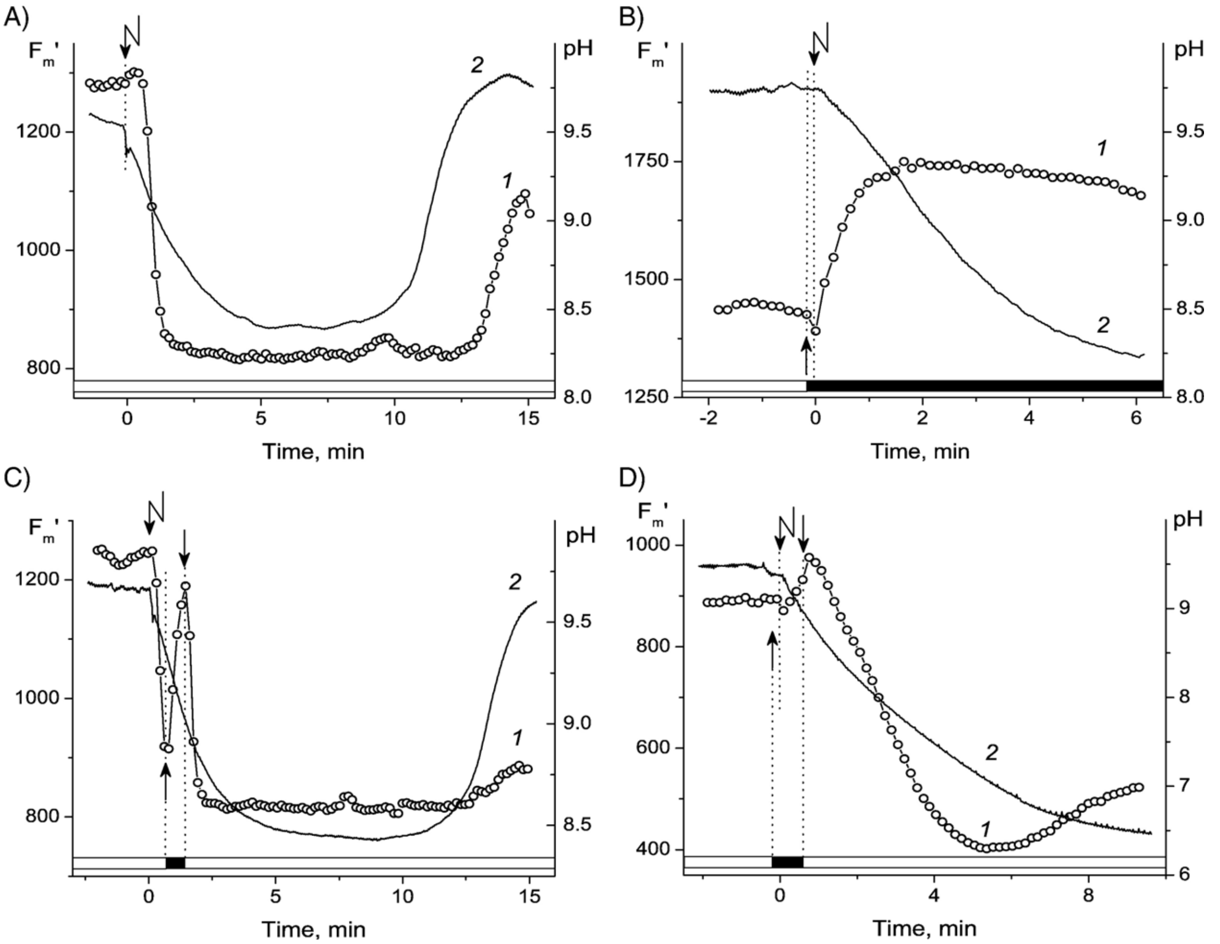

Fig. 2. Action potential-induced changes of F_m' in *Chara corallina* cells. (A) The decrease in maximum chlorophyll fluorescence F_m' (curve 1, circles) after action potential induction (AP) and a concomitant decrease of the external pH in the alkaline zone (solid line *2*). (B) Generation of an AP 10 s after darkening produces no quenching of F_m' (curve *1*, circles) but diminishes pH in the alkaline zone (solid line 2). (C) AP-induced quenching of F_m' is rapidly reversed in darkness and reappears upon subsequent exposure to light (circles, curve *1*). Curve *2* shows AP-induced pH changes measured externally in the same cell region. (D) Development of NPQ upon the return of *Chara* cell to weak light 30 s after triggering AP in darkness (circles, *1*); solid line *2* shows external pH changes. The light regime is indicated by the bars at the base of the graphs, where black bars indicate dark and white bars indicate light (28 μmol m^{-2} s^{-1} for A–C and 20 μmol m^{-2} s^{-1} for D). Zig-zag arrows in this figure and Fig. 3 mark the moment of AP generation.

During the dark exposure, a slight increase in F_m' occurred, in similarity with observations shown in Fig. 2B. However, an exposure of the pre-excited cell to light 30 s after AP caused a large drop in F_m'. It appears that triggering of AP in darkness produced some latent state, which was converted to the quenched state in response to weak light, even though such light was insufficient for quenching prior to cell excitation. The lifetime of the latent state was only roughly estimated: the intervals of 1 and 3 min between the AP generation and the exposure of cell to light were sufficient to produce light-induced NPQ during the post-excitation period (data not shown).

3.3. Action potential and Ca^{2+} ionophore induce similar shifts of NPQ light response curves

After cell excitation (AP generation), the NPQ curves shifted to lower light intensities (Fig. 3A, C). The NPQ curves for cells in the post-excitation period were measured in two ways. One procedure was to monitor AP-induced changes of F_m' and to record its minimal level attained after cell excitation. The amplitude of AP-induced F_m' changes depended on fluence rate, diminishing both at very low and high PFD. This is shown schematically by the length of vertical dashed arrows in Fig. 3A and illustrated with kinetic curves in Fig. 3B. This approach was convenient when the quenched state was stable as shown, e.g., in Fig. 2A, C.

The second procedure consisted in recording F_m' for 10–15 min after AP generation and in calculating time-averaged mean values and standard deviations (Fig. 3C). This procedure was needed in some cases, since AP generation was often followed by large-scale oscillations of F_m' with a period of about 3 min. Fig. 3D shows an example of AP-induced F_m' oscillations. The two procedures described above revealed the displacement of NPQ light response curves after cell excitation to the lower PFD range. This displacement was most pronounced in the alkaline cell regions but was also observed in the acidic regions (data not shown).

The prolonged recording of F_m' after AP induction clearly showed that F_m' oscillations were attributed to the ascending portion of NPQ curve and did not occur in the horizontal parts

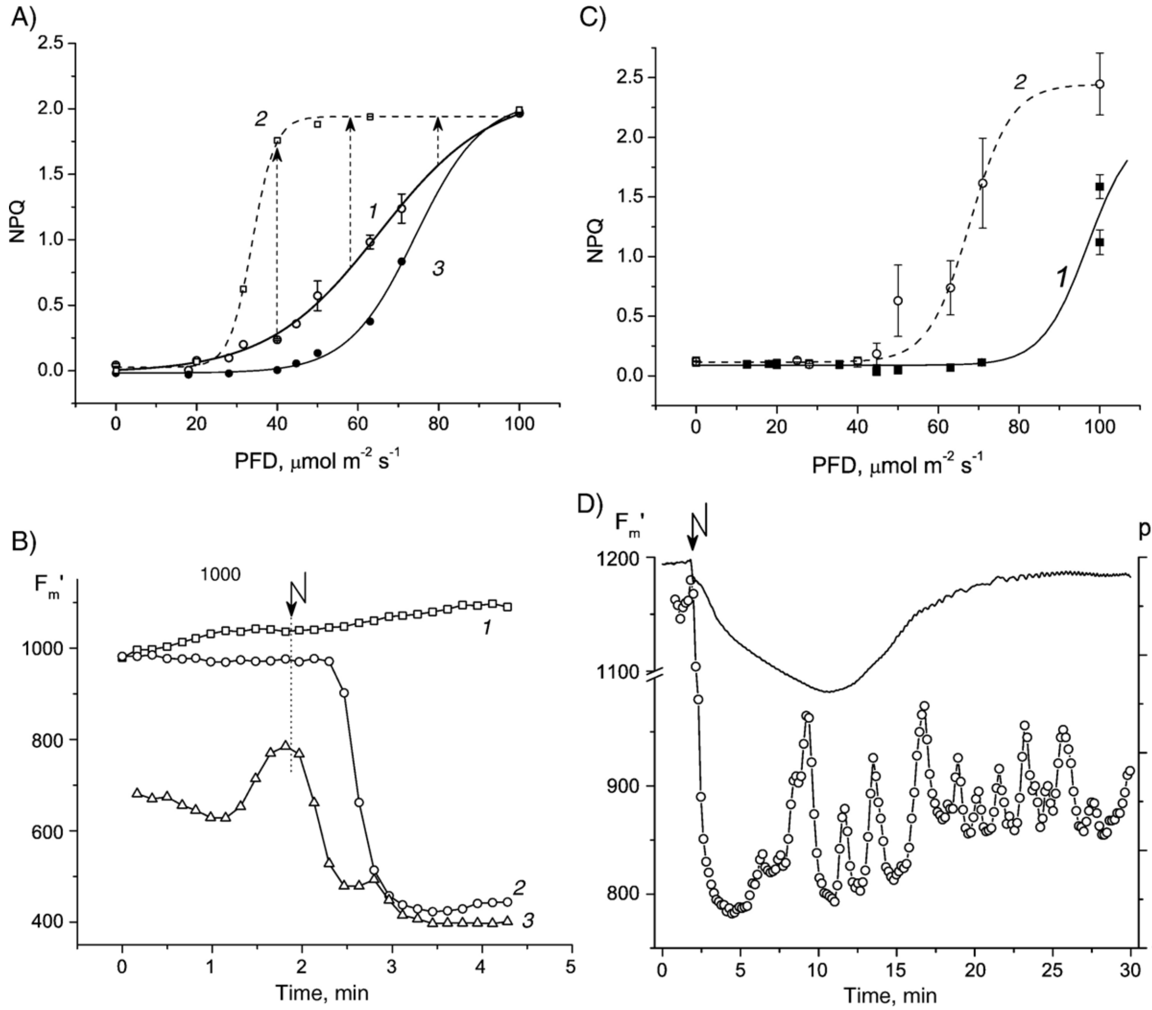

Fig. 3. Non-photochemical quenching (NPQ) as a function of photosynthetic flux density (PFD) before and after electric excitation of *Chara* cell and fluorescence phenomena related to cell excitation. (A) Light response curves for NPQ in alkaline cell regions before (curve *1*) and after AP induction (curve *2*) and in the acidic cell regions of a resting cell (curve *3*). Bars in curve *1* represent standard deviations obtained in experiments with two similar cells. Arrows designate the amplitude of AP-induced changes at various PFD. (B) Dependence of AP-induced F_m' changes on light intensity: (*1*) no excitation-induced F_m' response at low PFD (18 μmol m^{-2} s^{-1}); (*2*) strong AP-induced quenching at moderate PFD (40 μmol m^{-2} s^{-1}); (*3*) response of smaller amplitude at elevated PFD level (63 μmol m^{-2} s^{-1}). (C) Light intensity curves of NPQ before (*1*) and after cell excitation (*2*) in the alkaline cell region. Mean values of F_m' were obtained by time averaging over periods of 5–15 min; bars represent SD for time-averaged measurements. Note small SD values in the lower flat segment and high SD values for the ascending region of the curve where F_m' was subject to oscillations. (D) Quenching and subsequent oscillations of F_m' induced by AP generation (circles); solid line shows AP-induced pH changes in the external medium, measured at a PFD of 28 μmol m^{-2} s^{-1}. Zig-zag arrows in (C and D) mark the moment of AP generation.

of this curve. As can be seen from the error bars in Fig. 3C, the NPQ values were stable at low PFD, while NPQ oscillated at higher PFD, in the steep portion of the curve.

AP generation in plant cells is accompanied by transient changes in ionic composition of the cytosol: Ca^{2+} enters the cytoplasm from the external medium through voltage-gated Ca^{2+} channels [21,34,35] and is released from intracellular stores [36,37]. In order to check for a possible role of Ca^{2+}, we examined the effects of ionophore A23187 that ensures the transmembrane exchange of divalent cations for protons and is expected to increase the Ca^{2+} level in the cytosol. As can be seen in Fig. 4, the addition of A23187 to the external medium shifted the NPQ light curves to lower PFD in an apparent similarity with the effect of membrane excitation. This shift was observed for alkaline as well as acidic cell regions.

4. Discussion

Photosynthesis of leaves has been found to be sensitive to electrical or chemical signals, propagated from cell to cell through the plant, upon chilling, burning, or mechanical disturbance [23,38,39]. Transient inhibition of photosynthesis in individual cells is clearly evident upon AP generation in characean algae [19,22]. A strong AP-induced quenching of F_m' examined here indicates that the temporal depression of PSII electron flow after electrical excitation in *Chara* cells is largely due to the increase in thermal loss of chlorophyll excitation, provided that PFD is not too high to saturate F_m' quenching. The AP-induced quenching of F_m' rapidly reversed in darkness (Fig. 2C); this was most evident at light-limiting conditions when NPQ is small and was absent under high light conditions when a

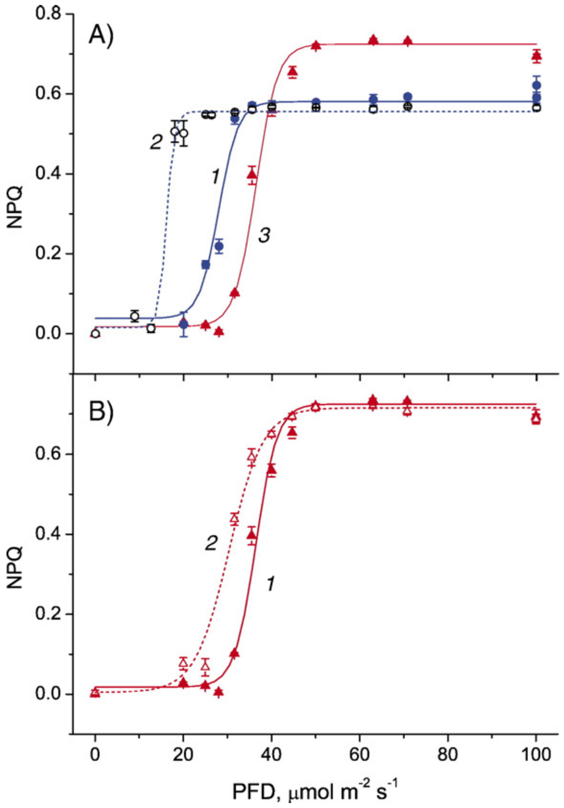

Fig. 4. Effect of the ionophore for divalent cations, A23187 (10 μM), on light response curves of non-photochemical quenching (NPQ) in the alkaline and acidic cell regions. (A) NPQ plots measured in the alkaline cell region before (*1*) and after (*2*) addition of A23187; the NPQ plot for the acidic region of the same cell is shown for comparison (thin line *3*). (B) NPQ plots measured in the acidic cell region before (*1*) and after (*2*) the addition of A23187.

large NPQ was pre-established. These properties of AP-induced quenching and the release of NPQ by the K^+/H^+ antiporter, nigericin, indicate that it represents energy-dependent (ΔpH-dependent) quenching q_E rather than alternative forms of NPQ (photoinhibition or “state transitions”) [3].

As can be seen from Fig. 3A, 3C (curves *1*, *2*), low NPQ was observed over a broader PFD range under resting conditions than during the post-excitation period. Hence, even low-intensity light was in excess of the cell’s capacity to utilize quanta for photosynthesis over a long period (10–15 min) after AP generation.

Several features are remarkable in the effects of AP on NPQ and PSII electron flow. (i) The effects of AP are at least three orders of magnitude longer than the duration of AP itself. This indicates the presence of an intermediary cascade that converts a short electric signal at the plasma membrane into a long-lived state. (ii) The light response curves for NPQ in the alkaline and acidic cell regions of unexcited cells are shifted (Figs. 1B, 3A), which accounts for the different AP-induced responses of NPQ in these regions at certain fluence rates. For example, AP-induced F_m' changes in the alkaline cell regions were particularly large at rather low PFD, at which no AP-induced F_m' changes were observed in the acidic regions. (iii) The AP-induced shift of the NPQ light-response curve to lower PFD range (Fig. 3A, C) may displace NPQ from the stable low position to the steep region of the NPQ curve where F_m' is subject to oscillations (see left vertical arrow in Fig. 3A).

Oscillations of chlorophyll fluorescence after sudden changes in PFD or CO_2 concentration have been repeatedly observed [15,16,40–42] and were interpreted as a consequence of imbalance between availability of NADPH and ATP for the dark reactions of photosynthesis. Although oscillations in whole leaves arise under particular conditions, fluorescence oscillations were commonly observed in individual cells or small cell groups and protoplasts [15, 40]. The F_m' oscillations described here originated in small (d = 100 μm) regions of a chloroplast layer of individual cells. Apparently, fluorescence oscillations are better manifest at a microscopic level than in whole leaves, since desynchronized oscillations of individual cells in spatially distant regions may disappear in the total signal. The spatial smoothing of locally heterogeneous fluorescence characteristics in large-size samples, together with attenuation of light intensity in thick samples, may explain why the transitions between non-quenched and quenched states in light-response curves of NPQ are much steeper at the subcellular level (this study) than for macroscopic objects (leaves [2,10,12]).

Our data demonstrate for the first time that induction of an AP induces fluorescence oscillations in the same way as sudden changes in light intensity. Considering, for example, Fig. 1B, we anticipate that a sudden change in PFD will cause oscillatory behavior provided that NPQ is displaced after such transition from the stable (flat) level to the steep region of the light-response curve. The AP-induced transitions toward the stable upper segment of NPQ curves can also give rise to oscillations. In this case, F_m' oscillations would not start immediately after AP; there will be a delay dependent on the dynamics of restoration of NPQ light response curves to their original shape and position. In this case F_m' oscillations will appear after a lag period, as in Fig. 3D.

Our results suggest that electric excitation of darkened cells transforms the chloroplast–cytoplasm system into a latent state, which is indistinguishable from the original state in terms of F_m. The existence of such a latent state is revealed under subsequent illumination, as it produces a large NPQ despite the ineffectiveness of such illumination prior to AP generation. The lifetime of the latent state estimated from data similar to Fig. 2D lasted at least for 1 min. The generation of AP had no effect on F_m' either at very high or low PFD, where NPQ curves merged for the resting and post-excitation conditions (e.g., Fig. 3A, curves *1, 2*). The F_m' responses of the largest amplitude were observed for low NPQ values at PFD of 30–40 μmol m^{-2} s^{-1}. Conversely, at high PFD when NPQ was large prior to the cell excitation, AP produced no additional quenching. The lack of AP-induced effects in very weak light (Fig. 3B, curve *1*) indicates that a critical electron transport rate is needed for AP-induced quenching.

The mechanistic aspects of the AP-induced F_m' changes and the nature of the latent state remain to be elucidated. The central event in membrane excitation of plant cells is the increase in cytoplasmic Ca^{2+} level owing to the opening of plasma membrane Ca^{2+} channels [34,35] and the release of Ca^{2+} from intracellular stores [36,37]. Calcium is known as a secondary messenger that regulates various enzymatic systems of the cell. Furthermore, the chloroplast envelope is endowed with a light-dependent Ca^{2+} uniport that ensures accumulation of Ca^{2+} in the stroma during illumination [43,44]. The stroma-located enzymes exhibit complicated dependence on Ca^{2+}. On the one hand, Ca^{2+} is needed for activation of the Calvin–Benson cycle enzymes and, on the other hand, it inhibits activity once the enzymes have been activated ([45,46] and references therein). The rise in cytosolic Ca^{2+} level during AP should be followed by accumulation of Ca^{2+} in the chloroplast stroma via the light-dependent uniport, which would suppress CO_2 fixation, thus increasing ΔpH across the thylakoid membrane and causing NPQ. This hypothesis is substantiated by observations that AP generation and the treatment with ionophore A23187 produced similar shifts of NPQ plots versus PFD. The effect of the $Ca^{2+}(Mg^{2+})/H^+$ antiporter, A23187, was strikingly different from the effect of the K^+/H^+ antiporter, nigericin (cf. Figs. 1B and 4). Although the ionophore, A23187, was capable of abolishing the thylakoid ΔpH in isolated chloroplasts [47], it did not release fluorescence quenching in *Chara* cells in vivo. It is more likely that A23187 affected the NPQ light response curves through the increase in cytosolic Ca^{2+} level. The ionophore-induced changes in cytosolic Mg^{2+} concentration are expected to be much less dramatic compared to Ca^{2+}, since the Mg^{2+} concentration in the cytosol (2–10 mM) is several orders of magnitude higher than the cytosolic Ca^{2+} concentration ($\sim 10^{-7}$ M). Based on the data obtained, one may assume that the latent state produced in darkness after generation of an AP corresponds to elevated Ca^{2+} level in the chloroplast environment.

We have shown in this paper that AP generation in excitable cells of a characean alga results in NPQ development at lower fluence rates compared to that in the resting state. This can be considered as an increase in NPQ sensitivity to PFD. This sensitivity was shown to depend on CO_2 availability, increasing in CO_2-deficient media [12]. These differences in sensitivity, mediated by variations of CO_2 content in the acid and alkaline zones, seem essential for spatial variations of NPQ in resting cells. At the same time, the effects of AP on fluorescence quenching cannot be explained by this mechanism. Although external pH is subject to large changes after AP generation (Fig. 2), availability of CO_2 is unlikely to decrease during this change and cannot account for the increased sensitivity of NPQ to PFD. Differential effects of the two ionophores, nigericin and A23187, lead us to favor the hypothesis of Ca^{2+}-mediated inhibition of the dark reactions of photosynthesis. Remarkably, similar shifts of NPQ light-response curves were observed following AP or A23187 treatment and for the alkaline cell regions compared to acidic regions. Therefore, we cannot exclude that cytosolic Ca^{2+} levels in the immobile ectoplasm of resting cells are subject to spatial patterning coordinated with the pH and photosynthesis patterns. These issues require further studies.

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