CHANGES IN MEMBRANE POTENTIAL ASSOCIATED WITH CYCLIC AND NON-CYCLIC ELECTRON TRANSPORT IN PHOTOCHEMICAL SYSTEM 1 IN Nitella translucens

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Received November 24, 1970

Summary

Light-induced changes in the electrical potential of cytoplasmic membranes of opposite polarity occur in single Nitella cells in the presence of DCMU with DCPIP and ascorbate, and with PMS. Relative efficiencies of 670 and 703 nm quanta, suggest an association with photo-chemical reactions in system 1. The light-driven depolarization in the presence of DCPIP + ascorbate which is inhibited by FCCP and stimulated by phlorizin, is suggested to be associated with H^+-movement coupled to non-cyclic electron transport. The light-driven hyperpolarization in the presence of PMS which is inhibited by FCCP and phlorizin, is suggested to be associated with phosphorylation coupled cyclic electron transport.

The electrical potential across the cytoplasmic membranes of giant algal cells like Nitella translucens responds to light (1,2), the light causing a depolarization of the membranes. Involvement of both photosynthetic light reactions, inhibition by low concentrations of DCMU and CCCP (1), and a high quantum efficiency (3), were found to be main characteristics of the photo-chemical process causing these changes. It has been suggested that electron transport coupled translocation of ions, probably protons, at the phase boundaries of the chloroplasts is the primary photochemical process involved.

In this communication experiments are described on light-induced changes in Nitella translucens, under conditions at which photochemical system 2 is made inoperative by DCMU for contributing to electron transfer from H_2O to NADP, and either cyclic or non-cyclic electron transfer in system 1 is made possible by adding PMS or DCPIP with ascorbate, respectively. Specific characteristics of the observed potential changes are given, and the effects of the uncoupler FCCP and the energy transfer inhibitor phlorizin thereupon are described.

Abbreviations: DCMU- 3,4 dichlorophenyl-N, N-dimethyl urea; CCCP- carbonyl cyanide-m-chlorophenyl hydrazone; FCCP- carbonyl cyanide- n-trifluoremethyoxy phenyl hydrazone; PMS- phenazine methosulphate; DCPIP- 2,6-dichlorophenol-indophenol.

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MATERIAL AND METHOD

Young, fresh grown Nitella cells of 5-8 cm length and about 0.6-0.8 mm diameter were taken from a laboratory culture in artificial pond water (APW) containing 1.0 mM NaCl, 0.1 mM KCl and 0.1 mM $CaCl_2$. The cells were grown at 15°C in a conditioned growth room under weak light in a light-dark regime of 12 hours each. Usually the cells were presoaked for a few days in a Ca^{++}-enriched medium, containing 1.0 mM NaCl, 0.1 mM KCl and 1 mM $CaCl_2$ (further referred to as Ca-APW). It was found that for our laboratory grown cells, presoaking in Ca-APW changed the physiological condition in such a way that, although variable for different cells, the potential response to light usually was higher than the one observed in cells directly taken from, and measured in APW. It has been reported that Ca^{++} ions drastically change the behaviour of the plasma membrane in acting as a K^+-diffusion barrier (4). There is evidence (5,6) that in the presence of Ca^{++}ions the membranes behave more as an H^+ diffusion barrier. The measurements were carried out at 20° in Ca-APW at pH = 6.

The membrane potential and changes thereof were measured by an electrode assembly essentially similar to that described in refs. 1 and 3. A glass capillary microelectrode was inserted into the vacuole of the cell at its midpoint and the reference electrode was in the external medium. This means that the potential responses discussed below are of the cell wall, plasmalemma and tonoplast in series. A small area of 8 mm in length of the central part of the cell was illuminated by a monochromatic light beam, transmitted by an interference fliter (band width 10-15 nm). A filter with maximal transmission around 670 nm was used for excitation of both pigment systems and around 703 nm for preferential excitation of system 1.

Relative efficiencies of quanta absorbed at 670 and 703 nm were calculated from the number of incident quanta, the approximate absorption of the cell at both wavelengths, and the rate of the potential change during the rising part of the potential response curve in the non-saturating 670- or 703 nm light. For each experiment the intensity of the incident actinic light is given, as it was measured by a calibrated thermopile. Usually young cells were used with an absorption of 0.5 - 0.6 at 670 nm. The absorption of the cell at 703 nm was estimated to be about 0.3 of the absorption at 670 nm.

RESULTS AND INTERPRETATIONS

Fig. 1 shows time courses of the light-induced changes in membrane potential occurring before and after the addition of 5 µM DCMU and 50 µM

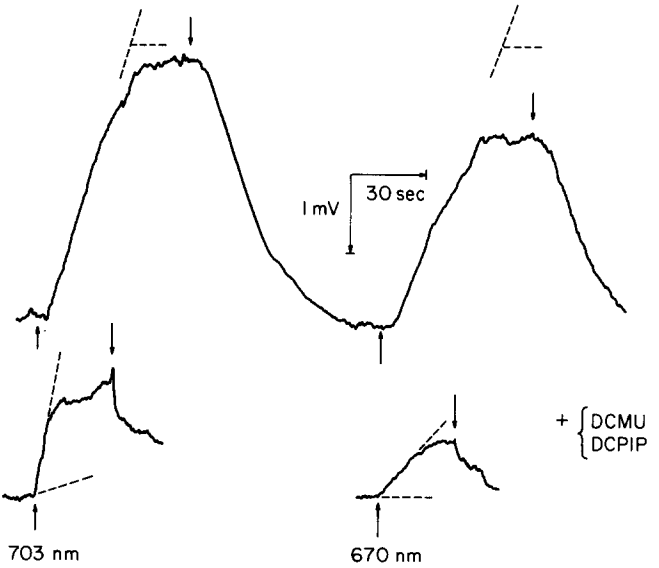

Figure 1. Kinetics of the changes in membrane potential in a single internodal cell of Nitella translucens, suspended in a Ca-APW with 1 mM ascorbate, in the absence (upper curves) and presence of 5 μM DCMU and 50 μM DCPIP (lower curves), occurring upon illumination with light of 703 and 670 nm (left and right hand curves, respectively). The intensities of 703 and 670 nm light were 3 and 0.6 nE/cm^{2}sec respectively. Upward and downward pointing arrows mark the beginning and the end of an illumination period. An upward moving curve means an increase in potential (depolarization). For comparison the slopes of the increases in potential in the light are indicated by the dotted lines drawn above, or in the curves. In the absence of DCMU and DCPIP the slopes and the number of absorbed quanta indicate that the efficiency of 703 nm-quanta for bringing about the potential changes is about 0.7 to 0.8 of the efficiency of 670 nm-quanta. In the presence of DCMU and DCPIP 703 nm-quanta are about 2.3 to 2.6 times as efficient as 670 nm-quanta. The membrane potential of the cell in the dark was -80 mV in the absence, and about -74 mV in the presence of DCMU and DCPIP.

DCPIP to the ascorbate containing Ca-APW (pH=6). The ascorbate (1 mM) was found not to affect the dark potential and the kinetics of the changes in the absence of DCMU and DCPIP. In the absence of DCPIP there was no light-induced change in potential in the presence of DCMU, as has been documented elsewhere (c.f. Fig. 1a in ref. 1). The figure shows amongst others that after the addition of DCMU and DCPIP the ratio of the rates of the potential changes in 703- and 670 nm light is increased by a factor of about 3.3. A calculation yields that in the presence of DCMU and DCPIP 703 nm quanta are 2.3-2.6 as efficient as 670 nm quanta. This suggests that the changes observed in the presence of DCPIP + ascorbate are associated with a photochemical reaction driven by pigment system 1. The

initial lag time upon onset of illumination, observed in the "physiological" system, is absent in DCMU and DCPIP-treated cells. The potential change in the light is completed within about 10 seconds. Upon darkening there is an initial short-lasting depolarizing spike followed by a relatively slow return of the membrane potential to the steady state dark potential. The spike can be seen more clearly in the expanded time recordings shown in Fig. 2b' and 2c'. The initial depolarization upon darkening took 0.25 sec for completion, as was observed by recording the phenomenon on an oscilloscope (not shown). In the recordings shown in Fig. 2b' and 2c' the rise time of the spike is a virtual one, due to the 1 sec response time of the recorder. The return phase of the spike is partly enhanced by illumination as can be seen from the left-hand recordings of Fig. 2b'. A reasonable explanation of this phenomenon cannot be given as yet. The

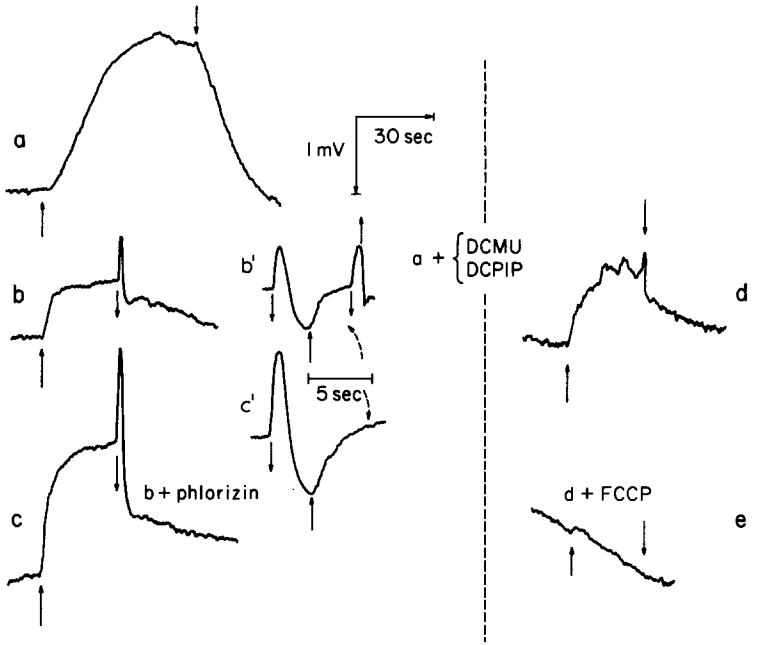

Figure 2. Kinetics of light-induced changes in membrane potential in single cells of Nitella translucens. (a) Cellsuspended in Ca-APW with 1 mM ascorbate, (b and b') same cell with 5 μM DCMU and 50 μM DCPIP, (c and c') same as b, after the addition of 2 mM phlorizin. The membrane potential in the dark of this cell was -100 (a), -90 (b) and -130 mV (c). (d) Another cell suspended in Ca-APW with 5 μM DCPIP and 1 mM ascorbate; (e) same as d, after the addition of 2 μM FCCP. The membrane potential in the dark of this cell was -80 (d) and -140 mV (e). The relatively large changes in the dark potential upon addition of phlorizin and FCCP took about 30 minutes for completion. The effect of the chemicals on the light response was observed after much shorter times, and was found to be independent of the change in the dark potential. Actinic light was of 703 nm of about 7 nE/cm^{2}sec intensity.

recordings of Fig. 2c and 2e, show that the energy transfer inhibitor phlorizin (2 mM) enhances the steady state potential changes in the presence of DCMU, DCPIP, and ascorbate, whereas the uncoupler FCCP (2 μM) is inhibitory.

Fig. 3 shows the results of experiments similar to those presented in Fig. 1, in the absence and presence of DCMU (5 μM) and PMS (10 μM). In the presence of PMS (+DCMU) light causes a decrease in the membrane potential (hyperpolarization), which in the dark reverses to the dark potential. The change takes about 30 sec for completion in the light. The high efficiency of 703 nm quanta relative to that of 670 nm quanta for

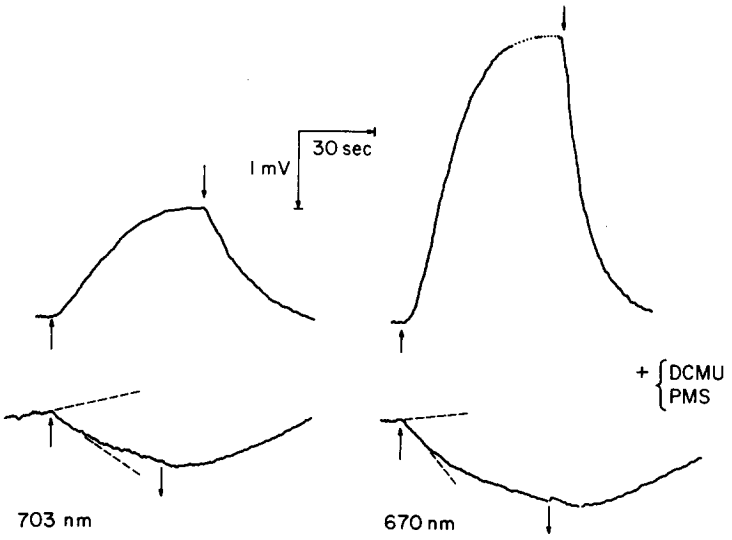

Figure 3. Kinetics of the changes in membrane potential in a single cell of Nitella translucens, suspended in Ca-APW, in the absence (upper curves) and presence of 5 μM DCMU and 10 μM PMS (lower curves). Left hand curves are for illumination with 703 nm light of 3 nE/cm^{2}sec intensity, right hand curves for 670 nm light of 4.5 nE/cm^{2}sec. The slopes of the curves and the number of absorbed quanta indicate that in the presence of DCMU and PMS 703 nm-quanta are about 3.2 times as efficient as 670 nm quanta for bringing about the membrane hyperpolarization. This value probably is too high, because the intensity of the 670 nm light was near to saturation. The dark potential of this cell was -105 mV in the absence and -95 mV in the presence of DCMU and PMS.

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causing the changes in the presence of PMS, as calculated from this and other experiments, suggests an association with a photochemical reaction driven by system 1. Fig. 4 shows that phlorizin (2 mM) and FCCP (1 μM) both inhibit the PMS-catalyzed potential changes. The relatively rapid dark decay of the potential as observed in the experiment of Fig. 4b was usually not observed. Usually the dark reversion, variable for different

cells, occurred with a half time varying from 5 to 30 sec.

In the presence of PMS with ascorbate it was found that light in addition to a decrease, also caused a phlorizin-insensitive increase in potential, with a same type of kinetics as those observed in the presence of reduced DCPIP. The small initial increase in potential upon onset of illumination (Fig. 4b, c and d) probably is a reflection of the opposing potential change with PMS. Also the light-off kinetics sometimes showed a further decrease in potential during the first seconds of darkness after illumination.

Therefore, it cannot be excluded that in the presence of PMS, as well as in the presence of DCPIP, the observed potential change is the resultant of two opposing changes, i.e., a depolarization, which is predominant with DCPIP, and a hyperpolarization, which is predominant in the presence of PMS.

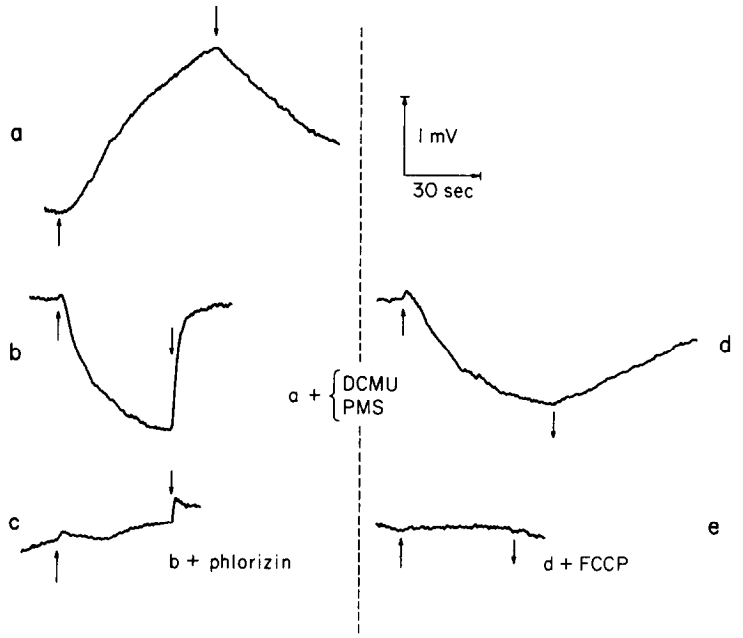

Figure 4. Kinetics of light-induced potential changes in single cells of Nitella translucens. (a) Cell suspended in Ca-APW, (b) same cell with 5 μM DCMU and 10 μM PMS, (c) same as b, after the addition of 2 mM phlorizin. (d) Another cell suspended in APW with 5 μM DCMU and 10 μM PMS, (e) same as d after the addition of 1 μM FCCP. Actinic light was of 703 nm of about 7 nE/cm2sec intensity. The original dark potential of the cells were -80 and -100 mV, respectively.

DISCUSSION

The present experiments show that changes in the electrical poten-

tial of cytoplasmic membranes occur in association with light-driven electron transfer in pigment system 1. Changes in membrane potential associated with electron transfer from H_2O to NADP, i.e., driven by both pigment systems working in series, have been described and interpreted before for Nitella (1), as well as for other plant cells (7). The difference in polarity of the system 1-driven changes observed with DCPIP (depolarization) and PMS (hyperpolarization), both in the presence of DCMU, suggests that different processes are involved. It is reasonable to assume that the changes observed in the presence of DCPIP + ascorbate in DCMU-inhibited cells are mainly associated with non-cyclic electron transport from reduced DCPIP to NADP. Reduced DCPIP has been shown to be a suitable electron donor for electron transfer in system 1 in DCMU-poisoned chloroplasts (8). There is also evidence that at least at low concentrations, it acts as a cofactor for cyclic electron transport (c.f. ref. 9). If our previously proposed hypothesis holds, that the potential changes are associated with electron transport-coupled proton uptake by the chloroplasts, it can be concluded that in the presence of DCMU and DCPIP with ascorbate, proton uptake takes place. Proposed model schemes on the coupling of proton movement to photosynthetic electron transport in isolated chloroplasts emphasize the existence of a coupling site near the reaction center of photosystem 1 with the primary reductant and oxidant at opposite locations in the thylakoid membrane (10). The observation that the light-induced depolarization in the presence of DCMU and DCPIP is at best about half of the change observed in untreated cells also is consistent with these schemes. The inhibition of the potential changes by FCCP can be understood in terms of the inhibition of the proton gradient across the thylakoid membrane, due to increased proton permeability. Two alternative explanations are possible for the stimulatory effect of phlorizin on the potential changes associated with electron transport in the presence of DCPIP: a. Inhibition of the potential change that is associated with DCPIP-catalyzed cyclic electron transport, under the assumption that the potential changes associated with cyclic electron transport, are of opposite sign as those associated with non-cyclic electron transport. The results with PMS, discussed below, give evidence for this assumption. b. Increase in membrane depolarization as a consequence of an increased proton gradient due to inhibition of concurrent ATP formation by phlorizin. A similar reasoning has been forwarded (11, 12) to explain the stimulatory effect of Dio-9 and phlorizin on CO_2 photoreduction during inhibition of light-induced kation uptake in intact algal cells. Phlorizin in acting as an energy transfer inhi-

bitor in the final sequence of ATP formation, is known to lower the ATP level in intact cells (13) and therefore to inhibit ATP requiring light-dependent cellular processes (c.f. ref. 10). The potential change observed in the presence of PMS suggests an association with a cyclic electron transport in pigment system 1. PMS has been shown to stimulate cyclic electron transport in intact cells of Anacystis nidulans and Schizothrix (14). The inhibitory effect of phlorizin would suggest that the potential changes are associated with, or are reflections of a process that is closely linked to phosphorylation coupled electron transfer. As such an involvement of ATP-requiring ion movements across the cytoplasmic membranes is possible. Evidence has been presented by several investigators for various species (c.f. refs. 15-17), that the light-stimulated active movement of alkali ions across the cytoplasmic membranes proceeds at the expence of energy delivered by ATP.

Our results are another demonstration of the fact that energy dependent cellular processes, notably those which result in changes in ion fluxes across the cytoplasmic membranes (17), are dependent either on a high energy intermediate, e.g., proton gradient, or on phosphorylated compounds such as ATP. Formation of both is coupled to photosynthetic electron transport in the photosynthetic apparatus in the chloroplasts.

The author is greatly indebted to Dr. C. Sybesma for the use of his facilities and for useful discussions. The work was made possible by a National Science Foundation grant 15777.

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