LIGHT-INDUCED CHANGES IN MEMBRANE POTENTIAL OF ALGAL CELLS ASSOCIATED WITH PHOTOSYNTHETIC ELECTRON TRANSPORT

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Summary

The negative membrane potential of Nitella translucens and Nitellopsis obtusa increases reversibly upon illumination. Relative efficiencies of 430-, 676-, 690- and 705-nm light quanta indicate that the changes are caused by light energy conversion in the photosynthetic apparatus. DCMU (1 μM) and CCCP (2 μM) inhibit the light effect. Lower concentrations of CCCP ($\geq$ 0.2 μM) cause an increase in the rate of the dark recovery of the membrane potential after illumination. The results are discussed in terms of a proposed model, in which changes in H^+ concentration in the cytoplasmic chloroplast layer, which are coupled with photosynthetic electron transport, are considered.

It has been reported that the membrane potential of giant algal cells responds to light (1-3), but so far only fragmentary evidence has been given, that the action of light is primarily due to energy conversion in the photosynthetic apparatus. A linkage between changes in the potential of the cellular membranes and photosynthetic energy conversion in these algal cells, would suggest that the cytoplasmic chloroplasts act as current sinks or sources in the light-activated intact cell. Recent results, obtained with chloroplasts *in vitro*, have evidenced (4,5), that ionic movements occur at the phase boundaries, which would not exclude a possible role for chloroplasts *in vivo*, in acting as current sources indeed.

This communication gives results on potential measurements with Nitella translucens and Nitellopsis obtusa. The effect of light of different wavelengths, in the presence and absence of chemical agents interacting with the photosynthetic electron transport chain, has been studied.

MATERIALS AND METHODS

Nitellopsis obtusa was collected from a local fresh water pool. A

batch of cells of Nitella translucens was kindly given by Dr. E.J. Williams, University of Edinburgh. The cells were kept in the laboratory, in shadowed northern daylight, in a standard artificial pond water (APW), containing 1.0 mM KCl, 0.1 mM N_aCl and 0.1 mM $CaCl_2$.

The experiments usually were done with one single cell of 8 - 12 cm length and a diameter of about 1 mm. It was controlled by microscopic observation that the selected cell(s) showed rapid protoplasmic streaming. The cell was fixed along its axis in a transparant lucite experimental chamber, filled with APW. The membrane potential was measured using a 3 M KCl-filled glass capillary microelectrode, inserted transversely into the cell, in contact with an Ag/AgCl wire via an agar-KCl bridge, and an identical Ag/AgCl wire in contact with the external medium. The microelectrode was selected such, that with both electrodes in the external medium, the potential difference was less than 5 mV. The high impedance source signal of the electrode system was fed into a unity gain solid state amplifier. The output of the amplifier was connected to a millivoltmeter or to an oscilloscope. In the experiments reported here the potential was recorded on a recorder, connected to the output of the millivoltmeter. Under these conditions the response time of the system was about 0.75 sec, and changes in membrane potential of about 300 μV could be detected with a signal to noise ratio of 10. The micro electrode usually was inserted at the middle of the cell. The tip of the electrode was in the central vacuole, which means that the recorded responses are of a complex series membrane system, composed of cell wall, plasmalemma and tonoplast. The cell was illuminated by a homogeneous monochromatic light beam from a modified 24 V DC, 250 W slide projector. The wavelength of monochromatic illumination was selected by placing interference filters (band width 10-15 nm) in the light beam. Light intensities were measured by a calibrated thermopile, and are given in nEinsteins/cm^2.sec. The maximal length of the illuminated area of the cell was 4 cm. The experiments were carried out at room temperature.

RESULTS AND INTERPRETATION

Figure 1,a shows the kinetics of the changes in membrane potential occurring upon illumination and upon darkening of a single Nitella cell. The increase in potential starts 1-2 seconds after the light is turned on. The lag time is shorter when the intensity of the actinic light is higher. Sometimes a small initial decrease in potential was observed during this lag time. Also after shutting off the light there is a short lag time (1-2 sec), before the potential reverses to the

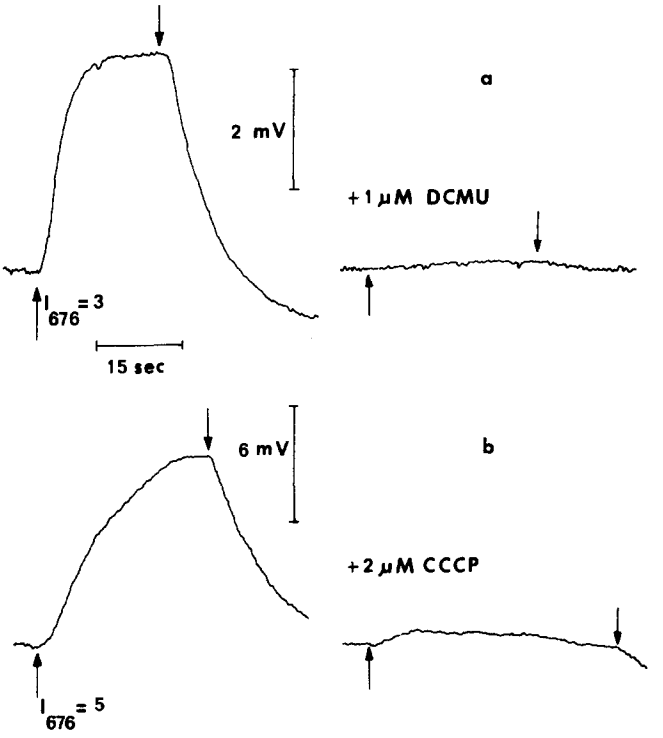

Figure 1. Kinetics of the light-induced changes in membrane potential in two different single internodal cells of Nitella translucens, in the absence and presence of 1 μM DCMU (a), and of 2 μM CCCP (carbonyl-cyanide m-chlorophenylhydrazone) (b). The membrane potential in the dark of the two cells was -130 mV (a) and -140 mV (b), respectively. In the presence of DCMU the dark potential was 2 mV more negative than in the absence, CCCP caused a negative shift of the potential in the dark of about 12 mV. Upward and downward pointing arrows mark the beginning and the end, respectively of an illumination period. The intensity (I) of the light (676 nm) is given by the numbers ($nE/cm^2 \cdot sec$). An upward deflection means an increase in potential.

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original dark level. The rate of the dark reversion of the potential was found to be variable for different cells of the same batch, with half times ranging from 6 to 30 sec. The kinetics of the changes were the same for light of different wavelengths, e.g. 430, 660, 676 and 705 nm, for each individual cell. The rate and the extent of the change in potential were measured at several intensities of actinic light of 676 nm, which is the maximum of the absorption band of the chlorophyll a. The rate of the change in potential in the light, i.e. the slope of the curve, 3-4 sec after the beginning of the illumination period, was found to be linear dependent on intensity, up to intensities of about 1.0 $nE/cm^2 \cdot sec$. Although variable for different cells,

the increase in potential at saturating intensities (7-10 nE/cm^{2}sec) was about 10 to 15 mV.

Action of light of different wavelengths. The relative efficiencies of quanta absorbed at 430, 663, 676, 690, and 705 nm to bring about a change in membrane potential in Nitella translucens were found to be 0.43, 1.0, 0.75, 0.85 and 0.88, respectively. This indicates that the potential changes are caused by, or associated with a photophysical or photochemical reaction in the chloroplasts, located in the cytoplasm.

Effect of chemical agents. Addition of 1 μM DCMU in the dark causes a very small, and probably insignificant change in membrane potential. The light-induced change in potential is totally inhibited (Fig. 1,a). The same observation was made with Nitellopsis. In the presence of 2 μM CCCP, the membrane potential in Nitella in the dark was about 20 mV more negative than it was in the absence of the uncoupler, and the light-induced change in potential was completely inhibited (Fig. 1,b). In Nitellopsis inhibition occurred at concentrations above 5 μM. Lower concentrations of the antibiotic (0.3 and 2 μM in Nitella and Nitellopsis, respectively) caused, that the rate of the dark recovery of the potential after illumination was substantially increased (Fig. 2). Dio-9 (5 μg/ml) neither effected the membrane potential in the dark, nor caused a measurable effect on the kinetics of the changes in membrane potential upon illumination and darkening.

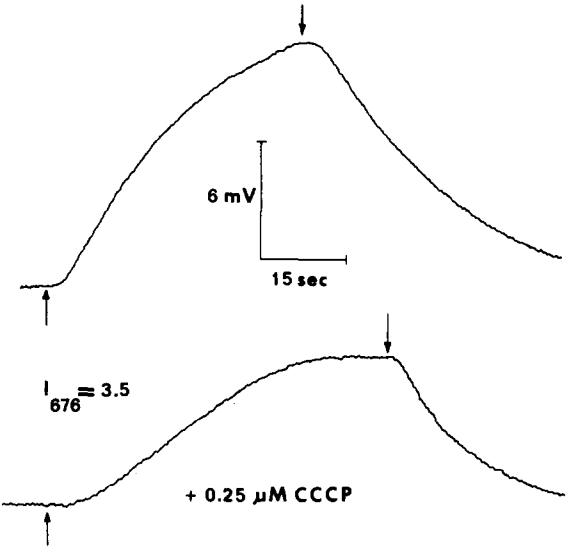

Figure 2. Light and dark kinetics of potential changes in a single Nitella cell in the absence and presence of 0.25 μM CCCP. The dark potential of this cell was -148 mV in the absence, and -154 mV in the presence of CCCP. Further explanations are in the legend of Fig. 1.

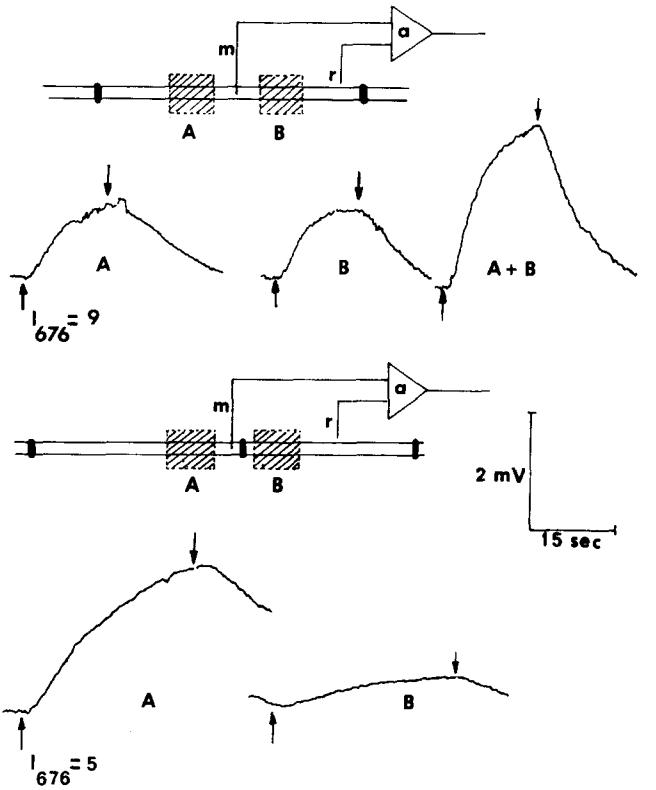

Figure 3. Upper part. Response of the membrane potential (-130 mV) of a single Nitella cell to illuminations at the areas A or B, each of 1 x 1 cm, at a distance of about 1 cm of the microelectrode m. Illumination of both areas A+B causes the response shown in the upper right hand trace. r is the reference electrode, a is the amplifier. Lower part. Response of the membrane potential (-147 mV) of a Nitella cell to an illumination at the area A, or at the area B in the neighbouring intact cell. Black partitions mark the nodes of the cell(s). Further explanations are in the legend of Fig. 1.

Figure 3 shows that simultaneous illumination of different parts of one single cell causes an additive effect on the change in potential, illumination of an adjoining cell causes a considerable lower change in the potential than the change which occurs when the measuring cell is illuminated with the same intensity.

DISCUSSION

The experiments reported here allow the definite conclusion, that photosynthetic energy conversion in the chloroplasts of giant vacuolar algal cells, like Nitellopsis and Nitella, causes a depolarisation of the cellular membranes. The fact that quanta absorbed at 676, 690, and

705 nm are about equally efficient, and with respect to quanta absorbed at 663 nm only slightly less efficient, in causing the membrane depolarisation, suggests that both photochemical systems (6) are involved in the reaction that gives rise to the observed changes. This suggestion is consistent with the fact that DCMU and CCCP both inhibit the light effect. It has been reported (7), that both chemicals at the concentrations used are effective inhibitors of CO_2 photoreduction in Nitella. The absence of the light effect in the presence of DCMU suggests that the reaction which is responsible for the potential change, is not primarily fed by ATP, generated by cyclic photophosphorylation. It has been shown (8,9), that the ATP-requiring light-induced transport of alkali ions still occurs in the presence of DCMU. The insensitivity to Dio-9 is in accord with this conclusion, however it cannot be taken for sure that the concentration of Dio-9 we used, was sufficiently high to be effective in inhibiting ATP formation in these algal cells (10). The question remains how the change in membrane potential is linked with the photosynthetic light reactions in the chloroplasts. The fact that a local illumination of a small part of the cell causes a change in potential of the whole membrane, indicates that current flows across the membranes towards the activated part of the cell, i.e. the illuminated area of fixed chloroplasts in the cytoplasm. It has been shown (11,12), that upon local current injection these cylindrical algal cells behave as a current-carrying coaxial cable, causing a change in potential of the membrane, due to a spreading of current along the cell axis with leakage across the envelopping membranes. In analogy with this, the illuminated chloroplasts in the cytoplasm are suggested to act as current sinks, or sources. It appears that there is a small current leakage from the illuminated cell towards the neighbouring cell (Fig. 3). At present no experimental evidence is available, which current carrying ions are involved in this proposed model. Current knowledge on light-induced H^+-uptake in isolated chloroplast (13) would predict, that illumination of chloroplasts *in vivo* results in a decrease in H^+-concentration in the cytoplasmic layer surrounding the activated chloroplasts. This would cause a flow of H^+-ions from the vacuole and the external medium towards the cytoplasm. The sign of the potential change is consistent with the conclusion, that in this model system the illuminated chloroplasts act as a current source, i.e. H^+-sink, indeed, with the major component of current across the tonoplast, i.e. H^+-flow from vacuole to cytoplasm. Preliminary experiments have indicated, that

also light-induced current flow from cytoplasm to external medium across the plasmalemma and the cell wall occurs (to be published). Consistent with the hypothesis that the light-induced changes in membrane potential are due to electron transport-coupled H^+-flow, is the observation (Fig. 2) that in the presence of low concentrations of CCCP, the dark recovery of the potential change after illumination is enhanced. The H^+-gradient, build up in the light across the chloroplast membrane, will reverse in the dark more rapidly in the presence of CCCP, due to increased H^+-permeability of the membrane (14), resulting in a faster re-equilibration of H^+-concentration in the cytoplasm and consequently in a faster decay of current, i.e. potential across the cellular membranes. Further experiments are in progress to establish,whether or not these potential changes affect the reactivity of chloroplast-bound constituents, participating in the photosynthetic electron transport chain. It has been suggested (15,16), that the emission yield of chlorophyll a_2, i.e. the quenching ability of the component Q (Q') of the reaction center 2, is dependent on changes in ionic concentration(s) in the cytoplasm.

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