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PHOTOSYNTHETIC ENERGY CONTROL OF AN ELECTROGENIC ION PUMP AT THE PLASMALEMMA OF *NITELLA TRANSLUCENS*

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SUMMARY

1. The kinetics of the light-induced changes in the potential across, and the resistance of the plasmalemma of *Nitella translucens*, as well as the changes in the membrane current–voltage (*I–V*) characteristics have been studied under a variety of energy conditions.

2. The so-called dark energy state of the cell, which is likely to be dependent on the concentration of as yet unidentified energy products of the photosynthetic (and respiratory) reactions, appears to determine the activity of an electrogenic ion pump, *i.e.* the membrane potential and resistance.

3. The energetic linkage of photosynthetic energy conversion with transport determining membrane parameters is shown to be triggered by at least two different processes. (i) A short-term light effect, completed within 0.5 to 2 min, primarily causes a decrease in the membrane resistance. This decrease causes the potential generated by an electrogenic pump to be decreased (membrane depolarization). (ii) A long-term light effect, completed within 2 to 20 min, causes a stimulation in the pumping rate of an electrogenic pump, due to which the membrane becomes hyperpolarized with a concomitant change in its differential resistance.

4. The results are discussed in terms of an electrical equivalent circuit for the plasmalemma as proposed by Spanswick (Spanswick, R. M. (1972) *Biochim. Biophys. Acta* 288, 73–89).

INTRODUCTION

In a recent publication from this laboratory[1] a method has been described which enables a simultaneous recording of the electrical potential across, and the resistance of plant cell membranes, as well as energy-dependent changes occurring therein. The kinetics of light-induced changes in the potential and resistance of the plasmalemma of *Nitella translucens*, which are nearly identical to each other in dark-adapted cells for short illumination periods, suggested that the observed initial membrane depolarization is due, at least for the main part, to a decrease in the potential generated by an electrogenic ion pump. This decrease occurs in response

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCMU, 3,4-dichlorophenyl-*N,N*-dimethylurea; DCIP, 2,6-dichlorophenolindophenol.

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to a decrease in the membrane resistance occurring in association with photosynthetic energy conversion. The pumping rate of the electrogenic pump was assumed to be constant in the short illumination period, during which the potential and resistance followed identical kinetics. It has been calculated that under the conditions used (*i.e.* cells adapted to darkness) the electrogenic pump activates a membrane current of about 1 to 3 pmoles/cm^2 per s. It was found that low concentrations of the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) inhibited the current generated by the pump in the dark. In preilluminated cells the kinetics of potential and resistance changes have been found to be completely different in *Nitella*[1,2], as well as in other characean cells[3,4]. Prolonged illumination of a *Nitella* cell caused an initial depolarization followed by a hyperpolarization, and a change in the resistance, the latter *via* a rather complicated kinetic pattern[1].

The present paper describes the results of experiments, which indicate, in addition to the relatively fast light-triggered reaction which causes a decrease in the membrane resistance, a second relatively slow light-dependent reaction, which causes a stimulation of the electrogenic ion pump at the plasmalemma. The photosynthetic control of the ion pump causes a hyperpolarization of the plasmalemma and consequently a change in its differential resistance.

The so-called energy state of the cell which is determined by products of photosynthetic energy conversion processes in the cell, appears to determine the activity of the electrogenic pump in the dark.

A current-scanning technique for measuring the current–voltage (I–V) characteristics of the plasmalemma is described, and essentials of comparative analyses of I–V curves, characteristic for the energy states of the cell, are outlined.

MATERIALS AND METHODS

Nitella cells were collected locally and maintained in the laboratory at 15 °C, as described previously[1]. Cells, usually of about 5 cm length and 0.06 cm diameter, were presoaked before each experiment in 1 mM NaCl, 0.1 mM KCl, 0.1 or 1.0 mM $CaCl_2$ and 0.1 mM $KHCO_3$ during 2 days with exposure to alternating light and dark periods of 12 h each. The medium (50 ml) in the measuring cuvette was kept continuously circulating along the length of the cell and aerated. The mixing time for added chemicals was about 1 min. In some cases the medium was withdrawn from the cuvette and replaced by a substituted medium. This procedure also could be completed within 1 min. Experiments were carried out at room temperature, routinely at a pH of 6.9.

A cell was illuminated perpendicularly over its total length by a homogeneous monochromatic light beam (wavelength band around 676 nm), from a modified lamphouse assembly of a 24-V, 250-W light projector. Light intensities were measured with an YSI Radiometer, Model 65.

Changes in the membrane potential, probed by the intracellular capillary electrode (a), with reference to the external electrode (b), were recorded on channel 1 of a multi-channel ultraviolet recorder (Fig. 1). Changes in the membrane resistance were recorded simultaneously on channel 2 of the recorder. For these recordings the switch S was in position 1. The signal on channel 2 was proportional to the amplitude of the potential modulations caused by square current pulses of 400 ms duration

and a constant low amplitude of 0.01 μA (ref. 1). The current pulses pass across the tonoplast, plasmalemma and cell wall in series through the internal current electrode (c) towards an external Ag wire (d). The distance between electrodes a and c was $0.42L$, in which L is the half length of the cell[5]. It has been shown[1,6] that the changes in potential and resistance observed are mainly, if not exclusively due to changes occurring at the plasmalemma. The pH of the medium, measured by a glass electrode e, was recorded on channel 3 of the recorder.

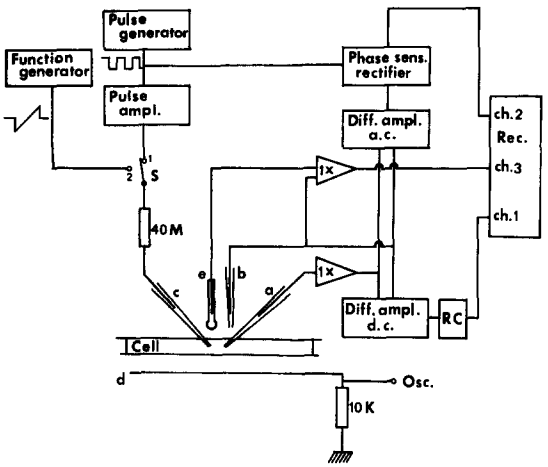

Fig. 1. Schematic diagram for the experimental arrangements for measuring the kinetics of energy dependent changes in the electrical potential and resistance of plant cell membranes (switch S position 1), and of the membrane current–voltage characteristics (S position 2). The potential is recorded on channel 1 (ch. 1) of the recorder, with a response time of about 1.5 s, selected by the RC circuit in the potential measuring part of the system. The resistance is recorded on channel 2 (ch. 2), with a response time of about 3 s. The recorder deflection on channel 2 is linearly proportional to resistance values of up to 120 k$\Omega \cdot$cm^2 (ref. 1). The pH of the external medium, probed by electrode (e), is recorded on channel 3 (ch. 3). Further explanations are in the text.

The current–voltage (I–V) characteristics of the plasmalemma were recorded by means of a rapid current-scanning technique, basically similar to that used by Coster[7]. The cell current circuit was connected to the output of a function generator by switching S in position 2 (Fig. 1). The function generator generates a voltage that changes with time at a uniform rate in a single sweep. With the 40-MΩ resistor in the current circuit, the current $i(t)$ $(=i_0+\alpha t)$ passing across the membranes changes from $i_0=0.34$ (inward current) to $+0.34$ μA (outward current) at a rate of 5.6 nA/s (2-min sweep) or, when faster recording was required, of 22.6 nA/s (30-s sweep). In some cases the 40-MΩ resistor was replaced by a 20-MΩ resistor, in order to increase the interval of the current sweep by a factor of 2. A simple calculation yields that for each time t' during the current sweep, with $t' > RC$, the voltage $V(t')$ across the membrane deviates from $V(i)$, with $i=i(t')=$constant, by an amount of $\alpha R^2 C$. R and C are the resistance and capacitance, respectively of the membrane. When R and C are assumed to be 200 k$\Omega \cdot$cm^2 and 1 μF/cm^2, respectively, this deviation amounts about 0.2 for α equal to 5. 6 and 0.8 mV for α equal to 22.5 nA/s. These deviations are small compared to the potential shifts caused by applied and electrogenic membrane currents. At the low K^+ concentration (0.2 mM) of the medium used, the

hyperpolarizing voltage responses upon inward currents were observed to be monophasic in the light as well as in the dark. A biphasic response was observed, consistent with observations of Kishimoto[8] and Spanswick[9], at concentrations above 0.4 mM. A distorting effect of such a response upon the resistance and *I–V* curves, as measured by our method, thus is absent at the K^+ concentration that was used.

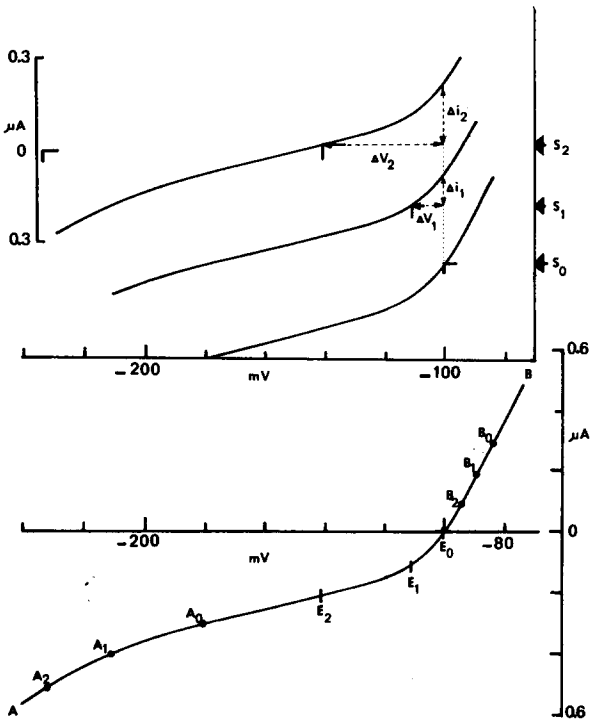

Fig. 2. Lower part: Hypothetical membrane *I–V* Curve AB, measured over an extended current interval −0.6 to +0.6 μA. A_0B_0, A_1B_1 and A_2B_2 are parts of the *I–V* curve, measured in a current interval −0.3 to +0.3 μA in the absence and presence of constant inward currents i_1 (0.1 μA) and i_2 (0.2 μA), respectively. E_0, E_1 and E_2 are the respective dark resting potentials in the absence and presence of these clamping currents, respectively. Upper part: Curves A_0B_0, A_1B_1 and A_2B_2, characteristic for the states S_0, S_1, S_2, plotted at equal vertical distances, after translation along a constant voltage coordinate from Curve AB. Further explanations are in the text.

Fig. 2 illustrate the essentials of the graphic method applied to compare the *I–V* curves measured in the dark. Curve AB (bottom part of the figure) is a hypothetical membrane *I–V* curve of a cell in a stationary condition or energy state S_0, measured over an extended current interval from −0.6 to +0.6 μA. Part A_0B_0 is the *I–V* curve of S_0, measured over the current interval −0.3 to +0.3 μA. The *I–V* coordinates of A_0, B_0 and the resting potential E_0 of S_0 are (−0.3, −180) (0.3, −85) and (0, −100), respectively. Let S_1 and S_2 be the states of the cell under a constant current clamp of 0.1 and 0.2 μA, respectively, and assume that the clamping currents do not cause a chemical or physical change in the membrane and in the cell

interior. Parts A_1B_1 and A_2B_2 are the *I–V* curves of states S_1 and S_2, respectively, measured over the current interval -0.3 to $+0.3$ μA, superimposed on the clamping currents 0.1 and 0.2 μA, respectively. E_1 (-0.1, -110) and E_2 (-0.2, -140) are the resting potentials of the cell in S_1 and S_2, respectively. The *I–V* curves A_0B_0, A_1B_1 and A_2B_2, which are characteristic for the states S_0, S_1 and S_2, respectively, have been plotted at equal vertical distances in the upper part of the figure. The plotting amounts to a linear translation of each of the curves from the main curve AB along a vertical line (constant voltage coordinate). The intercept of the small vertical and horizontal bars in each curve mark the point at which the "measuring" current is zero. The clamping currents in S_1 and S_2 of course remain at their values, and determine the coordinate of the respective resting potentials on the potential axis. Although different in shape around the resting potentials, the parts of the curves that fall within the same potential region are exactly parallel and, when translated vertically, will cover each other and constitute an extended *I–V* curve A_2B_0 of the membrane. As indicated by the dotted vertical and horizontal lines, the S_1 and S_2 states of the cell can be characterized, with respect to S_0, by the clamping membrane currents Δi_1 (0.1 μA) and Δi_2 (0.2 μA), and associated differences in the resting potential ΔV_1 (10 mV) and ΔV_2 (40 mV), respectively.

The *I–V* curves of *Nitella* cells, measured in the dark under a variety of experimental conditions (*i.e.* after preillumination, changes in pH (ref. 10) or after the addition of uncouplers like CCCP) were found to be similar to those characteristic for the S_0 and the current-clamped S_1 and S_2 states (Fig. 2). Thus these curves could be reproduced as was done in the example of the different S states in Fig. 2. Each curve, measured in a sequence of treatments, was reproduced at the appropriate coordinate on the potential axis, after a translation along the *I* and *V* axis, such that common parts of the various curves were completely, or almost completely, in parallel. As in the case discussed above (Fig. 2), the different *I–V* curves can be interpreted as being representative for conditionally induced states, which are characterized, with reference to a control state, by intrinsic ("clamping") membrane currents Δi_p, and associated difference in the resting potential ΔV. Usually the control state of a cell (S_0) is the one characterized by the lowest resting potential.

The following general remarks with respect to the method and the interpretations of the results can now be made. (i) In most cases it is observed that for a cell in a characterised hyperpolarised state, the membrane resting potential deduced from the graphic analysis outlined above, is more negative than the resting potential actually measured. The difference is presumably caused by the fact that the increase in the electrogenic current Δi_p, which causes (is associated with) the transition into the hyperpolarized stage, has caused a change in the passive membrane diffusion potential. (ii) One has to be aware of the fact that changes in the differential resistance, measured by any method, occurring during the transition of the cell into a higher energy (hyperpolarized) state need not to be due exclusively to changes caused by a direct interaction of photosynthetic energy products, or intermediates, with membrane constituents. According to the changes in the *I–V* curves, these changes are likely to be associated with changes in the pumping rate of an intrinsic electrogenic membrane pump.

RESULTS AND INTERPRETATIONS

Effect of preillumination of the cell on the membrane I–V characteristics

The *I–V* characteristics of the cell membrane have been measured in the dark after successive illumination periods. The results of a typical experiment are shown in Fig. 3. The condition, or energy state, of a cell, which is found to be dependent on the occurrences in the foregoing light and dark periods is designated by numbered S symbols. S_0 is the so-called low-energy state of the cell, which usually is observed after a long adaptive dark period. The *I–V* curve representative for state S_0 is the lower curve of Fig. 3. According to this curve, the resting potential in S_0 is -134 mV and the membrane resistance is about 100 $k\Omega \cdot cm^2$. The dashed line, marked 1, indicates the slope of the *I–V* curve at the resting potential in the light, as deduced

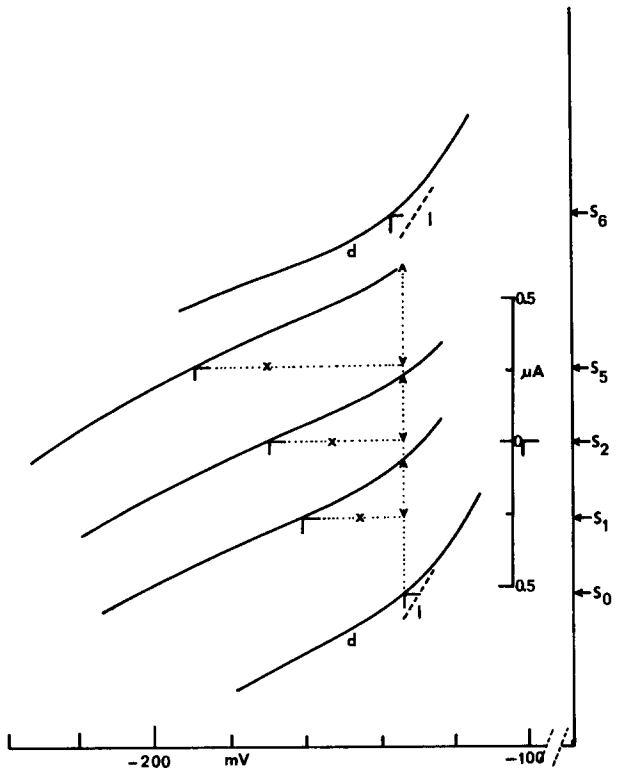

Fig. 3. *I–V* membrane curves of a *Nitella* cell, measured in the dark, after dark adaptation (S_0), a first (S_1), second (S_2) and fifth (S_5) illumination period of several minutes. (Pre)illumination periods were interrupted by dark periods, during which the *I–V* curves were measured in a 120-s sweep. The dark time between the measurements of the curves of S_5 and S_6 was about 60 min. The broken lines in the curves of S_0 and S_6, marked 1, are the slopes of the *I–V* curves at the rest potential in the light, measured 2 min after the onset of illumination. The dotted vertical and horizontal lines in the curves of S_1, S_2 and S_5 mark the membrane current and membrane hyperpolarization, associated with the transition of the cell into the respective energy states S_1, S_2 and S_5. The ×-marks on the horizontal dotted lines indicate the membrane potential actually measured in the respective energy states. Intensity of (pre)illumination (wavelength 676 nm) was approx. 2 nEinsteins $\cdot cm^{-2} \cdot s^{-1}$.

from the steady state changes in potential (ΔV=4 mV) and resistance (ΔR=46 k$\Omega \cdot cm^2$) after 1 to 1.5 min of illumination of the cell in state S_0. The state of the cell in the dark, after it has been illuminated with 676 nm light (intensity about 2 nEinsteins/cm^2 per s) during 7.5 min has been denoted as S_1. The 7.5-min illumination period in this particular case was interrupted after a first period of 3 min by a dark period of 19 min. The state of the cell at the end of this dark period has been defined as S'_0. S_1 is characterized by a dark resistance of about 172 k$\Omega \cdot cm^2$ (at the resting potential), a more negative membrane potential (−146 mV, indicated by the ×-mark on the horizontal dotted line in the figure), and by an appreciable shift of the *I–V* curve with respect to the one of S_0. According to the method outlined in the section Materials and Methods, the *I–V* curve representative for state S_1 is parallel to that of S_0 when the resting potential in S_1 is assumed to be −161 mV. The *I–V* curves, measured in the dark 2 min after a second (S_2) and fifth (S_5) (pre)illumination period of 3 and 2.5 min, respectively, are also shown. The ×-marks on the dotted horizontal lines coincide with the resting potential actually measured. The preillumination which induced the transformation of the cell into S_5 was started when the cell was in a dark state, similar to S_2. Each hyperpolarized state (*e.g.* S_1, S_2 and S_5) was found to be more or less stationary during a few minutes in the dark after an illumination period, after which it transferred slowly to the original dark state. This transformation is reflected by a slow membrane depolarization in the dark, at a rate of about 1 mV/min, and a change in the resistance of the membrane. Thus state S_6 (Fig. 3) was reached, after the cell in state S_5 was kept in the dark during about 1 h. S_6 appears to be approximately similar to S_0, as can be concluded from a comparison of the *I–V* curves in the dark (d) and the light (l), characteristic for both states.

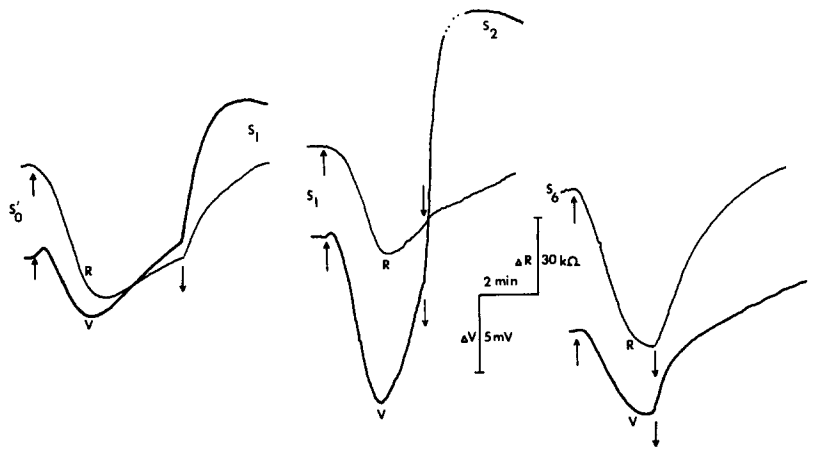

Fig. 4. Kinetics of the changes in potential (*V*) and resistance (*R*) upon illumination and darkening of a *Nitella* cell in the different energy states S'_0, S_1 and S_6 (Fig. 3). Upward and downward pointing arrows mark the beginning and end, respectively, of the illumination periods. Downward movements of the respective curves mean a membrane depolarization (*V*), or a decrease in the resistance (*R*). S'_0 is the energy state of the cell which, starting from S_0, was induced after an illumination period of 3 min, and a dark period of 19 min. Zero setting on channel 1 of the recorder was changed in the dark periods between the measurements of the curves. The resistance trace in the middle part of the figure is somewhat distorted, due to the fact that the dark resistance in this case (state S_1, *e.g.* Fig. 3) was 172 k$\Omega \cdot cm^2$, which is above the threshold of linearity of the resistance measuring system[1]. Further data are in the legend of Fig. 3.

The light and dark kinetics of the potential and resistance changes, measured in states S'_0, S_1 and S_6 of the cell, are shown in Fig. 4. Illumination of the cell in states S'_0 and S_1 has induced its transformation into state S_1 and S_2, respectively. The states S_1, S_2 and S_6 refer to the *I–V* curves of Fig. 3. It has been suggested[1] that the light-induced change in potential (depolarization) which in an S_0 or comparable energy state (*e.g.* S_6, Fig. 3) occurs with kinetics nearly identical to those of the resistance decrease, is due to a decrease in the potential generated by an electrogenic pump. The current, i_p, generated by this pump can be calculated from the ratio between the steady-state differences (light *minus* dark) of the potential (ΔV) and resistance (ΔR), respectively, in short illumination periods[1]. The current activated by the pump in the S_0 and S_6 state of this cell (Figs 3 and 4) is about 0.90 and 1.24 pmoles/cm^2 per s, respectively. As discussed in Materials and Methods, the different *I–V* curves of Fig. 3 can easily be interpreted in terms of differences (changes) in an active membrane current, Δi_p, generated by an electrogenic pump operating in the dark. Application of the described method shows that the current generated in the states S_1, S_2 and S_5, with respect to the one generated in the cell in state S_0, has increased with respective amounts of 0.19, 0.22 and 0.34 μA/cm^2, or 2.0, 2.3 and 3.5 pmoles/cm^2 per s.

The operative electrogenic pump thus apparently is light-dependent, *i.e.* stimulated by an energy product formed by a reaction during (successive) illumination periods, or in prolonged illumination. In the dark the energy stored in the light apparently is slowly consumed by the pump and/or other energy-dependent reactions, causing a slow decrease in the current generated by the pump.

I–V characteristics of the membrane in light and darkness

In Fig. 5 (bottom part) the *I–V* characteristics measured in the dark (full line, d) and in the light (dashed line, l), of the plasmalemma of a cell in an S_0 state (*i.e.* a cell kept in the dark for more than 30 min) are shown. The dashed curve was

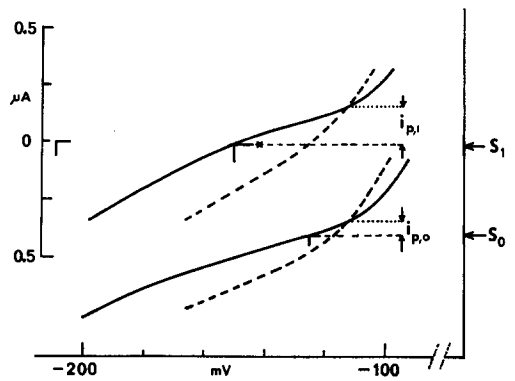

Fig. 5. *I–V* curves, measured in the dark (solid lines) and, 2 min after the beginning of the illumination period, in the light (dashed lines) of a *Nitella* cell. The lower curves were measured after the cell was kept in the dark for more than 30 min (S_0), the upper ones (S_1) after it was pre-illuminated for a few minutes with 676-nm light of an intensity of about 5 nEinsteins·cm^{-2}·s^{-1}. The upper solid curve has been reproduced according to the procedure, discussed in the section Materials and Methods, such that it is parallel, except in the far-hyperpolarized region, to the one of S_0. The ×-mark on the horizontal line marks the resting potential actually measured in S_1. Further details are in the text.

measured 2 min after the onset of illumination. It has been shown[1] (see also Fig. 4 in this paper) that in a dark-adapted cell the potential and resistance after 2 min of illumination both reach a quasi-steady state, *i.e.* a more depolarized potential and a decreased resistance. The figure shows that the shape of the *I–V* curve in the light is different from that measured in the dark. The light-induced change was found to be reversible provided that the illumination period did not exceed 2 to 3 min (see below). It can be concluded that at each potential, except in the extreme hyperpolarized region, the differential membrane resistance (which is the inverse of the slope of the *I–V* curve at the point given by the potential coordinate) is substantially decreased in the light. Thus, according to the curves of the S_0 state of this cell, the differential resistance at the resting potential in S_0 has been changed in the light from about 240 to 92 $k\Omega \cdot cm^2$. The resting potential has been changed from -125 to -117 mV. The *I–V* characteristics in the dark and the light of the same cell, transferred by preillumination into a hyperpolarized higher energy state S_1, are shown in the upper part of the Fig. 5. Except in the far-hyperpolarized region (-160 to -200 mV) where the differential resistance in S_1 is somewhat lower than in S_0, the *I–V* characteristics of S_1 and S_0 in the dark are parallel when the resting potential in S_1 is assumed to be -150 mV. The potential actually measured was -142 mV (indicated in the figure by the $\times$-mark). The *I–V* curve, measured in the light in state S_1 appears to be exactly parallel to that of S_0. In S_1, illumination has caused a depolarization of about 25 mV and a decrease in the resistance from 184 to 90 $k\Omega \cdot cm^2$. The *I–V* curve of S_1 in the light has been measured in a 30-s current sweep, started at a time about 30 s after the onset of illumination. At that time the potential and resistance had reached a quasi-steady state following an initial depolarization and decrease in resistance (*cf.* Fig. 4). The dark *I–V* curves show that the resting potential of the cell in S_1 is maintained at a hyperpolarized level by an active membrane current, $i_{p,1}$ which has increased as compared to the current ($i_{p,0}$) operating in S_0 with an amount of 0.10 $\mu A/cm^2$, or 1.1 pmoles/cm^2 per s. $i_{p,0}$ can be calculated as discussed before, from the ratio between ΔV and ΔR (light *minus* dark), but can be read also from the *I* coordinate of the intercept of the *I–V* curves in the light and in the dark. The absolute value of this coordinate is equal to the active current. The first method would yield that $i_{p,0}$ is about 0.53 $\mu A/cm^2$, the second method reads $i_{p,0}$ to be 0.65 $\mu A/cm^2$ (Fig. 5). These two values agree reasonably well. The potential coordinate of the intercept can be considered to be equal to the membrane resting potential in the absence, or after inhibition (see below) of the electrogenic pump. Note that these potentials in S_0 and S_1 are about the same (-112 mV).

Effect of CCCP

Fig. 6 shows the effect of CCCP on the membrane *I–V* characteristics of a cell, which after preillumination has been transferred from its dark-adapted S_0 state into a hyperpolarized state S_3. The *I–V* curves, characteristic for states S_4 through S_7 have been measured in the dark a few minutes after the addition of increasing amounts of the uncoupler to the cell in state S_3 through S_6. The curves have been reproduced according to the method outlined in the section Materials and Methods. The $\times$-marks indicate the (lower) dark potentials which were actually measured in states S_3 and S_4. The curves of S_0, S_3, S_4 and S_6 have the same shapes in the potential region between -125 and -190 mV which indicates that preillumination and CCCP, up to concen-

trations of 2 μM, have scarcely, if at all, affected the membrane permeabilities in the dark. The differential resistance of the membrane in S_3 and S_4 at potentials below -190 mV are somewhat smaller than in the other states. The reason for this effect is as yet unknown. The dashed curve in S_5 is the *I–V* curve of the membrane measured in the light (compare Fig. 5). In the presence of 4 μM CCCP (S_7) the shape of the *I–V* curve is different from the others. The change in the shape indicates an increase in the differential resistance, predominantly in the depolarizing region near the resting potential. The curves show, that preillumination of the cell has caused an increase

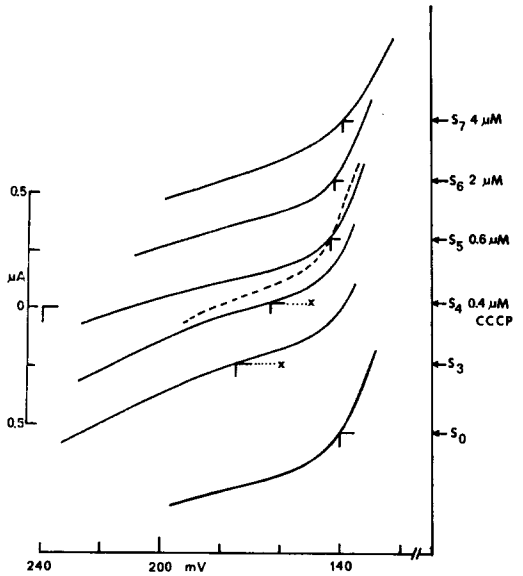

Fig. 6. *I–V* membrane curves of a *Nitella* cell, measured in the dark, after dark adaption (S_0), preillumination (S_3), and additions of increasing concentrations of CCCP (S_4 through S_7). The dashed curve in S_5 is the *I–V* curve measured in the light, 2 min after the onset of illumination (wavelength band around 676 nm, intensity approx. 5 nEinsteins$\cdot$cm$^{-2}\cdot$s^{-1}). The $\times$-marks coincide with the respective resting potentials measured in the dark states S_3 and S_4.

in i_p of approx. 0.25 μA/cm^2 (S_1 to S_3 transition). Addition of increasing amounts of CCCP in the dark has caused a decrease in i_p (S_3 to S_7 transition). A concentration of 0.6 μM of CCCP appears to be nearly completely inhibitory to the active current.

The kinetics of the light-induced changes in potential and resistance, measured in the different states of this cell (Fig. 6), are shown in Fig. 7. It shows that the light-induced initial membrane depolarization is higher in the S_3 state, as compared to S_0, and that it is inhibited by low concentrations of CCCP. In this cell a complete inhibition is effected at 0.6 μM.

The light-induced decrease in resistance is inhibited by CCCP at higher concentration, *i.e.* none at 0.6 μM and complete inhibition above 2 μM. Fig. 8 shows the CCCP titration curves of the membrane current (data from Fig. 6), light-induced change in potential and light-induced change in resistance (data from Fig. 7).

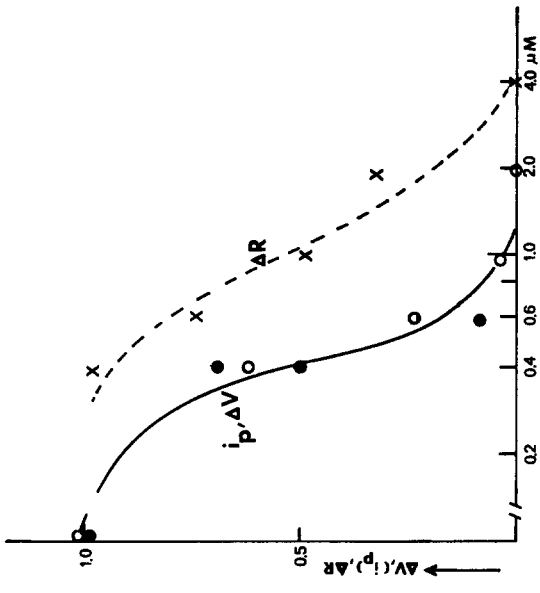

Fig. 7. Kinetics of the potential (left hand tracings) and resistance changes right hand tracings), measured simultaneously upon illumination and darkening of the *Nitella* cell (Fig. 6) in the different energy states S_0, S_3 through S_7. The change in the dark potential (depolarization), occurring upon the addition of CCCP at low concentration, is shown in the third curve from above; no concomittant resistance change was observed in this case (not shown). The resistance signal in S_4 is slightly distorted due to an alinear response of the measuring device at the high dark resistance (approx. 200 kΩ·cm²) in this energy state (*e.g.* Fig. 6).

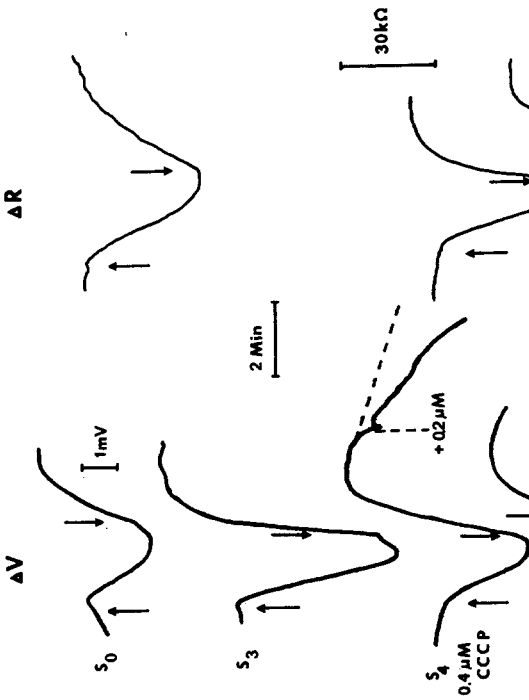

Fig. 8. Amplitude of light-induced membrane depolarization (ΔV, ●) and resistance decrease (R, ×), measured after about 2 min of illumination (Fig. 7), and active membrane dark current (i_p, ○) (*e.g.* Fig. 6) in a preilluminated *Nitella* cell, as a function of CCCP concentration in the external medium. Values of i_p, ΔV and ΔR are plotted as the fraction of their respective values in the absence of the uncoupler, *i.e.* measured in energy state S_3 (Figs 6 and 7).

DISCUSSION

The present results extend and confirm the conclusions reported in recent communications[1,11], that at least part of the dark resting potential of *Nitella translucens* is maintained by an electrogenic ion pump at the plasmalemma. The activity of the electrogenic pump, as is found now, depends on the so-called energy state of the cell. The energy state of cells in which the potential component maintained by the current generating pump is low, is designated as S_0. An S_0 state is induced, amongst others by keeping the cell in the dark for a long period. The membrane depolarization occurring in short illumination periods in S_0 cells was suggested to reflect a decrease in the potential generated by the electrogenic pump, due to a light-induced decrease in the membrane resistance[1].

The results of Figs 3–5 indicate that after prolonged illumination, or after successive illumination periods, interrupted by relatively short dark periods, the current generated by the electrogenic pump in the dark has increased appreciably. The increase was associated with a hyperpolarization of the plasmalemma and a change in the differential resistance. The magnitude and sign of this change in the resistance is determined by the shape of the *I–V* characteristics of the membrane. In cells in a more depolarized energy state (*e.g.* S_0) the hyperpolarization occurred parallel with an increase in the resistance; this is due to an increase in the rectification of the membrane. In the far-hyperpolarized region the (high) resistance decreased upon further hyperpolarization, which can be concluded from the downward bending of the *I–V* curve in this region, both in the light and the dark (*e.g.* Fig. 6). It has been observed indeed (Vredenberg, W. J., unpublished) that in prolonged illumination, the long-term hyperpolarization is accompanied by an increase in the resistance, which at high hyperpolarization is followed by a decrease in the resistance towards a lower steady state. Fig. 4 shows the increase in the resistance in the light after the initial decrease. Cells in which the activity of the electrogenic pump is higher than the pump activity of cells in the control state S_0 are called to be in a hyperpolarized higher energy state (S_i, in which i may indicate the number of particular pretreatments (illuminations, addition of chemicals, etc.)

The Figs 6–8 show that also in higher energy states of the cell the active membrane current i_p and the potential generated by it, are inhibited by CCCP in the concentration region between 0.2 and 0.8 μM, with 50% inhibition at about 0.4 μM. The inhibition of the light-induced change in the resistance occurs in the range between 0.6 and 4 μM, with 50% inhibition at about 1.0 μM. These results are similar to those formerly found for cells in an S_0 state[1,2].

The present results show that the ion transport processes at the plasmalemma are energetically linked to the photosynthetic reactions by at least two distinguishable processes (or products there of):

(1) A short-term (1 to 2 min) reversible light effect causes a decrease in the differential membrane resistance. This decrease is suggested to occur in association with a chemical or ionic change in the cytoplasm presumably caused by a relatively fast light-induced translocation of a reaction product, intermediate, or ions across the chloroplast enveloppe. As a consequence of the resistance decrease, the component of the membrane potential generated by the electrogenic pump decreases (membrane depolarization).

(2) A long-term (2 to 20 min) reversible light effect causes a stimulation of the electrogenic membrane current pump, which gives rise to a membrane hyperpolarization. The triggering reaction apparently proceeds with a relatively low time constant, both in the light and the dark. Presumably the triggering effector is a high-energy product, or intermediate of the photosynthetic reactions, which is translocated across the chloroplast envelope, and released into a cytoplasmic pool. Translocator systems, serving the transport of high potential phosphorylated compounds across the chloroplast enveloppe have been documented (*e.g.* refs 12 and 13). Our finding (Vredenberg, W. J., unpublished) that phloridzin did not affect the long term effect (nor the short term effect) suggests that ATP formed by photophosphorylation is not primarily involved.

Both photosynthetic light reactions are involved in the short term effect, causing the membrane depolarization[14]. The action spectrum of the long term effect has not been measured with sufficient accuracy so far. However, it has been found that 717-nm light is at least as effective as 676-nm light in causing the membrane hyperpolarization. The present results would be consistent with an hypothesis that the light-dependent reactions triggering the changes in the membrane resistance and the pumping rate of the electrogenic pump contribute mainly, if not exclusively, to the overall processes causing the changes in the potential observed in various algal and green plant cells (refs 2–5, 14–18). The system 1-driven changes in potential, observed in *Nitella* in the presence of 3,4-dichlorophenyl-*N*,*N*-dimethylurea (DCMU) and 2,6-dichlorophenolindophenol (DCIP), at which no light-induced change in resistance occurs[2], may reflect an exclusive change in the passive membrane diffusion potential, associated with primary ion translocations at the chloroplast membrane.

Electrogenic ion pumps, contributing to the membrane dark potential of *Nitella* and other green plant cell have been suggested by several authors (refs 1, 3, 11, 19–22). Spanswick[9,11] has proposed an explanatory equivalent circuit of the membrane, in which the electrogenic pump, with electromotive force E_p in series with an active resistance R_p, is connected in parallel with the (passive) diffusion potential E_m *via* the passive resistance R_m. According to this model,

$$E_m^* = E_m R_p/(R_m + R_p) + E_p R_m/(R_m + R_p) = [E_m^*]_{i_p=0} + i_p R_m \quad (1)$$

$E^*{}_m$ is the dark potential, measured across the membrane; i_p $(=E_p/(R_m+R_p))$ can be considered as the current generated by E_p through R_m and R_p in series, and $[E^*{}_m]_{i_p=0}$ $(=E_m R_p/(R_m+R_p))$ represents the dark potential in case $i_p=0$. A change in the membrane potential, due to changes in i_p and R_m will be equal to

$$\Delta E_m^* = \Delta[E_m^*]_{i_p=0} + \Delta i_p R_m + i_p \Delta R_m \quad (2)$$

The resistance measured when an external current is applied is equal to

$$R = R_p R_m/(R_m + R_p) \quad (3)$$

The reaction kinetics of the potential and resistance changes and the analyses of the *I–V* curves confirm that the short term membrane depolarization in S_0 cells and the energy-depende nthyperpolarization, associated with the transition of the cell into high-energy states can be quantitatively interpreted as being due to the respective

terms $i_p \Delta R_m$ and $\Delta i_p R_m$ on the right-hand side of Eqn 2. The assumption that in S_0 cells i_p remains constant in short illumination, during which a considerable change in R occurs (Fig. 4), would only be consistent with the proposed model, *i.e.* Eqns 1 and 3, when R_m is relatively small as compared to R_p in S_0 cells. According to the results shown in Figs 3, 5 and 6, it is likely that the passive potential term $[E^*_m]_{i_p=0}$ (Eqn 1) decreases, *i.e.* $\Delta [E^*_m]_{i_p=0} < 0$ (Eqn 2), in connection with the hyperpolarization $\Delta i_p R_m$, caused by the long-term light effect. This could be due to the increase in R_m of the rectifying membrane, occurring in association with the pump-driven hyperpolarization. The effect of course would be small if R_m in the dark remains small as compared to R_p in cells in higher energy states. Although variable for different cells $\Delta [E^*_m]_{i_p=0}$ amounts as an average to 10 to 15 mV, when R_m changes in the dark from 75 to 150 k$\Omega \cdot$cm^2 in association with $\Delta i_p = 0.2$ μA/cm^2. Thus the above effect could be accounted for by a change in R_m, when R_p is constant and about 375 k$\Omega \cdot$cm^2. This value of R_p would suggest that for cells in high energy states, in which $i_p \approx 1$ μA/cm^2 (ref. 10), E_p can be as high as 500 mV. An effect of an increase in R_m on $[E^*_m]_{i_p=0}$, as suggested here might alternatively explain the fact that the passive diffusion potential in the dark becomes more positive with respect to the potassium equilibrium potential at K^+ concentrations below 1 mM (*e.g.* refs 9, 23). It has been observed[9] that R_m increases considerably at K^+ concentrations below 1 mM.

In the presence of 0.6 μM CCCP at which $i_p = 0$ (Fig. 6), ΔE^*_m ($= \Delta [E_m]_{i_p=0}$) $=$ 0 and $\Delta R \neq 0$ for short illumination periods (Fig. 7). These results would fit in the model (*i.e.* Eqns 1 and 3) only when $R_m < R_p$. In summary, our results can be explained in terms of the parallel scheme proposed by Spanswick[9,11] if R_m is relatively small compared to R_p. The data would certainly be conflicting with this model if $R_p < R_m$, as suggested by Spanswick[9]. This suggestion, for instance, would predict that $E_p (\approx E^*_m) \neq 0$ in the presence of low concentrations of CCCP, unless a considerable change in R_m and/or R_p would occur upon the additions of CCCP. However, it has been found[1] that the dark resistance does not alter measurably upon the addition of low (0.4 μA) concentrations of CCCP.

The complex system of regulatory cellular energy reactions and the identity of the energy sources which control the membrane ion transport processes in plant cells (*e.g.* refs 2, 11, 19, 24–26) are still far from being known. The possibility to characterize the energy state of the cell in terms of an electrogenic ion pump seems promising in contributing to further experimental and theoretical approach.

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