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Rapid Effect of Light on the K^+ Channel in the Plasmalemma of *Nitella*

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Summary. Chlorophyll fluorescence, plasmalemma potential and resistance were measured simultaneously and subjected to a kinetic analysis. It was found that the light-induced changes of all three signals have two time constants in common. The faster one (τ_4 = ca. 20 sec) was assigned to the action of light-induced proton uptake across the thylakoid membrane on the plasmalemma H^+ pump. The slower one (τ_{5a} = 40 sec) is related to the light action of an unknown photosynthetic process on the potassium channel. The action on the K^+ channel was revealed from the reversal potential of the related effect on membrane potential. The comparison of the data with findings of other authors led to the hypothesis that the unknown photosynthetic mechanism is the depletion of $NADP^+$, which stimulates the uptake of Ca^{2+} from the cytosol, which is required for the NAD-kinase. The resulting change in cytosolic Ca^{2+} modulates the number of open K^+ channels.

Key Words Ca^{2+} · chlorophyll fluorescence · H^+ pump · K^+ channel · light · NAD-kinase · *Nitella*

Introduction

The effect of light on membrane potential of green plants has been known for about a century. Already Haake (1892) concluded from the absence of this effect in white tissue that photosynthesis is involved. Light-induced hyperpolarizations (Nagai & Tazawa, 1962; Nishizaki, 1968; Spanswick, 1974; Tazawa, Fujii & Kikuyama, 1979; Köhler et al., 1986) as well as depolarizations (Andrianov et al., 1971; Volkov, 1973; Findlay, 1982; Lucas, 1984; Smith & Walker, 1985; Felle & Bertl, 1986) have been observed. A major part of the research dealt with the light-induced hyperpolarization due to the stimulation of the electrogenic H^+ pump (Saito & Senda, 1973; Spanswick, 1974; Felle & Bentrup, 1976; Keifer & Spanswick, 1979; Bisson, 1986; Mimura & Tazawa, 1986*b*; Tazawa, Shimmen & Mimura, 1986).

However, until now the mechanisms of the interplay between the events in the chloroplasts and in the plasmamembrane are widely unknown. Many workers (Vredenberg & Tonk, 1973; Spanswick, 1974) tried to apply the hypothesis of MacRobbie (1966) that energy supply by ATP or NADPH should stimulate different transport systems (K^+ and Cl^- uptake, respectively). This hypothesis initiated much work based on inhibitor studies (Raven, 1969). Several workers (Keifer & Spanswick, 1979; Takeuchi & Kishimoto, 1983; Mimura, Shimmen & Tazawa, 1984) showed that the ATP level in the cytosol of different *Characean* cells does not change with light, and that the ATP level is saturated for electrogenesis (Kikuyama et al., 1979). Recently Mimura and Tazawa (1986*b*) tried to revive the ATP hypothesis, because they and Reid and Walker (1983) found small (but probably not sufficient) light-induced changes in ATP level in *Characean* cells.

Evidence of coupling via diffusible substances came from experiments in which the original chloroplasts of *Characean* cells were replaced by those from spinach (Tazawa & Shimmen, 1980) or from *Pisum* (Mimura & Tazawa, 1986*b*). However, Mimura and Tazawa (1986*a*) did not believe in the involvement of diffusible substances in the case of the rLPC (rapid light-induced potential changes), because they could not eliminate the light-induced responses by rapid perfusion. They assumed that surface charges at the chloroplasts may influence membrane transport and did not consider the possibility that perfusion might leave the narrow space between chloroplast and plasmalemma undistorted. Support for the involvement in the rapid light effects of diffusible substances, namely H^+ ions and Ca^{2+} ions, has come from our previous studies (Hansen, Kolbowski & Dau, 1987; Vanselow, Dau & Hansen, 1988; Vanselow, Kolbowski & Hansen, 1989*b*) and from the experiments reported here.

By means of kinetic investigations, Hansen (1978, 1980, 1985) could show that light acts via three parallel pathways on the electrogenic proton pump. One of these pathways is related to a quite

simple chain of events: light-induced uptake of protons into the lumen, subsequent pH increase in the stroma and in the cytosol, and a decrease of pump activity because of substrate depletion (Hansen et al., 1987; Vanselow et al., 1988). Felle and Bertl (1986), Steigner et al. (1988), and especially Remis, Bulychev and Kurella (1988) found the postulated light-induced alkalinization of the cytosol with a subsequent acidification. The relationship of the initial depolarization to transthylakoid H^+ fluxes is consistent with the finding of Lüttge (1973) that at least part of the photosynthesis-dependent H^+ uptake in barley and maize leaves is independent on concomitant CO_2 fixation.

A mechanism of physiological significance is assigned to the second (oscillatory) component, which is related to pH homeostasis (Hansen, 1980, 1985; Fisahn, Mikschl & Hansen, 1986). The slow acidification of the cytosol mentioned above may explain the third (hyperpolarizing) component of the light action on the H^+ pump. However, Fujii, Shimmen and Tazawa (1979) showed in tonoplast-free *Chara* that light-induced hyperpolarizations were induced even at pH_i below 6.2, even though they found that the H^+ pump has maximum activity at $pH_i = 6.9$. This question is not settled because the involvement of the light-induced closure of the K^+ channel and of the pH control loop related to the second oscillatory component has not been investigated.

In addition to this effect on the electrogenic pump, there is also an effect on passive transport, reported in this paper. This effect is extremely strong in *Eremosphaera* (Köhler et al., 1985, 1986; Thaler et al., 1987; Steigner et al., 1988). The existence of a light effect on passive transport in *Nitella* was shown by Keunecke (1974) and Hansen and Keunecke (1977), who found that the light-induced responses of membrane potential and of resistance displayed different time constants. However, the complexity of the response was too high for the curve-fitting routines available at that time. Thus, the first attempts of distinguishing between the effects on the pump and on the K^+ channel were not conclusive and partially wrong (Thiel, Keunecke & Hansen, 1984; Kolbowski et al., 1985).

The situation turned out to be simpler in the case of the temperature effect on plasmalemma transport. Fisahn and Hansen (1986) and Stein and Hansen (1988) succeeded in distinguishing between the effects on the pump and those on the channel by mathematical means, using the time constants of the responses as labels of the involved processes.

The new attempt to separate the light effects on the pump and on the K^+ channel could start from improved conditions: a more powerful kinetic analysis was achieved by using noise as an input signal (Vanselow, Kolbowski & Hansen, 1989*a*) supplemented by better curve-fitting routines making use of three output signals (chlorophyll fluorescence, F, plasmalemma potential, V, and resistance, R) and a much greater number of experiments. Thus, it became possible to separate the light effects on the electrogenic pump from those on the K^+ channel. This paper deals with the rapid component of the light effect on the K^+ channel, which probably corresponds to the rLPC of Tazawa and Shimmen (1980). The result is similar to that obtained in the case of the H^+ pump. It is not a sophisticated messenger system adapting plasmalemma transport to metabolic needs, but a simple side effect of the light-induced Ca^{2+} uptake into the chloroplasts, which mediates (at least the rapid phase of) the light effect on the K^+ channel.

Materials and Methods

The experimental setup is described in previous articles: chlorophyll fluorescence (Vanselow et al., 1989*a*), membrane potential (Vanselow et al., 1988, 1989*b*) and membrane resistance (Fisahn & Hansen, 1986; Stein & Hansen, 1988). Briefly, *Nitella flexilis* was purchased from R. Kiel in Frankfurt and kept in APW (artificial pond water containing 0.1 mol · m^{-3} KCl, 1.0 mol · m^{-3} NaCl, 0.5 mol · m^{-3} $CaCl_2$, no buffer) in the refrigerator at 10°C at a light intensity of 5 Wm^{-2} (16 hr/day). pH was adjusted to pH 6.8 by small amounts of HCl when the pH meter showed an increase of 0.5 pH units. In the experiments, APW was used if not otherwise mentioned. In order to increase chlorophyll fluorescene sometimes three to five ceils of *Nitella* of similar appearance were laid into the bathing medium. One of those was used for the electrical measurements.

Two Light Sources

Because chlorophyll fluorescence was measured as modulated fluorescence (yield), two light sources had to be employed. The status of the photosynthetic system was changed by the modulation of the intensity of the actinic light, which comprised the constant component $I_=$ and the variable component I_v. A superimposed measuring light I_m of lower intensity (*see below*) modulated by 1 kHz was used for the recording of the status induced by the actinic light. The light intensity I can be described by the following equations.

$$I = I_= + I_m + I_v \tag{1}$$

with

$$I_v = mI_= bn. \tag{2}$$

bn takes the values −1 or +1 in a random sequence (binary noise, clock of 6 Hz, Vanselow et al., 1989*a*). m = 45%.

Chlorophyll Fluorescence

For the measuring light of the fluorescence probe described by Vanselow et al. (1989*a*) a luminescence diode OL-SUR 150C (Oshino, 660 nm, modulated with 1 kHz between 0.01 and 4 W · m^{-2}) was used. The actinic light was provided by a halogen bulb (Xenophot HLX 150 W, Osram, blue filter Schott BG 38, intensity 33 W · m^{-2}). Crofon fibers (Schölly, FRG) led the chlorophyll fluorescence coming from the plant via a red light filter (Schott RG 9) to a photodiode (BPW 34, Valvo), which served as light detector. A dc-driven 40 W bulb provided a constant background illumination of 1.5 W · m^{-2} for the nutrition of the cells during the experiments. This light contributed little to the actinic light at the investigated spot because of the shadow of the probe.

Plasmalemma Potential and Resistance

The membrane potential measured by an inserted 1 kmol · m^{-3} KCl microelectrode is the sum of the voltages across the plasmalemma and the tonoplast. However, it is assumed that the effects reported in this article are mainly related to the transport processes at the plasmalemma. Firstly, there is the widespread credo of *Characean* workers that the contribution to the overall responses of the electrical events at the tonoplast is smaller than that of those at the plasmalemma (Findlay & Hope, 1964). This holds especially in the case of the changes in resistance, which are related to the light effect described in this paper: at $[K^+]_o$ = 0.1 mol · m^{-3} the overall resistance was between 20 and 50 kΩ · cm^2. This overall resistance would hardly be influenced by changes of the tonoplast resistance of about 1 kΩ · cm^2 (Smith, 1983; Tester, Beilby & Shimmen, 1987). Secondly, it is shown that the observed effects result from chloroplast-induced perturbations of cytosolic pH and *p*Ca. These perturbations can influence the nearby plasmalemma quickly, whereas dilution on the way to the tonoplast is expected to attenuate the induced responses of this membrane. Because of this, and in order to make a clear distinction between events at thylakoid membrane and at the plasmalemma, we call the potential measured by the inserted microelectrode "plasmalemma potential."

Resistance measurements were done by injecting a square-wave current of 1 Hz (±4 mV) into short cells (length 0.5 to 1.5 cm, diameter 0.2 to 0.4 mm) via a second microelectrode in series with a 50-MΩ resistor connected to a high-voltage amplifier (Burr Brown 3584JM), which could provide ±100 V. This setup acted as a current source as could be seen from the fact that the voltage at the output of the amplifier was more than 100 times greater than the induced changes in membrane potential. The 1-Hz signal recorded by the voltage electrode was detected by a phase-sensitive rectifier with a sampled integrator instead of the low-pass filter, which was used by Stein and Hansen (1988).

Short cells were selected in order to minimize interference from cable properties when the membrane potential was offset by an injected dc current. However, cable problems did not falsify the main result, namely the determination of the reversal potential of the component related to the K^+ channel, since this reversal potential was close to the resting membrane potential, and little current had to be injected.

Data Acquisition

Data acquisition (sampling rate 6 Hz) and the generation of the binary noise ($2^{15} - 1$ pulses, cycle time 91 min) were done by a personal computer. The kinetic analysis of the responses to the noisy input signal (Eq. (2)) was based on the evaluation of cross-structure functions (Schulz-DuBois & Rehberg, 1981), and is described in detail by Vanselow et al. (1989*a*).

Calibration

In order to ease the comparison of fluorescence data from different sources, we used $Y_=$ as a reference for relative units. $Y_=$ is the yield obtained at constant actinic light ($I_v = 0$ in Eq. (1)). Details of the calculation of the scaling factor are given by Vanselow et al. (1989*a*).

Results

Noise as a Means of Providing Linear Responses

Experiments were done under steady-state conditions, which yield linear responses. Linear responses are desirable because in a linearized system the responses of different processes are superposed without mutual interference and can be separated by curve-fitting routines (Hansen, 1985). The demand of linearity implies that dark/light transitions must not be employed. Instead, the cells were always exposed to constant background light consisting of the measuring light I_m and the mean value of the actinic light $I_=$ (Eq. (2)). The analysis was based on the evaluation of the pulse responses of chlorophyll fluorescence, F, plasmalemma potential, V, and plasmalemma resistance, R. The investigated pulse responses are basically similar to those which would be caused by a small flash of light superposed to the constant background light. Unfortunately, the photosynthetic apparatus turned out to be a very nonlinear system (Hansen et al., 1989).

Thus, the usual method of linearizing the responses, namely the reduction of the amplitude of the input signal (Eigen, 1968), would lead to very small signals, which would be covered by the noise of the recording apparatus. This problem can be circumvented if a signal is used, which enables the mathematical separation of linear and nonlinear components. Thus, the pulse response was not measured by stimulating the system by a real pulse (flash) of light, but by modulating the actinic light by binary noise (Eq. (2)). The responses to noise had to be analyzed by means of the calculation of the cross-correlation function, CCF, or by the cross-structure function, CSF (Schulz-DuBois & Rehberg, 1981; Vanselow et al., 1989*a*), which provide the linear part of the response to a flash of light. The

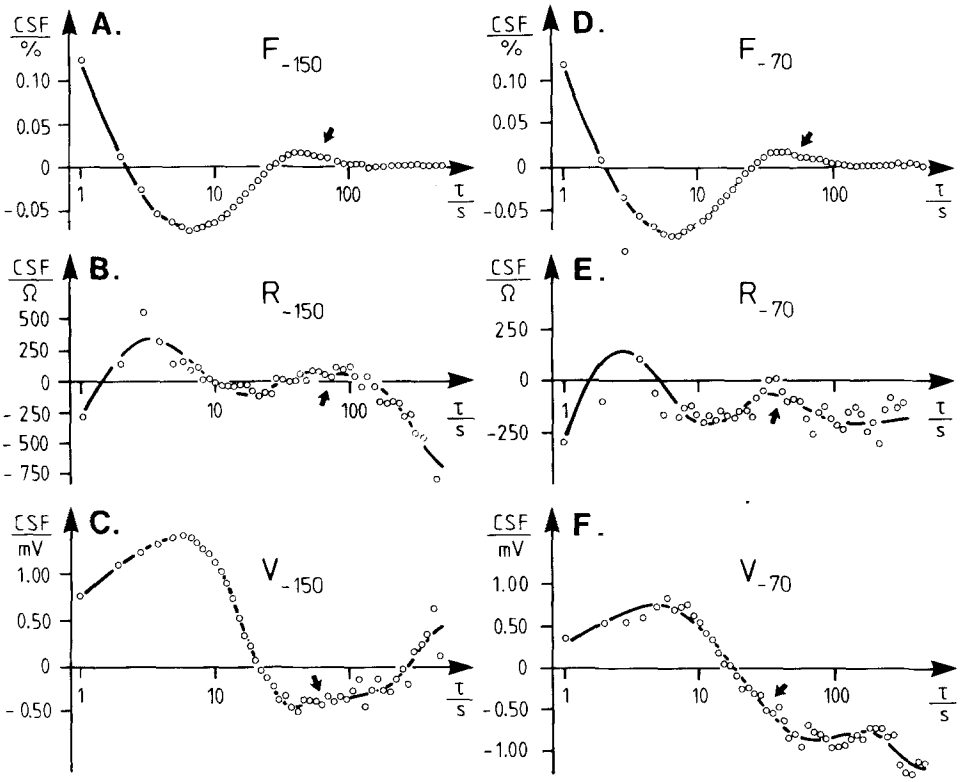

Fig. 1. Linear pulse responses of the light effect on chlorophyll fluorescence, F (A) and (D), on plasmalemma potential, V (C) and (F), and on resistance, R (B) and (E) as obtained from the stimulation by noisy actinic light (Eq. (1)) and evaluation by means of Eq. (3). The curves were fitted by means of Eq. (5), and the results are displayed in the Table. *Nitella* in 1.0 mol · m^{-3} NaCl, 0.1 mol · m^{-3} KCl, 0.5 mol · m^{-3} $CaCl_2$. Medium light intensity $I_= = 35$ W · m^{-2}. Membrane potential was offset from the resting potential = −110 to 150 mV (A,B,C,) or to −70 mV (D,E,F)

computer did an on-line calculation of the CSF

$$\mathrm{CSF}(t) = 1/T \int_0^T [y(s) - u(s - t)]^2 \, ds \tag{3}$$

with y being the output signal (F, V, or R) and u the input signal (light intensity), s the real time, t the correlation time, and T being the measuring time (100 to 200 min in our experiments). An on-line criterion for the minimum required measuring time is given by Vanselow et al. (1989*a*). The (linearized) pulse response $h(t)$ is calculated from the CSF

$$h(t) = 0.5[\mathrm{CSF}(t < 0) - \mathrm{CSF}(t)]. \tag{4}$$

The Linear Pulse Responses of F, V, and R

Figure 1 shows pulse responses of F (fluorescence yield), V (plasmalemma voltage), and R (plasmalemma resistance) measured at two different membrane potentials (offset by an injected dc current). The responses of all three signals show complicated curve shapes. In order to detect possible pathways of interaction, we have to solve the problem of how to resolve common components in these curves. From the theory of linear systems (Bode, 1964; Hansen, 1985), it is known that a linear(ized) pulse response can be fitted by a sum of exponentials plus oscillatory terms

$$h(t) = \sum_{i=1}^{N} a_i \exp(-t/\tau_i) \tag{5}$$

$$+ \sum_{j=1}^{M} \exp(-t/\tau_{dj})(a_{sj} \sin(t/\tau_{osj}) + a_{cj} \cos(t/\tau_{osj}))$$

with a_i being the amplitude factors and τ_i the time constants of the involved components. The data obtained from curve fitting these responses (smooth curves in Fig. 1) are displayed in the Table.

The Components of the Measured Responses

At first it is stated that the Table shows most of the components of chlorophyll fluorescence known from previous investigations (Hansen et al., 1987;

Table. The parameters of Eq. (5) used for fitting the curves in Fig. 1 at two different steady-state plasmalemma potentials

Signal		F		V		R	
Curve in Fig. 1		A.	D.	C.	F.	B.	E.
Plasmal. potential		−150 mV	−70 mV	−150 mV	−70 mV	−150 mV	−70 mV
Assignment for τ_i		τ_i/s	τ_i/s	τ_i/s	τ_i/s	τ_i/s	τ_i/s
F component							
Quencher Q	1	0.64	0.44	(3.8)	(2.4)	(1.1)	(0.9)
Unknown	3	3.1	2.8			(8.6)	(6.3)
Thylakoid ΔpH	4	19.5	19.1	17.8	17.3	18.1	17.4
Ca^{2+} influx	5*a*	35.3	33.9	29.9	34.6	33.1	32.7
Out of range	7	2705	3191	(1075)	(2192)	(1429)	(2465)
Cytosolic	*d*			514	334	338	
pH controller	*os*			534	214	372	
	a_i	$a_i/\%$		a_i/mV		$a_i/\text{K}\Omega$	
	a_1	0.4	0.5	−3.6	−1.6	−2.6	−2.8
	a_3	0.27	0.30			2.3	2.0
	a_4	−0.34	−0.33	7.1	1.0	−2.0	−2.4
	a_{5a}	0.16	0.16	*−2.8*	*1.8*	0.65	1.4
	a_7	−0.002	−0.001	3.1	−1.6	−1.4	−0.23
	a_s			−2.0	1.5	1.9	
	a_c			−2.8	0.13	1.3	

[a] The time constants given in paranthesis are not assigned to the mechanisms given in the first column. The oscillatory term of plasmalemma potential and resistance is described by: $\exp(-t/\tau_d) * (a_s \sin(t/\tau_{os}) + a_c \cos(t/\tau_{os}))$.

Dau & Hansen, 1988*a*,*b*; Vanselow et al., 1988, 1989*a*,*b*). The assignment of these time constants to reactions in the photosynthetic apparatus (as known from the investigations in spinach and *Aegopodium podagraria* mentioned above) is given in column 1. However, the time constant τ_2 assigned to the plastoquinone pool is missing in the responses from *Nitella* in the Table. This component was also absent from the responses of *Cygocactus truncatus* (Vanselow & Hansen, 1989). The absence of the τ_2 component does not mean that there is no plastoquinone pool. If reduction by PS II and oxidation by PS I are well balanced, the redox state of the pool is not affected by light-induced changes in the electron flow, and hence the component related to the changes of its redox state is absent from chlorophyll fluorescence. This effect becomes obvious when far-red actinic light is used instead of or in addition to blue light (Hansen et al., 1987).

A "new" time constant τ_{5a} (the indices 5*a* and 5*b* had to be introduced because only one of them was found in previous investigations, when τ_6 got its name) not discussed in detail in previous papers occurs in the Table. This is not a pecularity of *Nitella*, but a result of increased curve-fitting skill, which revealed this additional component also in spinach, *Aegopodium* and *Cygocactus truncatus* (Vanselow et al., 1988, 1989*a*,*b*; Vanselow & Hansen, 1989). This component and that related to τ_{5b} (which did not show up in the experiments in *Nitella*, but which is known from experiments in *Aegopodium podagraria* and in spinach, Vanselow et al., 1989*a*,*b*) are related to the so-called M_1-peak of chlorophyll fluorescence (Yamagishi, Satoh & Katoh, 1978; Schreiber & Bilger, 1987).

In this paper, the analysis is focused on the time constants τ_4 and the "new" time constant τ_{5a}. These time constants take equal values in F, V and R. The values are not exactly equal in the Table, because the data were determined in "free fits" (without the constraint to take equal values for corresponding time constants in "joint fits" of different output signals). Equal values could be obtained in joint fits of all three signals without increase of the sum of errors.

Dependence of the Kinetic Data on Plasmalemma Potential: Demonstration of the Involvement of the H^+ Pump and of the K^+ Channel

For the demonstration of the involvement of the H^+ pump and of the K^+ channel, we use the same method as applied by Stein and Hansen (1988) in the case of the temperature effect. Linearized pulse re-

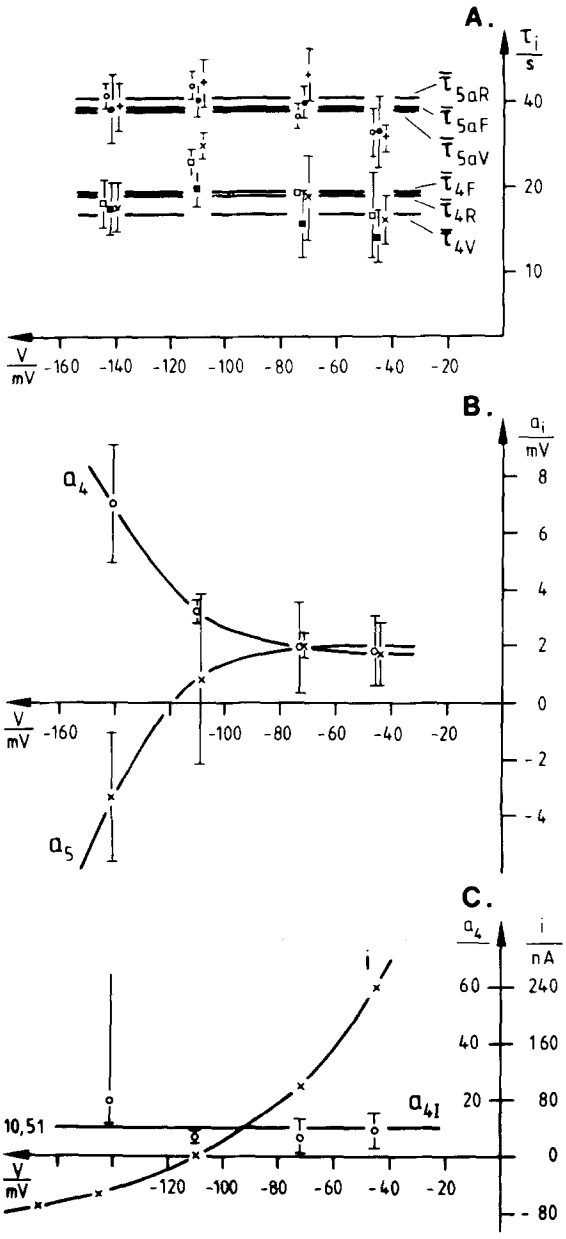

Fig. 2. Dependence of the time constants τ_4 and τ_{5a} (*A*) and amplitude factors a_4 and a_{5a} (*B*) on steady-state plasmalemma potential V_{ss}. Data are obtained from curve fitting of the responses from three to four measurements on the basis of Eq. (5). Four cells were placed in parallel in order to increase the fluorescence signal, but only one cell was penetrated by microelectrodes. Medium: APW of 1 mol · m^{-3} NaCl, 0.1 mol · m^{-3} KCl and 0.5 mol · m^{-3} $CaCl_2$ was used. (*A*). Dependence of the time constants on steady-state plasmalemma potential. The indices refer to the light effect on chlorophyll fluorescence (*F*, open symbols), plasmalemma potential (*V*, crosses), and resistance (*R*, filled symbols). (*B*). Dependence of the amplitude factors a_4 and a_{5a} of the light effect on plasmalemma potential on steady-state membrane potential. (*C*). Light-induced changes in membrane current *i* as calculated from Eq. (6), and the *IV*-curve (current-voltage relationship) as obtained from the currents (*i*) required to offset the membrane potential. The asymmetric error bars result from the usage of maximum and minimum pairs of a_{5aV} and R_v (Eq. (6)). Light intensity 35 W · m^{-2}, T = 19°C

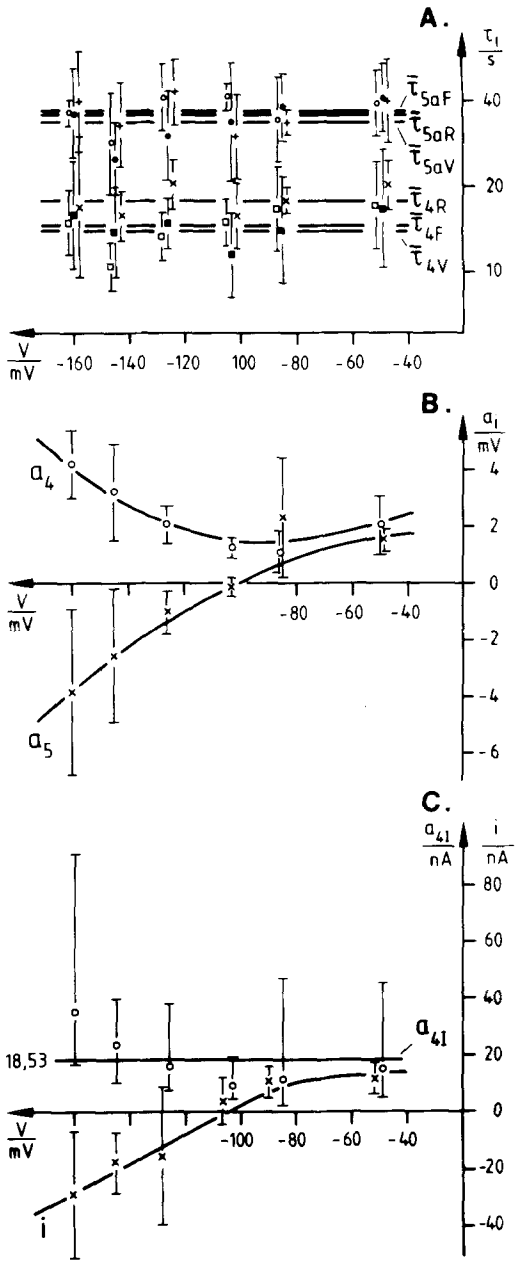

Fig. 3. Similar to Fig. 2, but the medium was APW of 1 mol · m^{-3} NaCl, 1 mol · m^{-3} KCl and 0.1 mol · m^{-3} $CaCl_2$. Average of four to six experiments

sponses similar to those of Fig. 1 were measured at different membrane potentials and at different outside concentrations of K^+. Membrane potential was offset by an adequate dc current injected via the microelectrode also used for the injection of the 1 Hz current for the measurement of resistance.

The feature, which is of major interest in this article, becomes obvious from a comparison of Fig. 1*A–C* and *D–F*. The responses of *F* and *R* are equal at both membrane potentials. There seems to be a

difference in the late responses of the R curves. However, the long-term decrease of R at a membrane potential of -150 mV is caused by drift without statistical significance. This drift is already outside the range of the time constants that are of interest here.

The real difference becomes obvious in the V traces in the regions marked by the arrows. Whereas the curve measured at -150 mV bends up, it continues to decrease if measured at -70 mV. This behavior indicates that one component has changed its sign. From curve fitting, we know that this range is dominated by the τ_{5a} component.

A quantitative analysis of this effect based on curve fitting (Eq. (5)) is shown in Figs. 2 and 3 for two different outside concentrations of K^+. The upper parts demonstrate what is mentioned above: averaging of several experiments reveals the coincidence of the time constants τ_4 and τ_{5a} in all three signals (F, V, and R). This occurrence of equal time constants in F and V was also found in other species, like spinach, *Aegopodium* and *Cygocactus truncatus* (Vanselow et al., 1988; Vanselow & Hansen, 1989). The coincidence of time constants is less good in the case of τ_4, but this is of no concern in the present study as this coincidence has been tested in previous investigations (Kolbowski, Keunecke & Hansen, 1984; Hansen et al., 1987; Vanselow et al., 1988, 1989*b*). Scatter of this magnitude occurs if free fits (without constraints to use equal time constants in different curves) are applied as done in the case of the data in Fig. 1.

The time constants, shown in Figs. 2*A* and 3*A*, do not depend on steady-state membrane potential, nor do they depend on external K^+ concentration. This is in line with the expectation discussed below that the related processes are located in the chloroplasts, and thus do not sense membrane potential.

The behavior of the amplitude factors a_4 and a_{5a} shown in Figs. 2*B* and 3*B* leads to a partial identification of the involved transport processes. From previous findings we know that the τ_4 component is related to the action on the electrogenic pump (Hansen et al., 1987; Vanselow et al., 1988, 1989*b*). As the pump is a current source with a reversal potential of about -450 mV, not the change in potential, but the change in current is the primary entity. Thus, the change in current is calculated from a_{4V} by means of the slope resistance, R_o, known from the resistance measurements. a_{4I} is the amplitude factor of the light induced current, which is calculated from a_{4V} by

$$a_{4I} = a_{4V}/R_o. \tag{6}$$

Figures 2*C* and 3*C* show that the amplitude of the light-induced current (which is the primary entity) does not depend on steady-state membrane potential.

This result supports the previous findings mentioned above, but is not the major issue of this article. The new results are obtained from the behavior of a_{5V}. Figures 2*B* and 3*B* show the dependence of a_{5aV} (a measure of the magnitude of the action of the τ_{5a} component on membrane potential) on the value of the steady-state membrane potential. In both figures, it is demonstrated that a_{5aV} changes its sign in the neighborhood of E_K. The dependence of the a_{5aV} reversal potential of the light-induced changes of the a_{5aV} component on external K^+ concentration is displayed in Fig. 4. The two data points from Figs. 2*B* and 3*B* are supplemented by a third data point obtained from the experiment shown in the upper part of Fig. 4. Experiments at higher K^+ concentration were not successful, because the cells in high $[K^+]_o$ could not stand long-lasting current injection for offsetting the membrane potential. However, already these three data points reveal the "Goldman" behavior of the reversal potential, with a dominating K^+ component.

Even though the measured reversal potential is not very close to E_K (this holds for the point at 1 mol · m^{-3} only) the evidence for a dominating role of the K^+ channel seems to be strong.

Firstly, there is no other candidate for a major transport system with a reversal potential in the range of -100 mV. The reversal potential of the chloride channel is positive; that of a sodium channel would not be more negative than about -60 mV. Cotransport systems should have reversal potentials far away (more than 60 mV) from the resting potential in order to work efficiently, and in the correct direction under all physiological conditions.

Secondly, the "Goldman" dependence is obvious in Fig. 4*B*. The slope and the saturation region at low K^+ concentrations are very similar to that of the binding constant of K^+ to the transport site of the K^+ channel in *Nitella* (Fisahn, Hansen & Gradmann, 1986). Thus, Fig. 4*B* displays the behavior expected for a light action on the K^+ channel. The origin of the saturation region is not quite clear. However, a shielding effect of the cell wall can be excluded, since Abe and Takeda (1986) observed the saturation also in "naked" *Nitella* protoplasts, and Köhler et al. (1985) found that during the strong dark-induced opening of the K^+ channel in *Eremosphaera* E_K was reached even at K^+ concentrations of 0.01 mol · m^{-3}, even though the resting potential shows the same saturation as that shown in Fig. 4*B*. The finding in *Eremosphaera* seems to indicate the involvement of other channels besides the K^+ channel rather than a limited selectivity of the K^+ channel. The relative contributions of these channels seem to depend on outside Ca^{2+} concen-

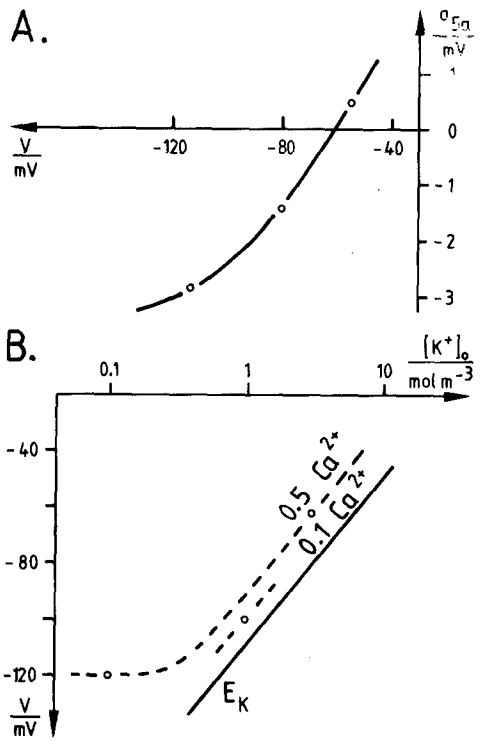

Fig. 4. Dependence of the reversal potential of the a_{5aV} component on external K^+ concentration. The upper part (*A*) demonstrates the determination of the reversal potential (average of three experiments). This is similar to Figs. 2*B* and 3*B*, but for a different bathing solution: 3 mol · m^{-3} KCl, 1.0 mol · m^{-3} NaCl, 0.5 mol · m^{-3} $CaCl_2$. The data points in (*B*) are taken from Figs. 2*B* and 3*B* and from the upper part (*A*) of this figure. The dotted lines connect the data measured in solutions with the same Ca^{2+} concentration. The solid line gives E_K as calculated from an internal K^+ activity of 70 mol · m^{-3} (Okihara & Kiyosawa, 1988)

tration, as indicated by the dotted lines in Fig. 4*B*. However, these dotted lines are not supposed to present a statement, but to stimulate further research.

Thirdly, the involvement of a high-G $K^+(Ca^{2+})$ channel (Tester, 1988*a*) fits very well into the model developed below.

Discussion

The data related to τ_4 support previous findings of the light action on the H^+ pump (Fig. 5, and Hansen et al., 1987; Vanselow et al., 1988, 1989*a*,*b*) and are not discussed here. The new information is related to the 5*a* component. Figure 4*B* indicates that this component can be assigned to the light action on the K^+ channel with a partial contribution of another transporter, probably the Cl^- channel, as discussed in more detail below. This situation is similar to that found by Stein and Hansen (1988) for the temperature effect on membrane transport in *Nitella*, and confirms the involvement of the same reactions in the light effect and in the temperature effect. Thus, τ_{5a} of this study is identical to τ_r of Fisahn and Hansen (1986) and Stein and Hansen (1988).

For the model shown in Fig. 5, we have to discuss the suggestion of Tester (1988*a*) that the involved transporter is a high-G $K^+(Ca^{2+})$ channel. The existence of this channel in *Characean* cells is indicated by the following findings:

1) Tester (1988*a*) tested a variety of drugs and found that the K^+ channel in *Chara* behaved very similar to the high-G $K^+(Ca^{2+})$ channel of animal cells (Vergara, Moczydlowski & Latorre, 1984).

2) The stimulating action of cytosolic Ca^{2+} has not been shown directly. However, Lühring and Tazawa (1985) found a decrease of resistance in perfused tonoplast-free cells of *Chara* with increasing Ca^{2+} concentrations in the perfusion medium. As membrane potential and pump activity (as measured by H^+ fluxes) stayed constant over a wide range, the reversal potential of the transporter responsible for the change of resistance had to be close to the membrane potential. The only candidate is the K^+ channel. In *Nitellopsis*, membrane potential also depolarized, indicating that a contribution of additional transport systems existed, and that this can be different in different species. Also, Mimura and Tazawa (1983) found a decrease in resistance without depolarization when internal Ca^{2+} activity was increased from 0 to 1 mmol · m^{-3} in perfused tonoplast-free cells of *Nitellopsis obtusa*.

3) Ba^{2+} is a well-known antagonist of Ca^{2+}. Ba^{2+} entering the cell closes the K^+ channel in *Chara* (Tester, 1988*b*) and in *Eremosphaera* (Thaler et al., 1987). Azimov, Geletyuk and Berestovskii (1987) showed the closure of K^+ channels by Ba^{2+} applied to the cytoplasmic side in single-channel recordings from the plasmalemma of *Nitellopsis obtusa*.

4) MacRobbie and Banfield (1988) showed that $^{45}Ca^{2+}$ and $^{86}Rb^+$ influxes were influenced by nifedipine in a parallel manner.

5) Kourie and Findlay (1989) have injected Ca^{2+} into the vacuole of *Chara*. Unfortunately, they do not show data. Instead, they report that "Ca^{2+} opens the chloride channels more significantly than the K^+ channels." Even though this is quite an indirect indication of the effect on the K^+ channel, it provides a hint to the other transport system probably involved.

The reversal potentials shown in Fig. 4*B* are more positive by 20 to 30 mV than the calculated E_K. This is due to a parallel effect on other transport system(s). Above, the experiments of Lühring and

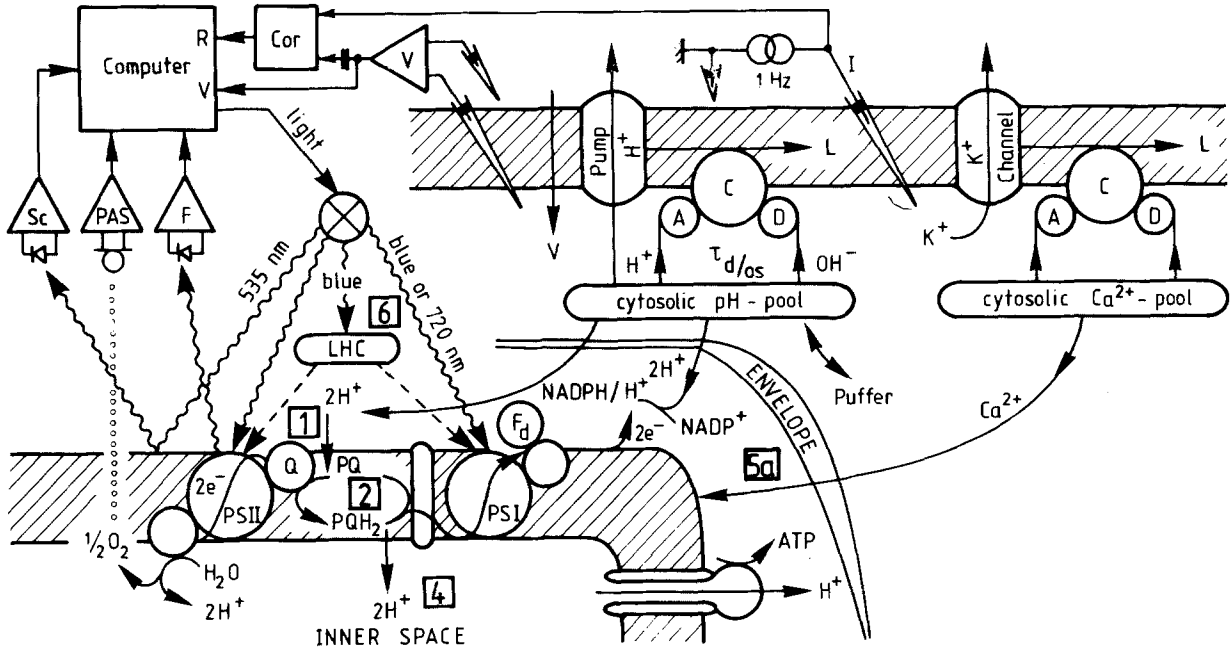

Fig. 5. An extension of the model of the initial effects of photosynthesis on plasmalemma transport (Hansen et al., 1987) including the action of light on the K^+ channel. The processes, which are identified (*see* Table), are indicated by the numbers of the related time constants (boxes). The transporters at the plasmalemma are deactivated and activated by transitions into and out of a lazy state. These transitions are mediated by a controller (*C*). According to a theory of biological control loops (Hansen, 1985) a controller needs separate sensors and substrates for activation (*A*) and deactivation (*D*)

Tazawa (1985) and Kourie and Findlay (1989) are mentioned showing that besides the K^+ channel other transporters are also influenced by changes in cytoplasmic Ca^{2+}. Similarly, Sanders, Hansen and Gradmann (1985) found a strong decrease in membrane resistance of *Chara* accompanied by strong depolarizations when *Chara* was perfused with solutions containing more than 1 mmol · m^{-3} Ca^{2+}.

A possible candidate is the Cl^- channel. Two different phenomena are related to Cl^- channels, the chloride influx during the action potential (Beilby & Coster, 1979; Lunevsky et al., 1983) and the increase of chloride fluxes at very negative potentials (Coster & Hope, 1968; Coleman, 1986). The Cl^- channel related to the action potential is activated by an increase in cytosolic Ca^{2+} (Lunevsky et al., 1983; Kikuyama, 1986; Shiina & Tazawa, 1987; Tsutsui et al., 1987). The phenomena observed in our investigations are not related to the action potential. However, Shiina and Tazawa (1988) came to the conclusion that the Cl^- channel in tonoplast-free cells of *Nitellopsis obtusa* is not opened by membrane depolarization, but by the increase of cytosolic Ca^{2+} concentration, as shown by Kikuyama (1986) who could initiate a Cl^- spike by microinjection of Ca^{2+}. Thus, the chloride channels could also be opened by a decrease of *p*Ca originating from other processes.

The chloride channel(s) may be closed at the prevailing membrane potentials. Thus, they would not sense a decrease in cytosolic Ca^{2+} activity. However, the noisy light comprises upward and downward steps in light intensity. The linear analysis cannot, per definition, distinguish between the responses to upward or downward steps. Thus, the measured responses can be caused by the dark-induced increase of cytosolic Ca^{2+}. The only problem is that the magnitude of the increase in $[Ca^{2+}]_{cyt}$ (caused by the downward steps in light intensity) has to be quite high for opening the Cl^- channel. Shiina and Tazawa (1988) found a value of 4 mmol · m^{-3} for half-maximum opening. It is difficult to estimate the changes in *p*Ca in our measurements, because the local changes in the narrow space between chloroplasts and plasmalemma may be much greater than those measured by Miller and Sanders (1987) in the bulk cytoplasm. In addition, the depolarizations measured in our experiments are very small (about 1 mV), and it is not known whether this slight opening of the channels can be brought about by a much smaller increase in cytosolic Ca^{2+} concentration.

As discussed below, we assume that the a_{5a} component is related to Ca^{2+} uptake into (upon increase of light intensity) or Ca^{2+} export (upon decrease of light intensity) out of the chloroplasts. Ac-

cording to the above considerations, the resulting change in cytosolic Ca^{2+} does not only influence the K^+ channel, but also the Cl^- channel, thus moving the overall reversal potential to more positive values. The K^+ channel is deactivated by increasing outside Ca^{2+} concentrations (Keifer & Lucas, 1982; Beilby, 1986; Sokolik & Yurin, 1986), whereas the Cl^- channel is activated (Shiina & Tazawa, 1987; Kourie & Findlay, 1989). In Fig. 4*B*, the dotted lines connect the data points obtained from experiments in 0.1 mol · m^{-3} Ca^{2+} and in 0.5 mol · m^{-3} Ca^{2+}, respectively. For a conclusive statement, much more of these extremely time-consuming experiments should be done. Thus, it can only be stated that the data in Fig. 4*B* are in line with the view that with lower outside Ca^{2+} concentration, the participation of the chloride channels becomes smaller, and the a_{5a} reversal potential moves closer to E_K.

The question of major interest here deals with the reaction in the photosynthetic apparatus, which causes the effect on the K^+ channel. From the comparison of the time constants in the Table, it has to be concluded that this reaction is identical to that labeled by τ_{5a} of chlorophyll fluorescence. This conclusion is based on the assumption that equal time constants in different output signals indicate the involvement of the same reaction. Of course, such a numerical coincidence of time constants may be fortuitous. However, the reliability is increased if the coincidence holds under different experimental conditions, especially under those that change the value of the common time constant. This has been found in the case of different light intensities by Vanselow et al. (1988, 1989*a*) and in different species, namely spinach, *Aegopodium podagraria* (Vanselow et al., 1988, 1989*a*,*b*), *Cygocactus truncatus* (Vanselow & Hansen, 1989) and in *Nitella* in the present investigation.

So far, we have no direct evidence of which reaction is labeled by τ_{5aF}. However, the comparison of our results with findings of other authors gives a quite clear picture. These findings are:

1) The involved K^+ channel is probably of the high-G K^+ (Ca^{2+})-type (*see* discussion above), which is activated by an increase in cytoplasmic Ca^{2+}.

2) Increase of external Ca^{2+} shifts the a_{5aV} reversal potential away from E_K (Fig. 4), thus indicating a Ca^{2+} action on the Cl^- channel as well.

3) A light-induced uptake of Ca^{2+} into the chloroplasts (Muto, Izawa & Miyachi, 1982; Kreimer, Melkonian & Latzko, 1985*a*; Kreimer et al., 1985*b*) and the resulting decrease of cytoplasmic Ca^{2+} concentration (Miller & Sanders, 1987) is well documented.

4) a_{5aR} in the Table is positive, indicating opening of the channel upon decrease in light intensity. This is expected if the channel is opened because of Ca^{2+} accumulation in the cytosol.

5) The uptake of Ca^{2+} is believed to be related to the activation of the NAD-kinase (Jarrett et al., 1982).

6) The Table shows that a_{5aF} is positive indicating an increase of chlorophyll fluorescence and thus a decrease of photochemical efficiency upon switching on photosynthetic electron flux.

The above findings lead to the hypothesis depicted in Fig. 5: the enhancement in electron flow by an increase in light intensity reduces $NADP^+$ and lowers the concentration in the $NADP^+$ pool. Consequently, photosynthetic efficiency is decreased, and F increases with the time constant τ_{5aF} (positive sign of a_{5aF} in the Table). The decrease in pool concentration activates Ca^{2+} uptake into the chloroplasts via an unknown mechanism. The increase in chloroplast Ca^{2+} concentration activates the NAD-kinase probably via calmodulin. The NAD-kinase causes a slow increase of the $NADP^+$ concentration (Muto et al., 1981; Jarret et al., 1982). Increasing $NADP^+$ concentration would increase electron flow and result in a decrease of F. Future research should test whether this decrease is related to the time constant τ_{5bF}, which was detected by Vanselow et al. (1988, 1989*b*) and by Dau and Hansen (1988*a*).

The resulting depletion in cytosolic Ca^{2+} concentration (and especially the enhancement caused by the downward steps in light intensity) is sensed by the K^+ (Ca^{2+}) channel, resulting in an increase (or decrease) of membrane resistance. The partial opening of the K^+ channels causes a hyperpolarization if the plasmalemma is depolarized to potentials more positive than E_K and a depolarization if it is more negative than E_K (Figs. 1–4).

This effect is different from that reported by Vredenberg and Tonk (1973), Spanswick (1974) or Bisson (1986). The decrease in membrane resistance after illumination measured by these authors is related to a slower time constant in the range of some minutes. This range is not covered by our experiments. We do not know whether or not this slow decrease in resistance is caused by a late release of Ca^{2+} from the chloroplasts. However, Miller and Sanders (1987) found that cytosolic Ca^{2+} concentration stays low in the light. This is expected from the measurements of Muto et al. (1982) showing an continuous light-induced uptake of Ca^{2+} into the chloroplasts for more than 20 min.

A very strong light-off-induced opening on the K^+ channel of *Eremosphaera* was reported by Köhler et al. (1985, 1986). This effect is similar to that reported here with respect to the time scale and the sign (opening upon reduction of light intensity).

Since the responses in *Eremosphaera* could be initiated by permeant acids, the first hypothesis was that the light effect in *Eremosphaera* is brought about by changes in cytosolic pH (Steigner et al., 1988). However, recent experiments of Thaler et al. (1987) dealing with the effect of Ba^{2+} indicate that changes in internal pCa induces the opening of the K^+ channel in *Eremosphaera*. This shows that the responses in *Eremosphaera* are not basically different from those in *Nitella*, but they are much more dramatic. The observed sensitivity to pH_i might be explained by the assumption that the K^+ channel is activated by Ca^{2+} as well as by a decrease in pH_i. However, the opposite effect of pH_i on a $K^+(Ca^{2+})$ channel was found in pancreatic β-cells (Cook, Ikeuchi & Fujimoto, 1984). Another effect, which may explain the action of pH_i on the K^+ channel, is a change in cytosolic Ca^{2+}-buffering capacity as observed by Zucker (1981) in neurones.

According to the model in Fig. 5, no messenger aiming at a physiological function is involved in the fast responses of V and R to a change in light intensity. The light effect on the K^+ channel related to τ_{5a} seems to be a side effect, and evidence for a role in metabolic control is not provided. This is similar to the model of the fast depolarizing effect of light on the electrogenic H^+ pump (Hansen et al., 1987), also shown in Fig. 5. Even though the model in Fig. 5 seems to be in line with present findings, further experiments are required in order to verify the involvement of the $NADP^+$ pool and of the NAD-kinase.

A "sophisticated" mechanism (i.e., a mechanism that is of relevance for the function of the cell) seems to be related to the complex time constants τ_d and τ_{os} (τ_{2V} and τ_{3V} in previous papers, e.g., Hansen, 1985) of the light action on membrane potential, namely, the involvement of a feedback loop controlling cytoplasmic pH (Hansen, 1978, 1980, 1985; Fisahn et al., 1986). It is not known whether the oscillatory components related to τ_{dR} and τ_{osR} are related to some homeostatic function. The Table shows that a lot of work remains to be done until all time constants are identified, and increased skill in curve fitting may reveal additional components.

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