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Patch-clamp studies of light-induced currents across the thylakoid membrane of isolated chloroplasts

Alexander A. Bulychev, Valerii F. Antonov and Elena V. Shevchenko

Biophysics Department, Biological Faculty, Moscow State University, Moscow (USSR) and Department of Medical and Biological Physics, Moscow I.M. Sechenov Medical Academy, Moscow (USSR)

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Light-induced currents through thylakoid membrane in isolated chloroplasts of *Peperomia metallica* were measured with suction electrodes under clamped membrane potential (MP). Comparison of the photocurrents and MP photoresponses on the same chloroplast indicated that the recording pipette had a low resistance access to a thylakoid lumen. Two components in the membrane photocurrent were distinguished. The fast inward current rose to an initial peak and declined within seconds to a steady level of about 50 pA. This component was not significantly affected by MP change in the range from −10 to +20 mV and seemed to reflect a light driven H^+ influx into thylakoids. The slow component was only evident under certain conditions, e.g., in the presence of 30 nM nigericin or substrate amounts of ATP or ADP with phosphate. It was directed either inward or outward depending on the sign of a set MP. With increasing length of light pulses both outward and inward currents increased in extent and exhibited a slowing down of decay kinetics. It is concluded that slow currents, irrespective of their direction, are carried through the same pathway by the same ion species. An apparent similarity was observed between photocurrents and MP photoresponses measured under voltage clamp and current clamp conditions. A strong sensitivity of photocurrent kinetics to variations in MP suggested that the baseline dark MP is an important factor affecting the time-course of the MP formation during illumination.

Introduction

Photosynthetic electron transport in chloroplast thylakoids is associated with an inward proton translocation and the formation of a transmembrane electric potential difference. The development of membrane potential (MP) under continuous light follows complex kinetics whose relation to primary and secondary ion fluxes has only been partially understood from the analyses of light-induced electrochromic absorbance changes [1], electric potential recordings with microelectrodes [2,3] and theoretical models of energy coupling [4].

The MP and pH gradient serve as a driving force for passive ion transport between thylakoids and chloroplast stroma. The ATPase couples the back flow of protons from thylakoids through H^+-specific gated channels to the synthesis of ATP. Effluxes of K^+ and Mg^{2+} together with an uptake of Cl^- occurs in thylakoid suspensions under illumination [5,6] and are probably mediated by ionic channels revealed in patch-clamp studies. A voltage-dependent chloride channel was characterized in swollen *Peperomia metallica* thylakoids [7] whereas K^+-specific voltage-dependent channels were observed in planar lipid membranes with fused spinach thylakoids [8]. Proton-selective channels activated by voltage were identified in lipid bilayers with reconstituted chloroplast ATPase [9]. More native systems are additionally required for studying ion transport across the thylakoid membrane.

One apparent manifestation of secondary ionic fluxes in electric potential recordings is the formation of a diffusion potential component under prolonged

Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; DTT, dithiothreitol; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid; MP, membrane potential.

Correspondence: A.A. Bulychev, Biophysics Department, Biological Faculty, Moscow State University, Moscow 119899, U.S.S.R.

illumination [6,10]. A postillumination decay of this component lasts from several seconds up to tens of seconds which exceeds the time constant of the membrane by one or two orders in magnitude. The diffusion potential of negative sign was seen in undamaged plastids and explained as a consequence of H^+ diffusion from acidic thylakoid lumen to chloroplast stroma [3,10,11]. Positive diffusion potentials were created in the light in the presence of valinomycin under low concentrations of K^+ and were interpreted as being due to K^+ diffusion from stroma to thylakoids [10]. However, positive postillumination potentials were also observed in the presence of ionophorous uncoupler A23187, an antiport exchanger of divalent cations against protons [6,10] which cast some doubt on the exclusive role of K^+ diffusion in the generation of these potentials. One possible interpretation to be considered is that secondary slow potential rise and a respective slow decline of the postillumination potential in the presence of ionophores results from H^+-pumping activity of ATPase under specific conditions promoting the ability of ATPase to hydrolyze ATP [4,12].

In order to clarify the origin of postillumination potentials and their possible relation to energy transduction it is desirable to measure transmembrane currents under controlled MP and initial symmetry of ionic environment in energized and uncoupled chloroplasts. Light-induced ionic currents were usually measured from the decay kinetics of field-indicating absorbance change [1,13] or by using permeable and impermeable pH-indicating dyes [1,14]. Patch-clamp technique has been applied recently to various cell membranes for recording single channel activities and whole cell currents [7,15,16]. In this technique, low resistance suction electrodes are used to form a tight seal with a membrane which permits the measurement of electrical currents through the membrane under clamped voltage [15]. In this study we applied the patch-clamp technique for recording light-induced currents across the thylakoid membrane of isolated chloroplasts and tried to distinguish components related to primary H^+ translocation and secondary ion fluxes on the basis of their different dependencies on the MP. This method allowed us to show the identity of mechanisms underlying postillumination potentials of a different sign.

Materials and Methods

Chloroplasts of *Peperomia metallica* were isolated from leaves as previously described [2,10] into a medium which contained 25 mM Tris-HCl (pH 7.8), 0.25 M sucrose, 25 g l^{-1} Ficoll 400, bovine serum albumine (1 mg/10 ml solution), 2 mM DTT and 50 mM KCl. DTT was added to promote the photosynthetic electron transport and CO_2 fixation in intact chloroplasts [17]. The ability of DTT to modulate H^+-ATPase in thylakoids [18–20] was also taken into consideration. Additions of ATP, ADP (sodium salts) and KH_2PO_4 were made when required as specified in the text and legends to figures.

Chloroplasts of *Anthoceros sp.* were used for MP recordings in situ. Sections of thalli were bathed with 0.1 mM KCl, 1.0 mM NaCl, 0.5 mM $Ca(NO_3)_2$, 5 mM Hepes-NaOH (pH 7.0) [11]. Glass microcapillary electrodes filled with 1.0 M choline chloride were used for MP measurements.

Patch-clamp recordings were performed according to a method previously described [15]. Micropipettes were pulled from Pirex capillaries in two steps in order to produce steep tapers. After heat polishing micropipettes were mounted in a holder with an internal reference Ag/AgCl electrode and an outlet for an application of suction. Micropipettes were filled with a medium that was used for chloroplast isolation. An external Ag/AgCl electrode was placed into the measuring chamber. Electrical currents were measured with a patch-clamp amplifier (Bioinstruments, Puschino, USSR). It was possible to switch the recording circuit from a voltage clamp mode to a current clamp mode for monitoring the potential difference between the pipette and an external electrode. Currents and voltages were displayed on an oscilloscope and recorded on a pen recorder. Resistances of suction electrodes were about 10 MΩ. After making contact with a chloroplast by suction, seal resistances from 50 up to 100 MΩ were obtained. Gigaohm seals were not observed, presumably because of a high permeability of an outer envelope membrane that contacted inevitably with a tip of a suction pipette. Low resistance pipette-membrane seals (of about 100 MΩ) proved to be applicable in studies of other plant cell membranes and were employed, for example, in recent patch-clamp experiments with sweet corn protoplasts [21]. The recording configuration was supposedly similar to whole cell recording with an essential difference that the thylakoid folded system played the role of a whole cell.

The measured currents were filtered with a low pass filter having a cutoff frequency of 1 kHz. Holding MP, also termed clampled voltage, represents the potential of the micropipette in contact to thylakoid lumen with respect to an external electrode. Membrane potentials were counted in relation to the dark MP level that was taken as zero.

Preliminary manipulations with a specimen were done under dim green light. After a seal was achieved, a chloroplast was illuminated with pulses of white light of full (500 W/m^2) or attenuated intensity. Durations of light pulses varied in the range from 0.1 to 10 s and were usually 2–3 s. A water filter was inserted in the light path in order to prevent heating by long light

pulses. A narrow excitation beam (50–100 μm in diameter) was used. It was possible to make recordings from several chloroplasts with one micropipette, although the first seal was most stable. Suctions were done from a flat side or an edge of a lense-shaped chloroplast, in the vicinity of photosynthetic lamella. Suctions made from the convex surface neighbouring a stromal compartment did not lead to photocurrent recording. Dark periods between consecutive light pulses were usually about 0.5 min long.

Results

Description of a photocurrent

When a micropipette was pressed against an isolated chloroplast and a negative pressure was gently applied a small portion of a chloroplast with underlying photosynthetic lamella was sucked into the tip of the recording pipette and formed there an Ω-shaped vesicle. At this stage a photocurrent usually appeared; otherwise additional suction was applied in order to obtain large photocurrents. It was assumed that the envelope and thylakoid membrane inside the pipette became permeabilized or disrupted as a result of a pressure-induced deformation and a low resistance access to a thylakoid lumen was established. Fig. 1a shows a typical photocurrent sygnal measured in the absence of DTT or other additives at a holding potential of 0 mV. The photocurrent was directed inward the pipette. The peak value was usually 100–200 pA and the steady state current was about 50 pA. In some cases peak currents exceeded 300 pA. A small outward current was often seen after darkening (dashed line in Fig. 1a) which was interpreted as a ΔpH-driven outflux of protons from thylakoids.

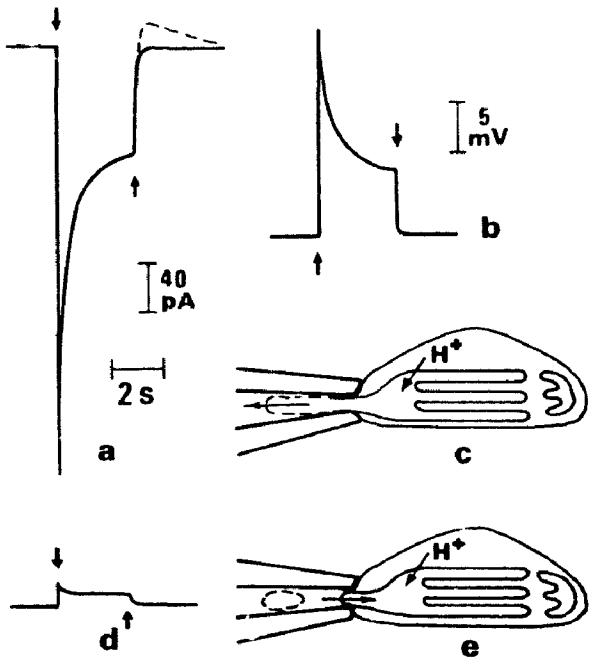

Fig. 1. Measurements of the photocurrent (a, d) and the MP photoresponse (b) with suction electrode on a chloroplast of *P. metallica* and diagrams of supposed recording configurations (c, e). (a) Photocurrent (inward current downward) measured in the absence of DTT at a holding MP of 0 mV. (b) The MP photoresponse of the same chloroplast. (c) A supposed patch-clamp configuration illustrating the origin of an inward photocurrent. (d) Inversion of a photocurrent after tearing off the tip-localized vesicle. (e) A diagram explaining the origin of a small outward photocurrent after the vesicle in a pipette was torn off. Arrows mark the onset and end of illumination.

When the measuring circuit was switched from a voltage clamp mode to recording MP in the same experiment, a typical light-induced electric potential change was observed (Fig. 1b). This result suggested that the recording pipette has a low resistance access to the thylakoid lumen and that the inward current reflected an influx of protons into the thylakoid. A possible recording configuration is represented schematically in Fig. 1c. It corresponds to a 'whole cell' mode which originated after permeabilization of an Ω-shaped invagination inside the pipette tip.

By applying further excessive negative pressure it was possible to tear off the vesicle in the pipette. That was accompanied sometimes with a great reduction of a photocurrent, at least an order in magnitude, and a simultaneous reversion of its direction (Fig. 1d). The supposed recording configuration in that case is shown in Fig. 1e. Under these conditions the photocurrent flowed out of the pipette through a resealed surface into the thylakoid. There were occasional records with an apparent noise in a photocurrent in the absence of noise in darkness. It is conceivable that such records arise from incomplete sealing, when the tip opening contacts both the thylakoid lumen and extrathylakoid space. Only photocurrents of large magnitude without noise were considered as meaningful.

Fig. 2a shows a schematic diagram of a basic recording configuration and its equivalent circuit. The main elements are a thylakoid membrane resistance R_m in series with an electromotive force E_m and a source of a photocurrent in the thylakoid membrane. R_l' and R_l'' are leak resistances which provide shunting between a pipette and the medium and between a chloroplast stroma and the medium, respectively. R_e is the resistance of the envelope membranes. Membrane capacitances are not included for simplicity because capacity currents are negligible under voltage clamp conditions. A required pipette resistance R_p is very small compared with a parallel resistance of R_m and R_l'.

In Fig. 2b is a simplified equivalent circuit redrawn for the case where R_l'' is much less than R_m. Under this reliable assumption, parallel resistances R_l'' and R_e can be omitted. This reduced scheme is also valid for the case of damaged envelope membranes, i.e. when R_e is small and can be considered as a short. As can be seen from Fig. 2b the total current flowing

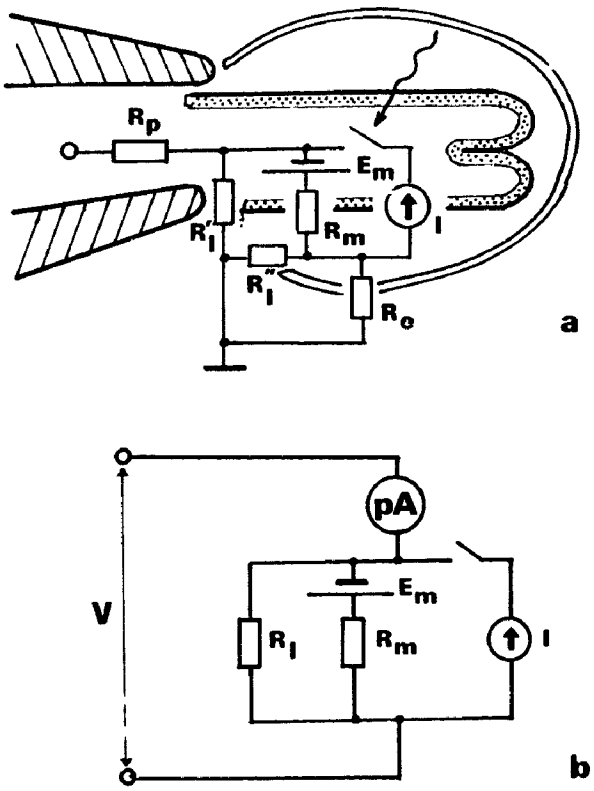

Fig. 2. Schematic diagram of the recording configuration and its equivalent circuit. (a) Schematic view of a chloroplast and a pipette tip. R_p, R_m, R_e and R_l denote resistances of the pipette, thylakoid membrane, envelope membranes and shunting pathways, respectively. A current source, I is switched on by illumination. E_m is the electromotive force which may equal zero or change in the course of ion redistribution between thylakoids and chloroplast stroma. (b) A simplified equivalent circuit of the thylakoid membrane with a shunting pathway. A current-voltage convertor is shown as a picoammeter; V signifies the holding membrane potential.

through an ammeter (I-V convertor) is composed of currents through a shunt resistance and through a thylakoid membrane. The light-induced current fluctuations measured under voltage clamp conditions might result from light-driven currents (e.g. H^+ pump as a current source) as well as from light-induced changes in membrane resistance R_m or from a change of electromotive force E_m associated with a redistribution of ions across the membrane. A leak current does not change during illumination unless leak resistance is modulated by light. In these experiments a linear leak current contributed a large portion to the steady dark current (results not shown). A leak current, as approximated by steady dark current, had inward or outward direction depending on the sign of clamped potential and it was absent at potentials about 0 mV. In contrast, photocurrent had the same inward direction at various clamped potentials and it did not disappear under conditions of zero leak current. Therefore one might conclude that a photocurrent was independent of a leak current.

Effect of the membrane potential

Photocurrents monitored in the absence of ionophores or other additions did not change their shape significantly when the holding MP was set at different levels in the range from −10 to +20 mV. Attempts to set MP beyond that relatively narrow range were mostly unsuccesful because the dark current or a photocurrent exceeded saturation limits of the amplifier.

More complex kinetics of the photocurrent were observed in the presence of nigericin. Electroneutral K^+/H^+ exchange mediated by nigericin leads to a diminished pH gradient and a reversal of light-induced K^+ flux from normal outward to inward direction [6]. In Fig. 3 are shown the time-course of photocurrents under various MP in the presence of 30 nM nigericin. The initial peak of the current had always inward direction but subsequent changes depended on the voltage applied. Postillumination currents of different signs were clearly seen at positive and negative MP.

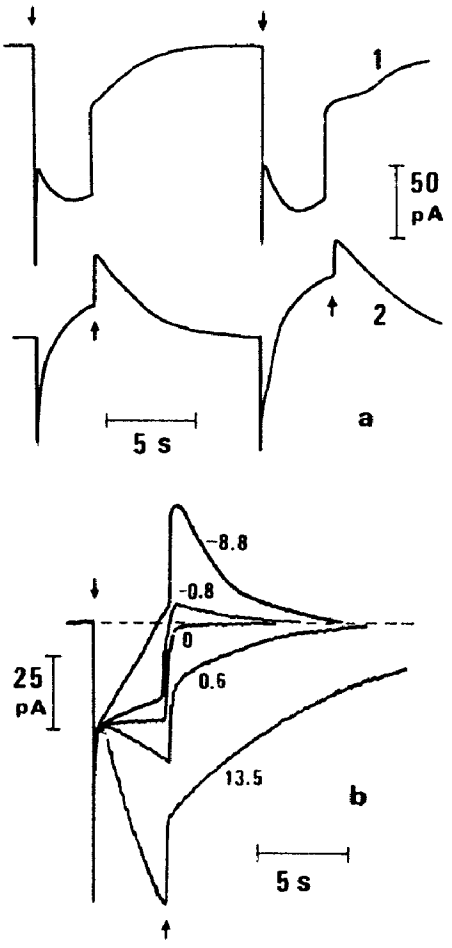

Fig. 3. Kinetics of the photocurrent in nigericin-treated chloroplasts. (a) Photocurrents induced by two 3 s light pulses at MP of 7.1 mV (1) and −3.3 mV (2). (b) A reversal of postillumination currents at zero MP; the numbers indicate the clamped MP in millivolts. The standard experimental medium was supplemented with 30 nM nigericin and 10 mM KH_2PO_4. Downward and upward arrows indicate the light on and light off points, respectively.

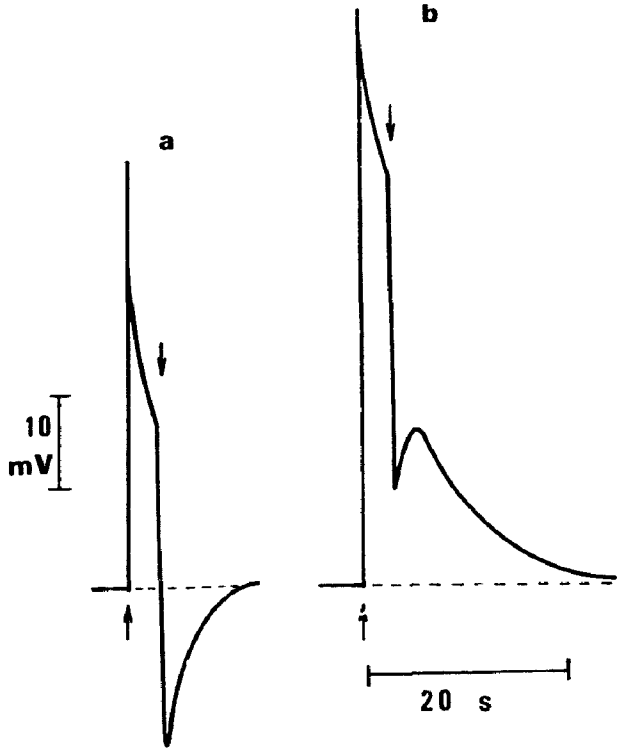

Fig. 4. Kinetics of MP changes in *Anthoceros* chloroplasts in situ during and after illumination. (a) Control. (b) In the presence of 10 μM nigericin. The beginning and the end of illumination are marked with arrows.

The postillumination currents were defined as inward or outward depending on their direction with respect to the steady level of the dark current. According to this definition a postillumination current had an inward direction at positive MP and an outward direction at negative MP. Complex kinetics of photocurrents were fairly reproducible without prolonged dark adaptation (Fig. 3a). This is in apparent contrast to a significant effect of the dark adaptation on the MP photoresponse in situ in the absence of uncouplers [22].

As shown in Fig. 3b, a slow component of the photocurrent and postillumination currents exhibited a reversal of sign at zero MP. Different kinetics of the photocurrent at positive and negative MP could be observed even at very small displacements of the holding MP from the dark level.

The effect of nigericin on photocurrents in isolated chloroplasts was compared with its effect on the MP formation. The results obtained with chloroplasts of two different plant species were similar, although they are most clear in chloroplasts of *Anthoceros*. Typical light-induced MP with a transient postillumination undershoot were seen in the absence of the uncoupler (Fig. 4). After a treatment of a plant preparation with 10 μM nigericin, the potential undershoot was suppressed and a positive post-illumination potential appeared. Although an external concentration of nigericin was large, its concentration in the chloroplast stroma was expected to be low due to barrier properties of the plasmalemma and the envelope membranes of plastids. Fig. 4 indicates a similarity between MP changes in the presence of nigericin in *Anthoceros* and the transmembrane currents in nigericin-treated chloroplasts at positive MP. Postillumination potentials induced by nigericin were inhibited by further addition of 50 μM DCCD.

Dependence on light duration

Voltage-dependent components of the photocurrent in isolated chloroplasts were often seen in the absence of nigericin if the medium was supplemented with 1 mM ADP with 5 mM phosphate or 1 mM ATP alone or a mixture of 0.5 mM ATP and 0.5 mM ADP with phosphate. A reversal of the postillumination current under a change of the holding MP from about +10 to −10 mV served as a sensitive indicator for occurrence of slow voltage-dependent components. Sometimes the reversal of postillumination currents was achieved after several light exposures whereas in some cases no effect

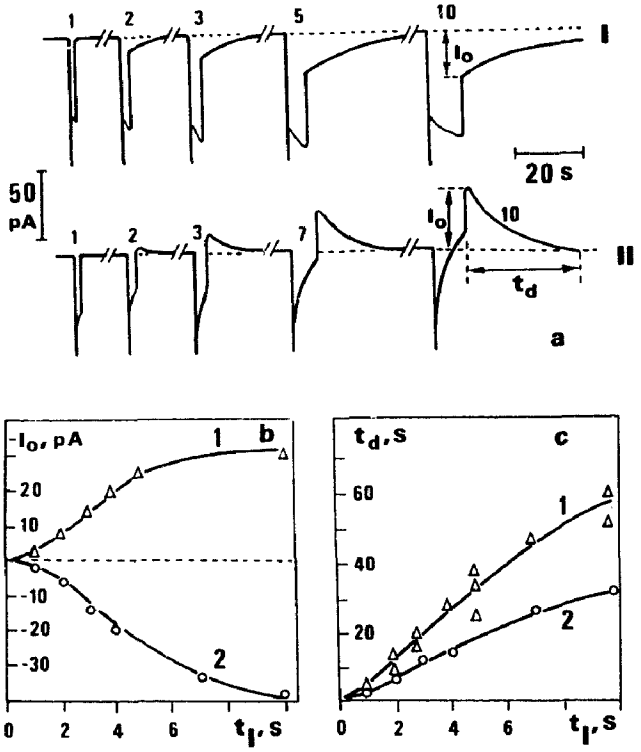

Fig. 5. Membrane currents induced by various light exposures in the presence of 0.5 mM ATP, 0.5 mM ADP and 10 mM KH_2PO_4. (a) Kinetics of currents at holding MP of +11.8 mV (*I*) and −2.5 mV (II). Numbers over the curves indicate the lengths of light pulses (t_l) in seconds. (b) The magnitude of postillumination currents as a function of light duration at 11.8 mV (1) and −2.5 mV (2). (c) Decay time, t_d of postillumination currents as a function of light duration at 11.8 mV (1) and −2.5 mV (2).

of MP on postillumination current was apparent. Nevertheless, additional characteristics of postillumination currents were obtained under these conditions.

Fig. 5 shows current responses induced by light pulses of various duration under positive and negative MP in the presence of 0.5 mM ATP, 0.5 mM ADP and 10 mM phosphate. The plots are also shown for the initial magnitude of the postillumination currents and full times of their decay as a function of a light pulse length. The dependencies obtained at negative and positive MP looked similar which suggested a common origin for inward and outward slow currents. The decay of these currents was completed within several seconds after short light pulses but lasted tens of seconds after long pulses. The duration of current changes after illumination was dependent on the magnitude of the holding MP.

Tetraethylammonium was reported to block the conductance of K^+ channels in thylakoid membrane [8]. An addition of 18 mM tetraethylammonium bromide to ATP-containing medium did not prevent the voltage-dependent reversal of postillumination currents after half an hour incubation but inhibited postillumination currents, perhaps unspecifically, after longer periods (results not shown).

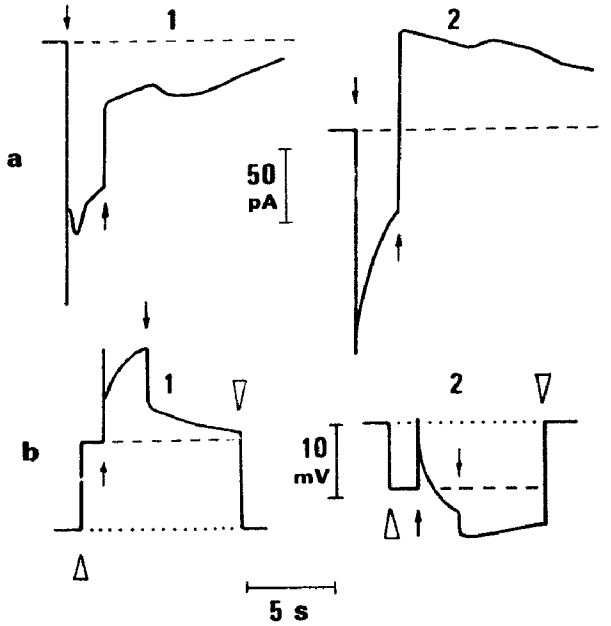

Fig. 6. Light-induced current and MP signals recorded in a voltage clamp and current clamp modes, respectively. (a) Membrane currents at MP of 11.0 mV (1) and −6.1 mV (2). (b) Kinetics of light-induced MP changes in the same chloroplast under passing outward (1) or inward (2) current with an intensity of 0.3 nA. Triangles mark the moments of switching the current on and off and arrows indicate the beginning and the end of illumination. The medium contained 1 mM ATP.

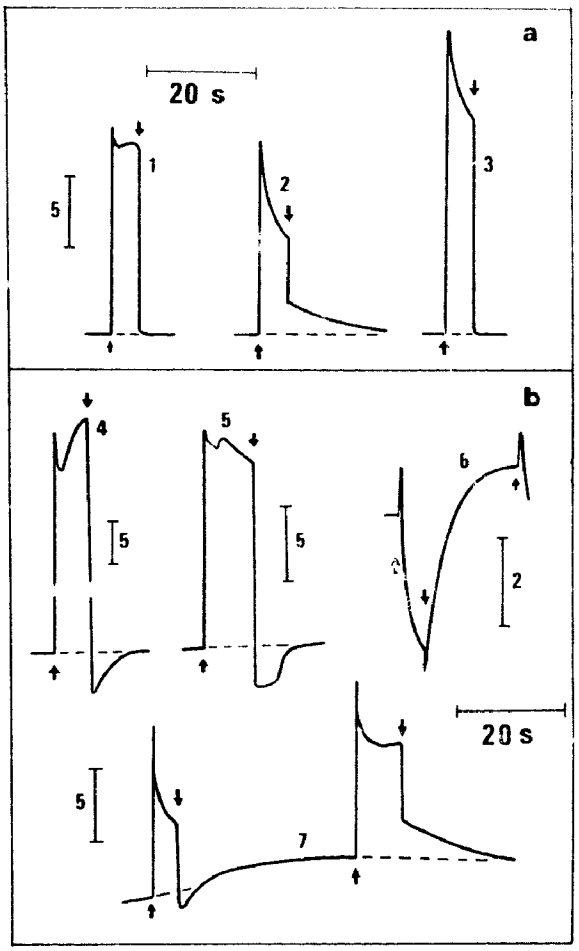

Fig. 7. Membrane potential photoresponses of *P. metallica* chloroplasts in the presence of ADP (a) or ATP (b). The medium was supplemented with: 1 mM ADP (1), 1 mM ADP and 10 mM phosphate (2), 1 mM ADP, 10 mM phosphate and 50 μM DCCD (3), 1 mM ATP (4–7). Vertical bars with figures signify the scale for membrane potentials in millivolts. Upward and downward arrows mark the beginning and the end of illumination, respectively.

Comparison with light-induced membrane potentials

In Fig. 6 are presented light-induced currents and MPs recorded from the same chloroplast when the measuring circuit was switched in a voltage clamp or current clamp mode. Passing an outward current (0.3 nA) shifted the dark MP to the positive side and brought about a MP photoresponse with a secondary potential rise and a slow postillumination decay. An inward current produced the negative shift of the dark MP and brought about a photoresponse with a secondary potential decrease and an obvious potential undershoot. It appears that postillumination currents and potentials are related manifestations of the same phenomenon.

The kinetics of light-induced MP formation in the presence of ADP or ATP were measured on isolated plastids with conventional microelectrodes (Fig. 7). In the presence of 1 mM ADP, MP returned rapidly after

darkening to the initial level in the absence of phosphate but declined slowly in its presence. The postillumination potentials were abolished after an addition of 50 μM DCCD.

A variety of MP photoresponses were measured in the presence of 1 mM ATP. Most of them corresponded fairly well to MP changes of intact *Anthoceros* chloroplasts in having a secondary peak of the potential in the light and a transient potential undershoot after darkening. In rare cases photoresponses consisted of fast positive and slow negative potential changes as was described for *P. metallica* chloroplasts in situ [2]. There were also recordings with spontaneous transitions from negative to positive postillumination potentials The variations in photoresponses are presumably related to an elevated heterogenity of chloroplast preparations in the presence of ATP. Such a heterogeneity becomes evident when individual chloroplasts in a suspension are probed and their electrical photoresponses are compared.

Taking into account the effect of MP on photocurrents and photopotentials (Fig. 6) one could find a certain consistency in a seeming variety of photoresponse kinetics. Various types of MP photoresponses could be attributed to different background voltages at thylakoid membrane.

Discussion

Our results show that patch-clamp technique can be applied for studying light-dependent ion transport across photosynthetic membranes. Light-induced currents were unrelated to a leak current which indicated that they did not flow through a shunt resistance. These currents could not arise at the envelope membranes. They were never observed when suctions were made to a chloroplast envelope at a distance from photosynthetic lamella. A spontaneous destruction of a chloroplast envelope in some experiments did not lead to a disappearence of the photocurrent.

Suction electrodes have a low resistance due to a specific geometry of a micropipette tip. Because of this advantage the MP of a thylakoid membrane can be controlled within a limited range and a sum of ionic currents during and after illumination can be measured. Routine measurements of photoelectrical responses with suction electrodes are even easier to perform as compared with microelectrode recordings because they do not require an additional pipette for chloroplast holding. Suction electrodes provided stable and reproducible recordings which lasted up to half an hour.

A thylakoid sack attached to a pipette tip differs in some aspects from intact thylakoid. Under physiological conditions at a steady state, the H^+ influx is compensated for by a passive efflux of protons and no net current passes the thylakoid membrane. Under patch-clamp recording as it is shown in Fig. 2a, the main route for protons pumped into a thylakoid lumen is their removal into a pipette. This is so because H^+ diffusion in water proceeds with a much higher rate as compared with a backward diffusion through the membrane. The active influx of protons uncompensated by a passive H^+ efflux is stoichiometrically related to an electron transport rate.

According to a simplified model of a thylakoid membrane, a stoichiometric ratio $H^+/e^- = 2$ [1], a concentration of P700 is about 10^{-13} mole cm^{-2} [4,12] and a steady electron flux through one electron transport chain is 100 s^{-1} [3]. Then a steady current of about $2 \cdot 10^{-6}$ A cm^{-2} in the light is expected. Comparing the latter value with a measured steady current of 50 pA one would estimate the thylakoid membrane surface involved as $2.5 \cdot 10^{-5}$ cm^2 which is equal to a surface of a sphere with a diameter of 30 μm. Space clamp requirements are usually fulfilled for cells of such dimensions [23] but still could be questionable for chloroplasts in view of a complex geometry of a folded membrane system. If a partial recycling of protons in the membrane is taken into consideration then the above estimate gives a lower limit for the membrane surface involved. An estimated area fits in with dimensions of blebs that are formed in hypotonically swelled chloroplasts of *P. metallica* [7].

There was an apparent similarity between the kinetics of light-induced currents and potentials as measured with suction electrodes and microelectrodes, respectively. A kinetic similarity was also observed between current and MP photosignals measured under voltage clamp and current clamp conditions (Fig. 6). Slow components of both signals were especially sensitive to a baseline level of MP. Such sensitivity indicates that a dark MP level is an important factor influencing a time-course of the MP photoresponse. It is known that in the dark there exists a fairly large Donnan-type MP in thylakoids [24]. Possible effects of the Donnan potential on the light-induced ion fluxes and MP formation were considered using a simulation model [12], although no dramatic modifications in MP photoresponses were noted. A damage or a disruption of a chloroplast envelope should result in a change of a dark thylakoid MP and lead to alterations in the MP photoresponse kinetics (see Fig. 7b and Ref. 25).

Two superimposed components were distinguished in the light-induced currents in the presence of nigericin or adenine nucleotides (Fig. 8). The initial fast component (F-current) appeared as a sharp peak of an inward current at any clamped MP. This current ceased rapidly after darkening. The other component was slow (S-current) and had either inward or outward direction depending on the sign of the holding MP. The F- and S-components showed different dependencies on the

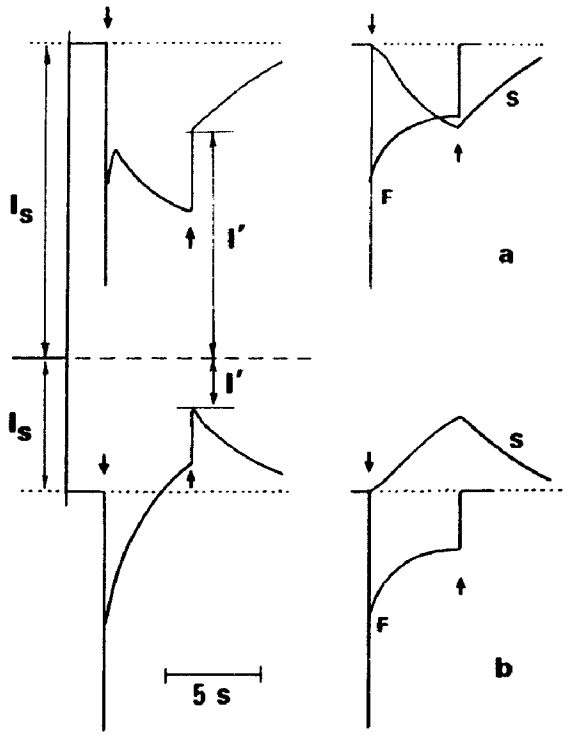

Fig. 8. Schematic decomposition of light-induced membrane currents into fast (F) and slow (S) components at positive (a) and negative (b) membrane potentials. Broken line in the middle is a zero current level. Symbols I_s denote steady dark currents at clamped MP and I' are instant dark currents measured after switching off the light at positive (upper row) and negative (bottom row) holding MP. Photocurrents are defined as changes of the current with respect to a steady dark level shown by dotted lines. In the right panel, fast and slow components of a photocurrent are presented.

voltage thus giving rise to a variety of photocurrent kinetics.

A model of a thylakoid membrane is represented by a light driven proton pump in parallel with a quasi-reversible H^+-translocating ATPase and passive pathways for H^+, K^+, Cl^- and other ions [4,12]. A fast inward current may be attributed to functioning of light-driven pumps whereas slow component and postillumination currents may be related to modulated fluxes through other pathways. It would be expected that the magnitude of the fast photocurrent is dependent on the polarity of the holding MP. Such a dependence has not been demonstrated clearly because a comparatively narrow range of clamped potentials was used.

A phenomenon of the reversal was observed for S-currents. Such a phenomenon is generally typical for passive ionic fluxes. A peculiar feature of S-currents is that their direction is opposite to what is expected for currents through open channels. Formally, it can be taken as evidence that a certain component of the dark current is reduced by light to different degrees, depending on the length of a light exposure. A light-induced decrease of the membrane or shunt conductance would explain the fact that the absolute value of the current after darkening (I' in Fig. 8) is less than the steady dark current I_s at any polarity of MP. A decrease of the conductance for cations during illumination might occur, in principle, in association with surface potential changes on the lumenal side of the membrane [26]. However, experimental data obtained so far suggest that the membrane conductance increases in the light under both phosphorylating and nonphosphorylating conditions [27].

Alternatively, transients of a passive current could be caused by a change of a driving force due to creation and dissipation of a concentration gradient for some ion. Such gradients could be created by secondary ion fluxes and 'scalar' protons released or consumed in stroma-localized reactions. Interpretation of S-currents is inconclusive at present because little is known about the extent and dynamics of ATPase dependent H^+ fluxes in the dark and in the light under conditions employed. As DTT was present, it was possible that ATPase dependent H^+ fluxes contributed to the dark current and changed during illumination due to a shift in the activation state of the enzyme and perturbations of the phosphorylation potential.

A possible relation of postillumination currents and potentials to ATPase is in line with an inhibitory effect of DCCD on postillumination potentials, although this inhibition might have been caused by unspecific interactions of DCCD with membrane-bound carboxyl groups. The direction of postillumination currents (inward at positive potential in the pipette) also may suggest an active cation influx driven by H^+-ATPase. With this view, a deceleration of the S-current decay at increasing MP would be explained as a direct or indirect (mediated by a distribution of ions or charged substrates) stabilization of H^+-transporting ATPase by membrane energization. However, additional assumptions would be needed to explain a reversal of S-currents under small changes of the holding MP. One may suggest, for example, that the membrane system attached to a pipette, due to its complexity, contains areas with opposedly oriented ATPases. In that case, a portion of enzyme complexes would be activated at positive MP and another portion would become active at negative MP.

From similar effects of varying light exposures on outward and inward S-currents one may conclude that these currents are carried through the same pathway and perhaps by the same ion species. Protons are likely candidates because H^+ fluxes, in contrast to fluxes of other ions, are sustained for many seconds after darkening [4,12].

It is noteworthy that S-currents were not seen in the presence of DTT and phosphate unless nigericin or adenine nucleotides were added. This may indicate that S-currents arise under conditions when pH gradi-

ent is reduced by ionophores or by substrates for photophosphorylation. In addition, ADP and ATP could act as chelators for Mg^{2+} and interfere ATPase functioning [28]. On the other hand, nigericin at low concentrations (10–30 nM) stimulates photophosphorylation [29,30] and may thereby activate the same ion fluxes as do the substrates for phosphorylation.

Further studies with application of specific blockers of ion channels are required to identity particular ion currents flowing through thylakoid membranes in darkened and illuminated chloroplasts. Suction electrodes may provide a useful tool for studying the effects of membrane voltage on electron transport and associated processes.

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