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Patch-Clamp Study of Vascular Plant Chloroplasts: Ion Channels and Photocurrents

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This article presents direct measurements of large-conductance cation channel ($G = 730$ pS in 100/50 mM KCl; $P_{K^+}/P_{Cl^-} = 2.8$) and light-induced current (photocurrent) in chloroplasts from C_4 plants, *Amaranthus hybridus* and *Zea mays*, using the conventional patch-clamp technique. It was shown that preillumination of chloroplast gave rise to a fast decaying transient ($\tau \cong 6$ ms) in the light-induced current for the second and following light pulses. This transient increase of photocurrent was interpreted as a consequence of photoreducing of the redox pool between photosystems.

KEY WORDS: Chloroplast; patch-clamp; ion channel; light-induced current; *Amaranthus hybridus, Zea mays*.

INTRODUCTION

Light-driven electron transfer in photosynthetic membrane causes active H^+-pumping into the thylakoid lumen. The pH gradient built up across the thylakoid membrane is used by vectorial proton-translocating ATPase for ATP synthesis, while stroma alkalinization is necessary for the activation of key enzymes in the CO_2 fixation cycle (Junge and Jakson, 1982; Heldt *et al.*, 1973; Baler and Latzko, 1975).

The formation of electrochemical gradient for protons across the thylakoid membrane in light has been previously explored by a set of indirect methods including measurements with pH- and potential-sensitive dyes (Auslander and Junge, 1974; Schuurmans *et al.*, 1978), monitoring of electrochromic absorbance changes of photosynthetic pigments at 515 nm (Junge and Jakson, 1982), and measurements of delayed fluorescence intensity (Bulychev *et al.*, 1985). Only two plant species, *Peperomia metallica* and *Anthoceros sp.*, which have abnormally large chloroplasts, allowed direct recordings using microelectrode techniques (Bulychev *et al.*, 1972; 1976, 1985; Vredenberg, 1976; Remiš *et al.*, 1986). Recently, Bulychev *et al.* (1992) showed the applicability of standard patch-clamp technique for measurements of light-induced current across the photosynthetic membrane of *P. metallica*. The advantage of the patch-clamp method is the possibility to work with smaller objects, say of a few micrometer in size. Accordingly, the patch-clamp technique has been successfully applied to study the ion channels in the intact envelope of chloroplasts from Charophyte alga *Nitellopsis obtusa* (Pottosin, 1992, 1993), although the activity of the chloroplasts envelope channels was also investigated in different reconstituted systems (Flügge and Benze, 1984; Keller *et al.*, 1988; Schwarz and Wagner, 1992; Mi *et al.*, 1994).

In this paper we describe the procedure of application of the patch-clamp technique to study the ion channels in the envelope as well as the photocurrents in the thylakoid membranes with intact chloroplasts from two C_4 plants. We found a significant difference of photocurrents in dark- and light-adapted preparations. Its possible origin is discussed in relation to the function of the photosynthetic electron transfer chain.

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MATERIALS AND METHODS

Leaves were collected from the field-grown plants early in the morning and kept at 4°C during the day of the experiment to avoid starch formation. Chloroplasts were obtained mechanically as described in Bulychev *et al.* (1972). Briefly, the pieces of the leaf were placed in a solution containing (mM): KCl, 50; Sorbitol, 250; EGTA, 0.5; and HEPES-KOH, 5 (pH = 7.2). Cuticle was gently removed using a sharp razor blade. Then the tissue was scraped so the leaf cells were broken and chloroplasts were released in the medium. The preparation was filtered to remove cell debris, and an aliquot containing chloroplasts was transferred into the experimental chamber filled with the same solution. For the patch-clamp measurements, intact unbroken chloroplasts of 10–15 μm size from *Amaranthus hybridus* and 6–8 μm from *Zea mays* were selected. Microscopic analysis of leaf sections showed that these were approximately twice as large as compared to the average size chloroplasts and were preferentially located in the bundle sheath cells. Microelectrode were made from soft or hard glass, hematocrit or 7052 AM-system, respectively, by two-step pulling. Soft glass electrodes required further intensive heat polishing to decrease the tip diameter. Patch pipettes were filled with a solution filtered through 0.2 μm filter and containing (mM): KCl, 100; EGTA, 0.5; $CaCl_2$; 1.5; and HEPES-KOH, 5 (pH = 7.2). After insertion of the patch electrode into the solution, the electrode offset potential was corrected by electronic means via the patch-clamp amplifier we were using, and tested by patch disruption at the end of the experiment (drift less than ±2 mV). The successful recordings (n = 142) were obtained with an electrode resistance of 11 MΩ as an average. Soft glass in most cases showed better adhesion to the envelope membrane (82% of total successful recordings). The standard patch-clamp technique in attached and inside-out modes was used for ion channel recording. Sequential disruption of envelope and thylakoid membranes gave access to the thylakoid lumen, and it was possible to measure light-induced current across the thylakoid membrane. This recording configuration was considered similar to whole-cell recording. The amplifier was a homemade one designed according to F. J. Sigworth "Electronic Design at the Patch Clamp" (in *Single Channel Recording*, B. Sackmann and E. Neher, eds., Plenum Press, New York, 1983).

Photocurrents were induced by short flashes (maximal light intensity measured at the sample position, $E = 1388\ W/m^2$; full-width at half-height of 1 ms) or long pulses of white light ($E = 8.2\ W/m^2$). The duration of continuous light stimulus (1–1000 ms) was controlled by means of the exposure controller and the shutter of a Minolta XG-1 camera. Flash and light intensity could be decreased by a series of neutral dark filters (ND2 and ND4). The response to the flash was 90% of maximum when the flash intensity was reduced to 6.5% of maximal intensity (S.E. = 1.3%, n = 3).

Voltages given throughout are in reference to the intrachloroplast side, and negative currents are the movement of positive charges into the chloroplast. Records were filtered at 1 kHz, digitized and stored in hard disc, and/or displayed and photographed from the oscilloscope screen. Analyses were carried out in part using p-Clamp 5.1 acquisition software and Plot50, as well as manually.

Blebs (swollen thylakoids) were obtained by a procedure similar to the one described in Schönknecht *et al.* (1988). Chloroplasts isolated in a solution containing (mM) KCl, 20, Trizma, 10, $MgCl_2$, 5 (7.2 pH), as described above, were osmotically shocked to rupture the envelope. After a while (~ 15 min) blebs started to appear; measurement of diameters began approximately 15 min after the first blebs appeared.

RESULTS

General Observations

The tight seals usually were formed in two steps. First, after the application of light suction the resistance increased up to 50–100 MΩ. Then, it spontaneously increased further up to 0.5–10 GΩ, reaching the sustained level. However, in many cases the application of positive holding potential (around 30 mV) or pulsed suction was helpful in improving the resistance at the second stage. The percentage of successful recordings of single-channel activity in the intact envelopes of chloroplast obtained from 4–5 weeks old *Amaranthus* plants was around 20%. No significant changes in the channel activity were observed upon the patch excision, and the inside-out patches were usually stable unless large (> 30 mV) negative steps of potential were applied.

An example of single-channel recording at different potentials obtained with *Amaranthus hybridus*

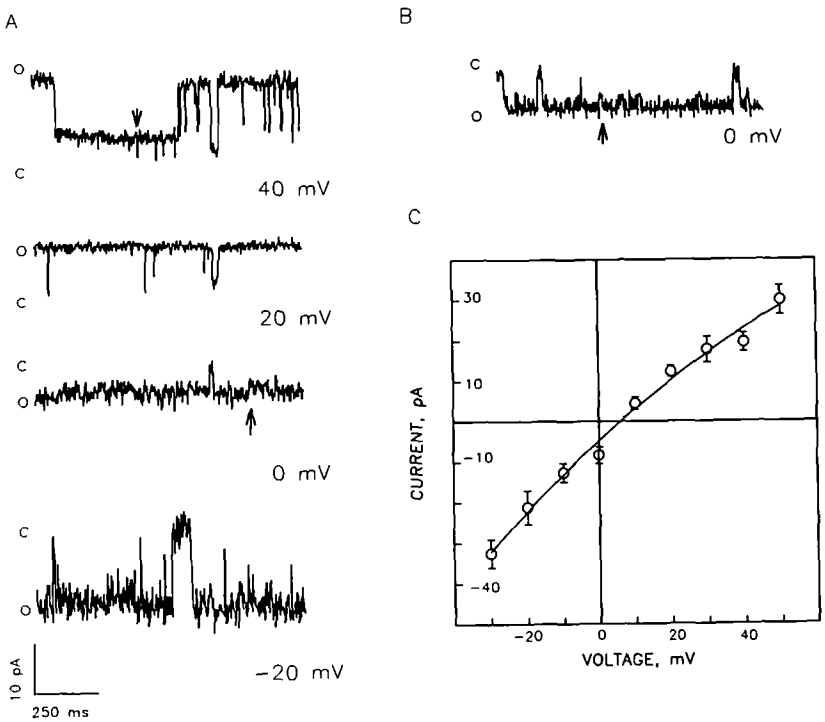


Fig. 1. Single-channel currents in the chloroplast envelope membrane. A and B: Records of ion channel activity in chloroplasts of *Amaranthus hybridus* and *Zea mays*, respectively. O and C correspond to completely open and close states, respectively. Arrows indicate the channel low-conductance states. C: I/V plot for the open state of *Amaranthus* channel. Means obtained by averaging data collected from 10 samples, bars are S.E. Solid line is low-order polynomial fitted to the data. 100 mM KCl in patch pipette and 50 mM KCl in the bath. Nernst potential for K^+ was +17 mV in these ion conditions. The reversal potential was +7.8 mV, indicating a weak K^+ over Cl^- selectivity. The sign of the voltage defined as positive corresponds to intrachloroplast side made more positive; the current corresponds to the influx of cations into the chloroplast.

chloroplast is presented in Fig. 1A. Besides the fully open state, substates of smaller current amplitude were detected. With long incubation, the occurrence of the fully open state diminished irreversibly, while a substate with a conductance of around 130 pS in 100/50 mM KCl was stable in time. Similar kinetic behavior (i.e., closure to substate) was observed when the potential was stepped up to large positive values (> 40 mV), with a slow (within seconds) recovery of the kinetics when the voltage was returned to lower values. Steps to negative potentials had a generally smaller effect on channel's kinetics, but the increase of current noise (flickering) in the open state was recorded at these potentials (Fig. 1A).

The current–voltage (I/V) relationships of the channel in the open state were analyzed in 100/50 mM KCl gradient to access the channel selectivity (Fig. 1C). The reversal potential of the I/V curve, V_r, was 7.8 ± 1.4 mV ($n = 10$), which implied slight selectivity for K^+ over Cl^-, $P_{K^+}/P_{Cl^-} = 2.8$ as calculated using the Goldmann–Hodgkin–Katz equation. Slight inward rectification of the I/V curve was observed, which probably dealt with the larger cation flux from the side with higher salt concentration. Linear regression of the I/V curve (not shown) gave a chord conductance of 730 ± 40 pS for the fully open state in these ion conditions.

With maize chloroplasts we never obtained a seal resistance > 100 MΩ. These chloroplasts generally showed much worse adhesion to the patch pipette, and we were unable, in most cases, to suck a sufficient envelope area inside the electrode to improve the seal; at least the leak resistance and capacitance artifact remained unchanged during the suction pulse. Finally, the application of strong suction led to the breakdown of the envelope and lysis of plastid. In three out of more than 100 attempts, however, we were able to detect the single-channel activity with

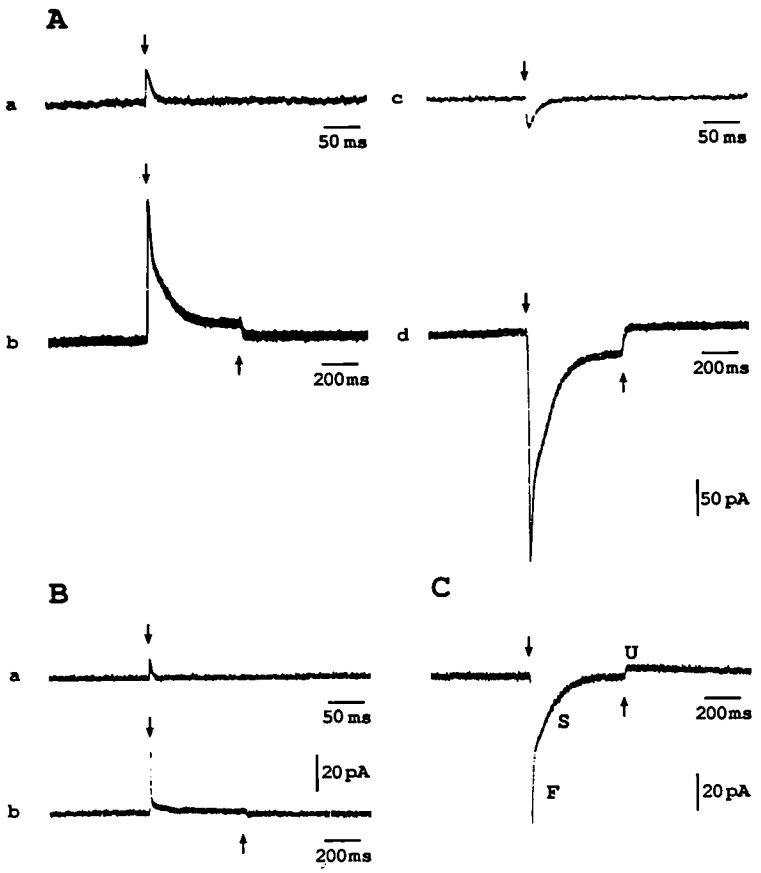


Fig. 2. Different types of photocurrent responses in chloroplasts. Holding potential is 0 mV, ionic composition of solutions as in Fig. 1. A: Different recording configurations for the same chloroplast of *Amaranthus hybridus*; (a) and (b) before, (c) and (d) after disruption of thylakoid membrane by short suction pulse; (a, c) and (b, d) are responses to short saturative flash and pulse of continuous light, respectively. B: Photocurrents in *Zea mays* chloroplast; (a) flash induced response, (b) response to continuous light. C: Complex kinetics of photocurrent in *Amaranthus*: fast (F) and slow (S) components of the light transient, (U) upstroke of current on the offset of illumination. Arrows indicate onset and offset of light. Light pulse duration was 0.5 s.

low-resistance seals (50–100 MΩ). An example of such a recording made at zero voltage is present in Fig. 1B. The direction and magnitude of the single-channel current were close to that obtained with *Amaranthus* chloroplasts at 0 mV. Therefore, these two channels probably have similar conductance and selectivity, although we were unable to prove this as the ion currents in maize chloroplasts could be studied only in a narrow range of potentials close to zero.

Forming of the initial 50–100 MΩ contact of the patch pipette with the chloroplast envelop did not necessarily give rise to a further increase of resistance. Instead, in many cases, the resistance spontaneously decreased to 20–50 MΩ. This change was accompanied by the increase of capacitance artifact, which likely reflects the breakdown of envelope and access of pipette to the chloroplast interior. No photoresponse was registered during this step. However, additional short suction usually produced a positively directed response to the light (Fig. 2A, B). Then, a short and intense suction pulse was applied which caused inversion of the photocurrent polarity. The magnitude of the photocurrents usually diminished 2- to 3-fold in the first few minutes of recording, but responses of magnitude as in Fig. 2C and Fig. 4A–E could be typically recorded for a longer time (up to 30–90 min).

Table I. Kinetics of photocurrents

	Continuous light					Flashes	
	Peak current amplitude (pA)		Steady-state current (pA)	Light transient (ms)		Amplitude (pA)	Decay time (ms)
Parameter	Dark adapted	Preilluminated		τ fast	τ slow		
Mean	14.6	40.7	7.4	5.5	110.9	44.9	120.0
S.E.	2.1	5.4	0.6	0.7	9.5	6.7	1.60
Number of experiments	18	18	35	18	35	50	50

Chloroplasts from older plants (8–10 weeks) generally gave more stable photocurrents. However, with these chloroplasts it was in some cases difficult to invert the current; large voltage pulses (50–100 mV) accompanied by strong suction were helpful in this case. Only a few recordings were obtained with maize chloroplasts (example in Fig. 2B); the responses were much smaller in magnitude as compared to the photocurrents measured in *Amaranthus*, and we were unable to invert the photocurrent by applying large voltage and strong suction pulses.

Only inwardly directed photocurrents in *Amaranthus* were used for further analysis. The reason for doing so was that this recording configuration was more stable in time, while for positive currents the polarity was usually spontaneously inverted in the first few minutes even without additional suction pulse. The kinetics of photocurrent was complex: (1) partly resolved rapid rise, (2) biphasic decay in the light to a steady-state level, and (3) further decay in the dark. The latter was often accompanied by a small upstroke of current better seen with a freshly prepared sample, with a slow decay (in a time scale of seconds) to the initial dark level (Fig. 2C).

The kinetic parameters of the photocurrents are given in Table I. Those parameters for light- and dark-adapted preparations are measured 10–50 min after electrode insertion into a thylakoid; for those preparations for which the sample was kept in the dark over 1 min or more, the amplitude of the fast component ($\tau \cong 6$ ms) of the light transient, as estimated by fitting the decay time course by a double exponential function, did not exceed 25% of maximal (see next section). It was found that the amplitudes of the slow component of the photocurrent ($\tau \cong 100$ ms) as well as the steady-state current decreased upon sample aging (in several 10 min intervals). The amplitude of the fast component also decreased, although this change was relatively small. However, in addition, the increase of the nondadaptable fraction of the fast transient (i.e., the fast component present both in the light- and dark-adapted preparation) was found under these conditions.

The time course of flash-induced photoresponse could in most cases be fitted by a monoexponential function (see τ in Table I). The amplitudes and τ varied greatly from sample to sample (7–300 pA and 1–60 ms, respectively). The amplitude of flash-induced response decreased during the flash series (flashes were given 2–3 s apart, not shown), and the amplitude of the response was partly restored when the sample was kept over a few minutes in the dark. However, at very long incubation times (several 10-min intervals) an irreversible decrease of the amplitude of the flash-induced response and an acceleration of its decay kinetics (in some cases, severalfold) were observed. Hence, only the first flash in each experiment was taken into account, and the average equivalent charge transferred per flash for all experiments ($n = 50$) was calculated as

$$q = \tau \times I_{peak} = (3.84 \pm 0.57) \times 10^{-13}\ \mathrm{C}$$

Effect of Pre-illumination on the Kinetices of Light-Induced Current Transient

As shown in Table I, the magnitude of the initial current response to continuous light differs, on average, by 64% in dark-adapted and preilluminated chloroplasts. This occurred because in the dark-adapted preparation the amplitude of the fast component was usually small; the second pulse given 0.5–1.0 s apart produced a large increase of the fast component

Fig. 3. Effect of preillumination on the kinetics of photocurrent at 0 mV. Two 0.25-s light pulses are separated by 0.6-s dark interval. Before the experiment sample was kept in the dark for several minutes. The ionic composition of solutions is as in Fig. 1.

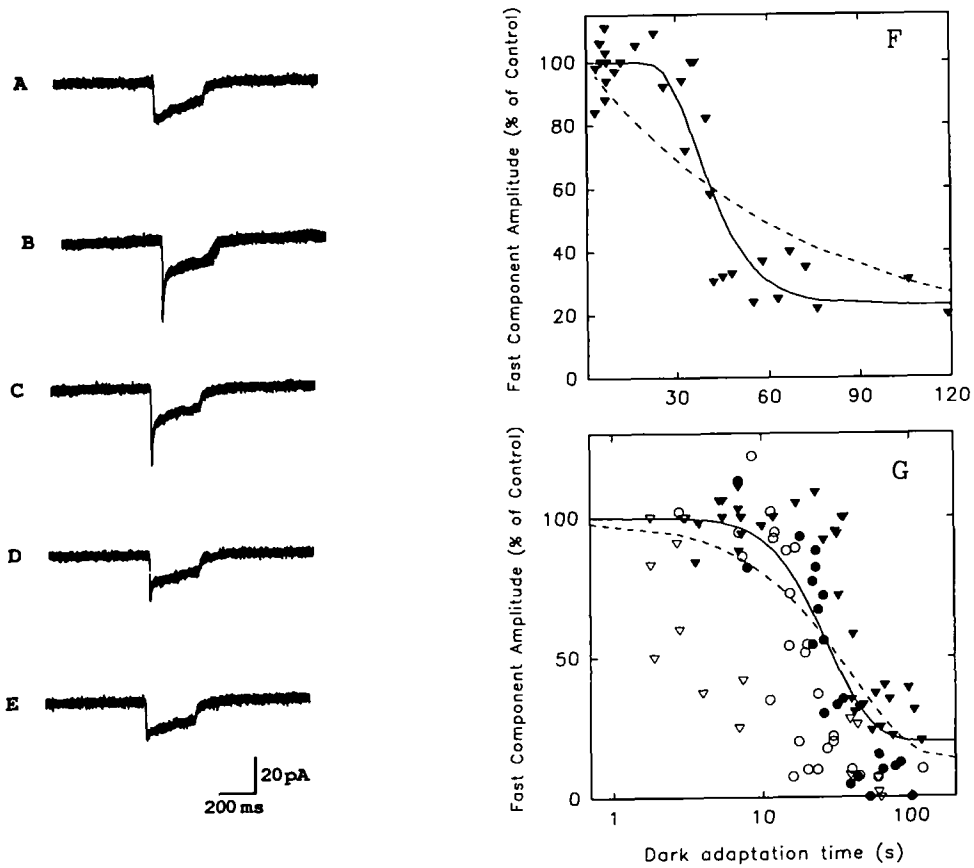


Fig. 4. Effect of dark adaptation on the amplitude of fast component of photocurrent transient. The duration of dark interval preceding the pulse in sequence was (in seconds): (A) 62, (B) 8, (C) 22, (D) 26, and (E) 39. F: The amplitude of fast component expressed in percentage of control (test pulse given 0.5–1.0 s apart preceding one) vs. dark interval duration. Dashed line is best fit to the data by monoexponential function, $\tau = 80$ s. Solid line is best fit for exponential power function, $n = 35$, $\tau = 10$ s, and $C = 25\%$. G: Summary of four individual experiments as the one in (F), lines as in (F). For monoexponential function $\tau = 39$ s, and for power function $n = 3.1$, $\tau = 16$ s, and $C = 20\%$. See text for explanation of parameters. Other experimental conditions are as in Fig. 3.

amplitude (Fig. 3). In contrast, the amplitude of the slow component was smaller for the second pulse, while a complete restoration of the slow-component amplitude required several seconds of dark adaption. At the same time, the fast-component amplitudes measured in 0.5 s and after a few seconds in the dark were usually the same. For longer adaptation times (10–30 s), however, an abrupt decrease of the fast-component amplitude was observed (Fig. 4B–E). With 18 out of 25 samples the fast-component amplitudes diminished to less than 25% of maximal or were even completely abolished by keeping the sample in the dark for 30–60 s. The effect of dark adaptation on the fast component amplitude was studied in detail with four samples (Fig. 4F–G). The time coursed showed a sigmoid shape, i.e., no change of the amplitude for the first 10–30 s, and an abrupt fall to a low sustained level (0–20% of maximum) which remained unchanged up to 10 min of dark adaptation. For very long incubation (> 1 hr) the fraction of "nonadaptable" fast transient increased irreversibly; thus, the experiment was stopped when it approached a 30% level.

The pooled points from all experiments were better fitted by the exponential power function

$$f = C + (100 - C)(1 - (1 - e^{t/\tau})^n)$$

rather than by the monoexponential function. Here, C is the fraction of the nonadaptable fast component, in percent. The best fitted values of τ and n were 16 s and 3.1, respectively.

Because of variations in τ with different samples,

the transition region for the average curve is smeared. As a result, τ was overestimated, while n was underestimated, as compared to individual samples. As an example, in Fig. 4F, the best fit was obtained with $n = 35$ and $\tau = 10$ s.

The other objective of this study was to estimate the duration of preillumination pulse necessary to produce the maximum amplitude of the fast component. We found out that the short saturative flash was not sufficient in all cases, at least when the test pulse of continuous light was given 200–1000 ms after the flash ($n = 7$). Strong white light of variable duration was insufficient unless prepulses longer than 8 ms were used. Thus, a time delay in the development of the fast transient as a function of the preillumination period was observed (Fig. 5A). In two out of five experiments the transition region was analyzed more carefully (i.e., all the time points in the interval where the amplitude of fast transient changed from 10 to 90% were repeated at least three times with each sample). This allowed an accurate fitting of the observed dependence by a model curve. In both cases the monoexponential function gave poor fit to the points, while the exponential power function

$$f = 100(1 - e^{-t/\tau})^n$$

provided good fit to the data with $n = 7.8$ (9.5) and $\tau = 12$ (20) ms for the first and second samples, respectively. When the data of five individual experiments were pooled, an apparent increase of τ (22 ms) and a decrease of n (1.6) were found, which is likely a result of smearing of the transition region due to the variation of τ for different samples.

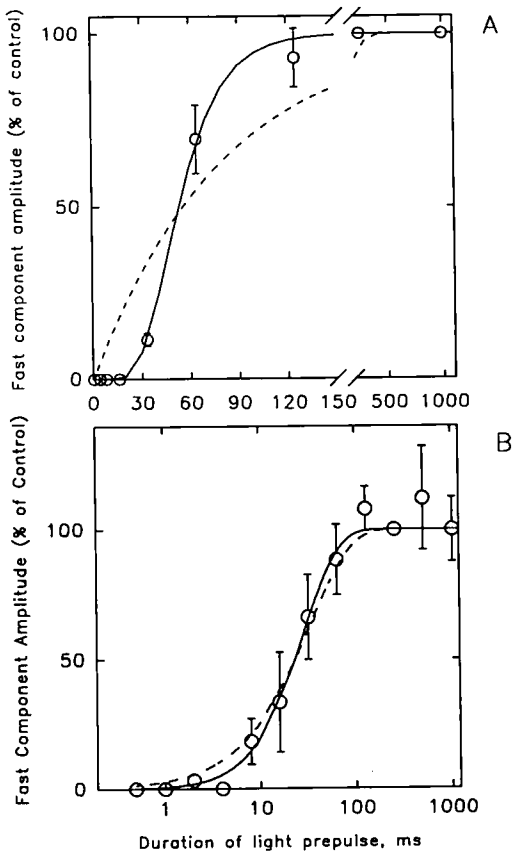


Fig. 5. Dependence of fast component amplitude on the prepulse duration. A three-pulse protocol was used: first pulse for dark adapted preparation was of variable duration, second and third of 250 ms each. Dark interval between pulses in sequence was 0.5–1.0 s. The amplitude of fast component measured for the second pulse was taken relative to the one obtained for the third pulse (in percent). A: Plot of single experiment; bars are S.E. for three values, solid line is best fit with exponential power function, $n = 9.5$ and $\tau = 20$ ms; dashed line is best fit with a monoexponential power function, $\tau = 80$ ms. B: Summary of five experiments as the one in (A); S.E. are for means obtained in different experiments. Lines as in (F). Parameter values for exponential power function and monoexponential function are $\tau = 22$ ms, $n = 1.6$, and $\tau = 33$ ms, respectively. See text for explanation of parameters. Other experimental conditions as in Fig. 3.

DISCUSSION

General Observations

The large-conductance cation channel described in this paper closely resembles the 1-nS (in symmetrical 100 mM KCl) cation channel found in *N. obtusa* chloroplast envelop (Pottosin, 1992), with a minor difference in the K^+/Cl^- permeability ratio and a slightly smaller conductance (730 pS in 100/50 mM KCl), which is likely to be a consequence of lower salt concentration. Both channels were mainly in the fully open state at negative (chloroplast inside) potentials, while at positive potentials they switched to lower conductance states. The plot of time-average conductance as a function of membrane potential (Fig. 6) for *N. obtusa* and *Amaranthus* envelope channels shows a similar pattern that implies that these channels might be an obligatory component of the chloroplast envelope studied *in situ*. This would favor the influx of cations in chloroplast through the channel.

Recent examination of detergent-solubilized chloroplast envelopes reoconstituted into a planar lipid bilayer revealed voltage-independent cation channels with a conductance of 120–180 pS (Schwarz and Wagner, 1992; Mi *et al*, 1994). These

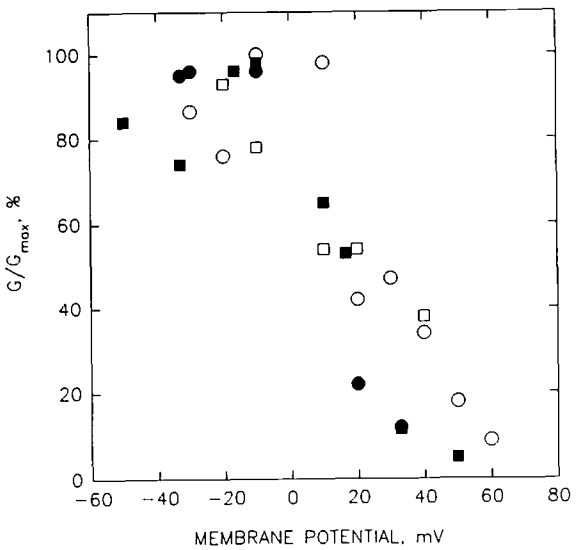
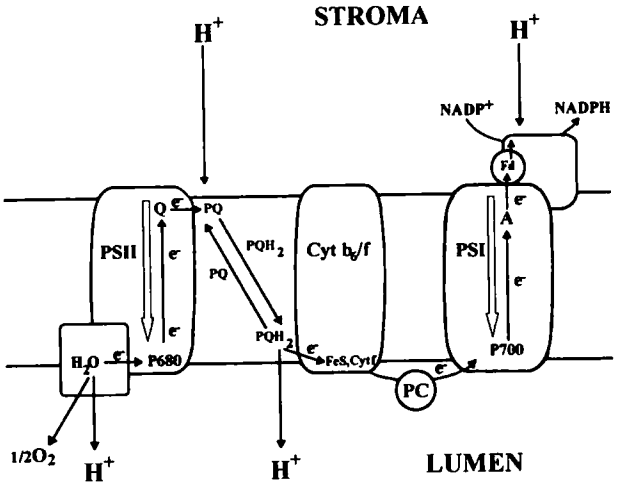


Fig. 6. Plot of time-averaged conductance for large cation channels in *Nitellopsis obtusa* and *Amaranthus hybridus* chloroplast envelopes. The conductance was 1020 pS (100/100 mM KCl) for *Nitellopsis* and 720 pS (110/50 mM KCl) for *Amaranthus* channels, respectively. Each point represents the channel current averaged over 10–15 s of successful recording at given potential taken relative to maximal amplitude of single channel current at the same potential. Filled and hollow symbols are for different samples. ●, ■: *Nitellopsis*. ○, □: *Amaranthus*.

properties are reminiscent of the behavior of the 130-pS substate of the large-cation channel recorded here, which was the most prominent upon preparation aging. A similar large-conductance multistate channel ($G = 1–1.3$ nS) in the inner membrane of mitochondria was proposed to be formed by a number of pores in the inner membrane and a large pore in the outer one (Kinnally *et al.*, 1992). This hypothetical structure implied a certain degree of flexibility, with a variety of conductance levels observed to be dependent on the integrity of a contact site. A similar model may serve as an attractive explanation for the changes of the conductance pattern in the chloroplast cation channel both upon aging and upon reconstitution in artificial systems.

Our results confirmed the recent finding of Bulychev *et al.* (1992) that the standard patch-clamp technique could be applied to study light-induced currents in photosynthetic membranes. The application of this technique for measurements of photocurrents may be very general, with the possible exception of bundle-sheath chloroplasts in advanced C_4 plants (Panicoid grasses) which do not contain a well-developed thylakoid system (Coombs and Greenwood, 1976; Staehelin, 1986). Accordingly, in this study we obtained only a few recordings of photocurrents with chloroplasts of *Zea mays*, while with bundle-sheath chloroplasts of a dicotyledon C_4 plant *Amaranthus* possessing well-developed grana (Fisher and Evert, 1982), the recordings were made more readily and in a highly reproducible manner.

To our knowledge, the chloroplasts of C_4 plants have been examined by means of electrophysiological techniques only for the first time. Thus, it was interesting to find a qualitatively similar kinetics of photocurrents as compared with patch-clamp recordings obtained by Bulychev *et al.* (1992) who used giant chloroplasts from the C_3 plant *Peperomia metallica*. These authors compared the kinetics of photocurrents obtained with conventional microelectrode and patch-clamp techniques as well as in current- and voltage-clamp modes, and found them to be very similar. Their results suggested that the patch-pipette had a low-resistance access to a thylakoid lumen, and the electrogenic transfer across the thylakoid membrane could be measured. We confirmed this notion by verifying the sequence of steps that finally led to the registration of an inwardly directed photocurrent in our preparation (Fig. 2). A positively directed photocurrent was considered to be recorded in the thylakoid-attached configuration, and a negative one in the whole-thylakoid mode. As the current amplitude in both modes should be proportional to the membrane surface, the relatively large current measured in the thylakoid-attached mode as compared to the current measured from the whole-thylakoid configuration (Fig. 2A: b and d) needs to be explained. The thylakoid surface could be measured by swelling thylakoids in hypotonic medium, which results in formation of large spherical vesicles (blebs). Our experiments with *Amaranthus* revealed blebs with an average diameter of 13.1 μm ($\pm$1.2, $n = 55$) or a membrane surface of around 540 μm^2. The fraction of membrane surface pulled into the pipette could be estimated only indirectly as intact thylakoid membrane forms an intensively folded system. The distance between neighboring bilayers in the thylakoid stack is around 0.02 μm while the average diameter of the stack is 0.5 μm (Junge and Jakson, 1982). The thylakoids could be pulled as far as 10 μm inside the patch-pipette, as it could be seen in the light microscope. Thus, the membrane area in the pipette could be at least 100 μm^2, providing a single string of stacks. This should be an underestimate, however, while at a distance of 10 μm from the tip the pipette becomes



Scheme 1

substantially wider and several stacks could be placed in parallel.

Under voltage-clamp conditions any charge translocation across the membrane should be detected. The light-induced electrogenic reactions in the photosynthetic membrane take place in transmembranously located complexes of photosynthetic reaction centers of photosystems I and II (Junge and Jakson, 1982). According to the simplified Z-scheme of photosynthesis (Witt, 1979; Junge and Jakson, 1982), the light-induced electron transfer in the reaction center of PSII results in the release of H^+ into the thylakoid lumen and reduction of plastoquinone molecule (PQ) which takes H^+ from the outside and delivers them in electroneutral form (PQH_2) to the inner side of the membrane (Scheme I). These protons are released upon PQH_2 reoxidation in b_6f complex (a rate-limiting step), while electrons are transferred to the reaction center of PSI, thus supporting its electron transfer capacity.

By measurement of charge transfer across the thylakoid membrane induced by a short saturative flash of light, one would obtain a direct estimate of electrogenesis in the reaction centers of PSI and II during a single turnover. The number of reaction centers of PSI and PSII is 1200–1700 and 850–1300 per μm^2, respectively (Staehelin, 1986), which transforms to $1.2–1.7 \times 10^6$ reaction centers for a 572.6-μm^2 thylakoid membrane surface. The charge translocated at a single turnover should then be $1.8–2.7 \times 10^{-13}$ C, which is close to the value of 3.9×10^{-13} C measured here.

Under continuous light the electron-transfer reactions in PSI and II are coupled at the site of PQH_2 reoxidation in b_6f complex, which is a rate-limiting step in the intersystem electron transfer. The rate of this reaction is slowed down upon accumulation of H^+ in the thylakoid lumen, with a typical value of 15–20 ms at neutral pH and a linear deceleration with a factor of approximately 2 per pH decrease by one unit (Junge and Jakson, 1982; Tikhonov *et al.*, 1984; Van Kooten *et al.*, 1986; Joliot *et al.*, 1992). With the number of photosystems in the thylakoid membrane given above, the transmembrane electron transfer will result in a photocurrent in the range of 9–18 pA. The value of 14.6 pA for the dark-adapted preparation obtained here (Table I) fits these limits, while the smaller value of 7.6 pA measured at the end of the long light pulse could be a result of the "back-pressure" effect of protons accumulated in the thylakoid lumen at light. Upon microelectrode insertion the acidification of the thylakoid lumen should not be as large as in intact thylakoids (by 3–4 pH units, Junge and Jakson, 1982). However, a decrease of pH by up to 2 pH units was measured also after microelectrode impalement (Remiš *et al.*, 1986).

Effect of Preillumination on the Light-Induced Current Kinetics

The most intriguing finding of this study was the effect of preillumination on the photocurrent. The amplitude of the photocurrent in preilluminated chloroplasts transiently increased by a factor of 3 (Table I). Previous studies of electrogenesis by PSI and PSII separately and in series showed that the transient and fast increase of photoresponse is determined by PSI (Remiš *et al.*, 1981). This is a consequence of faster turnover of the PSI reaction center, providing the electron supply by the PSI donor pool (FeS Rieske protein, cytochrome f and plastocyanin, Scheme I) is not limited (Junge and Jakson, 1982). However, this pool becomes empty after a few turnovers because of the existense of the rate-limiting transfer step before it, and the turnover rate of PSI at a steady state should be limited by PQH_2 reoxidation. Thus, the transient component of the photocurrent can be explained by answering the following question: why is the fast transient of the photocurrent observed only in preilluminated chloroplasts? Previous measurements of photoelectrogenesis in *Peperomia* chloroplasts under conditions where the electron transfer between photosytems was shunted by an external electron acceptor showed the absence of a fast transient in dark-adapted chloroplasts

(Remiš *et al.*, 1981). After preillumination of the chloroplast the fast transient reappeared, providing the dark interval after preillumination was short. This was interpreted as patial photoreduction of PSI donors. We think that the effect of preillumination on photocurrent in *Amaranthus* chloroplasts has the same origin. The plots in Figs. 4 and 5 show that the magnitude of the fast transient depends both on the dark interval between pulses and on the duration of the light prepulse. The dependence on light prepulse duration correlates with the time course of photoreduction of the PQ pool under saturative light (Joliot *et al.*, 1992). The re-oxidation of the total intersystem redox pool was found to be a pseudo-first order process with a characteristic time of around 25 s (Renger and Schulze, 1985). The dependence of the fast transient amplitude on the dark interval was fitted by an exponential power function with a characteristic time of 16 s, close to the above value (Fig. 4). The reduction of PSI donors by PQH_2 is thermodynamically favorable and takes place both in the light and in the dark. Therefore, the oxidation of PSI donors in the dark should be seen only after complete re-oxidation of PHQ_2, which could explain the observed delay in the time course presented in Fig. 4. Because of the large variation of the parameter n from 3 to 35, no direct comparison with the size of the PQ pool could be made here. However, the value of 12 equivalents per PSII reported by other authors (Joliot *et al.*, 1992) is at least in this range.

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