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Light-induced potential changes across the chloroplast enclosing membranes as expressions of primary events at the thylakoid membrane

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SUMMARY

Some characteristics of light-induced changes in the membrane potential of chloroplasts in mesophyll cells of plant leaves are presented. It is suggested that these changes are reflections of light-driven primary electrochemical events at the thylakoid membranes.

There is increasing evidence that the primary photochemical energy conversion reactions in algae and green plant cells are triggered by an electron transport coupled generation of electrical and ionic gradients across the thylakoid membranes of chloroplasts[1-4]. Up to date, the energization of the membranes of cellular (sub) units with electron transport activity has been deduced from indirect measurements only, namely from absorption and fluorescence changes of native pigments or substituted chemical probes in the thylakoids or chromatophores[5-7], as well as from changes in delayed light emission[8-10]. Direct measurement of the electrical potential across thylakoid membranes by conventional glass microcapillary electrodes is impossible because of the microscopic dimensions of these structures. However, Bulychev *et al.*[11, 12] have succeeded in determining the electrical potential across the membrane of intact chloroplasts in mesophyll cells of leaf sections of *Peperomia metallica*. These potentials were shown to undergo rapid changes upon illumination with light absorbed by the photosynthetic pigment systems.

In this communication we confirm the occurrence of fast light-induced potential changes across the enclosing membranes of *Peperomia* chloroplasts and

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report on some hitherto unrecognized characteristics thereof. On the basis of a kinetic analysis we suggest that they are caused by primary field-driven ion translocations at the thylakoid membranes.

For the measurements a 20 mm^2 leaf section was cut at one edge at an angle of about 70° with a sharp razor blade, and clamped in position in a specially constructed sample holder on the stage of an inverted microscope (Wild M50). The specimen was kept in a circulating medium, after White[13], containing, in mM: $CaNO_3$, 6; KNO_3, 4; $MgSO_4$, 15; Na_2SO_4, 7; NaH_2PO_4, 0.7; sucrose, 118; morpholinopropane sulphonate–Tris buffer (pH 6.8), 1. Temperature was controlled by thermostating the circulating medium outside the sample holder. The mesophyll cells at the cut edge were illuminated by light from modified lamphouse assemblies of a 250 W light projector using two flexible light guides (1 mm in diameter). One guide was positioned perpendicularly to the plane of the

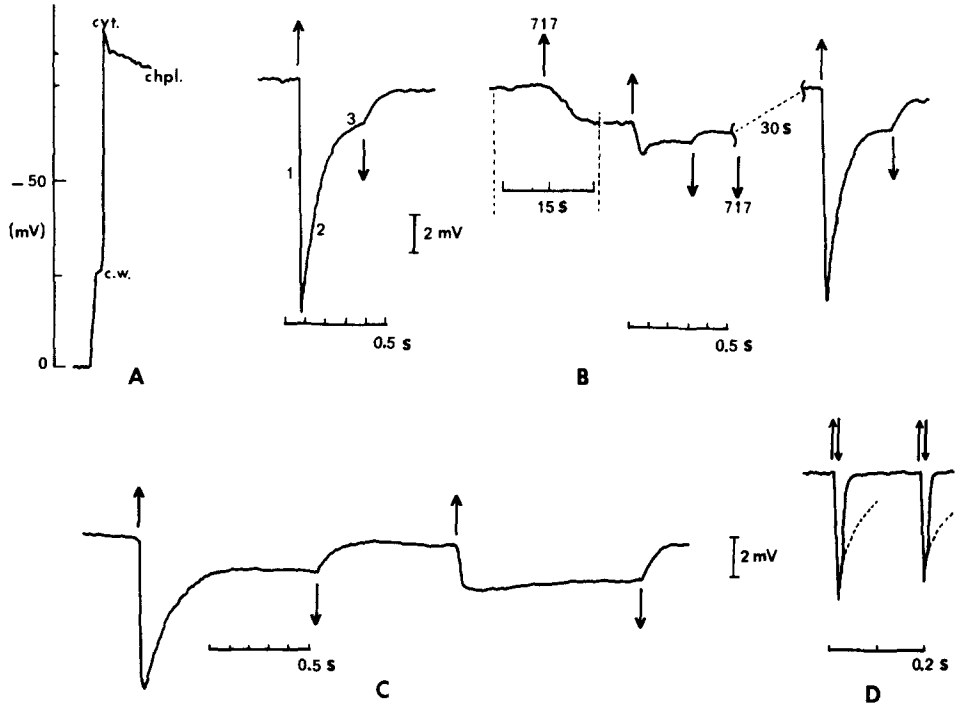

Fig. 1. (A) Recording of the potential response upon impalement of an electrode into a chloroplast (chpl.), after passing the cell wall (c.w.) and cytoplasm (cyt.) of a mesophyll cell of *Peperomia metallica*. (B) Recording of the potential change of chloroplast envelope in a light period of 300 ms without and with continuous 717 nm background light. Note the different time scale between the dotted vertical lines. (C) Different cell, potential response in two sequential light periods. (D) Different cell, potential response in two sequential light flashes of 8 and 10 ms, respectively; the dotted lines represent the potential change in an extended light period, as estimated from the observed rate of hyperpolarization after the initial depolarization in the light. Upward and downward pointing arrows mark the beginning and end, respectively, of an illumination period. Unless indicated otherwise illumination was with white light of approx. 800 kergs/cm^2·s intensity; the intensity of 717 nm light was approx. 50 kergs/cm^2·s. A downward moving trace means a depolarization of the membrane.

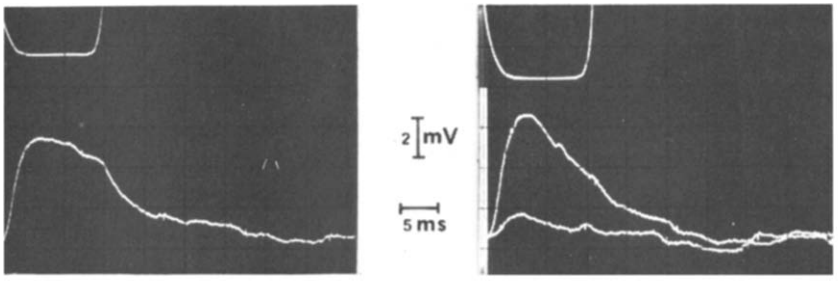

Fig. 2. Time course of the initial potential change of the chloroplast envelope at 24 °C and, for a different chloroplast, at 5 °C in a 10 ms flash of white light of approx. 800 kergs/cm²·s intensity. The lower curve in the figure at the right hand side gives the response upon the same flash with continuous 717 nm background light of approx. 5 kergs/cm²·s. An upward-moving trace means a depolarization of the membrane. The opening and closing kinetics of the optical shutter, as probed by a photodiode in the light beam, can be read from the upper curves.

edge. Monochromatic illumination was obtained by placing interference filters in the light beams between lamphouse and input of the light guide. The indicated light intensities are approximate only, because the intensity at the cell surface could not yet be accurately measured. The chloroplast potential and its fast light-induced changes were measured with conventional 3 M KCl-filled glass microcapillaries, in contact with an Ag–AgCl wire *via* an agar–KCl bridge[14]. A similar electrode in the suspending medium served as a reference. The potential changes were monitored, after impedance matching by a unity gain amplifier, on a galvanometric recorder with a time resolution of 16 ms, or on an oscilloscope when fast kinetics were studied. In the present experiments the time resolution of the set up was limited by the opening time (3 ms) of the mechanical shutters which were used in the light beams.

When the potential changes during the insertion of the electrode into a cell and a suitable located chloroplast were monitored at 20 °C (Fig. 1A), three distinct successive steps were noticed: (i) A drop of the potential to about −20 to −30 mV; this level was found not to change during illumination and may represent the potential difference between the cell wall and the surrounding medium. (ii) A level of −80 to −125 mV, which likely is the potential of the cytoplasm at 20 °C; its relatively slow changes during illumination resembled those described previously for *Nitella* cells[14,15]. (iii) A potential 10 to 25 mV more positive than that of the cytoplasm was ascribed to the chloroplast stroma phase because it underwent extremely rapid changes in the light. These latter potential changes will be described in detail below.

Upon illumination, we observed a rapid depolarization (phase 1) of variable magnitude, in some cases by as much as 30 mV. This depolarization was followed by a

slower hyperpolarization (phase 2) to a steady state level (phase 3) slightly above the dark potential (Fig. 1B). The potential change in phase 1 varied from chloroplast to chloroplast, but for any one chloroplast its magnitude and rate were positively correlated with the light intensity. At sufficiently low intensities of white, as well as of 676 or 717 nm light, the steady-state phase 3 was reached without a transition through the decay phase 2. Surprisingly, when strong white light was given in addition to such background illumination, hardly any further change was elicited, *i.e.* phases 1 and 2 were considerably suppressed (Fig. 1B). A monophasic change to the steady-state level in strong light was also seen shortly after a previous light period had induced the steady-state phase 3 (Fig. 1C). Many measurements suggested that the decay phase 2 was faster at higher light intensities. However, as said above, the light intensity also affected the magnitude and rate of phase 1. Conclusive evidence for the light dependence of phase 2 was obtained by use of two sequential light flashes. A short flash was fired which permitted completion of phase 1, and of a small portion of phase 2. A second flash followed after 160 ms darkness. As can be seen (Fig. 1D), both flashes elicited nearly identical responses. Taking into account that, because of technical difficulties, the intensity in the second flash was slightly lower than in the first one, it appeared that phase 2 had not proceeded during the dark period, but that a regeneration of the original state had occurred. This experiment and similar ones, indicated that in the light the 'dark resting state' of the chloroplast is transformed into a different, light induced condition from which it is reversed slowly in the dark. Phase 2 is a reflection of this transformation at high light intensities. Consequently, the extent of phase 1 and phase 2 depended on the degree of this transformation from one condition into the other, and was maximal in the dark state.

With the available facilities, the exact kinetics of the rapid phase 1 were difficult to measure. In particular, the opening time of the shutter limited the resolution of the rise time at high light intensities. At 24 °C, maximal signal was not attained until shortly after complete opening of the shutter. Hence, we estimated a rise time of approximately 5 ms. We never observed any significant change in the time constant when the temperature was lowered to 5 °C (Fig. 2; the apparent difference in the decay kinetics during the light period were unrelated to the temperature).

The rapidity of phase 1 revealed its association with one of the primary events in the photosynthetic apparatus. Experiments of Witt and co-workers (*cf.* ref. 2) have indicated that an electric field across the thylakoid membrane is generated within 10^{-8} s upon excitation, due to a primary charge separation in the photosynthetic electron transport chain. The decay of this field in less than 10 ms was shown (*cf.* ref. 2) to be accompanied by a binding of protons on the outside, and a release on the inside of the thylakoid membrane. Such a process would be expected to generate a Nernst potential across the inner enclosing chloroplast membrane, provided it functions in the dark as a diffusion barrier to protons. We suggest that phase 1 of the observed light-induced potential changes is a direct expression of this event.

Subsequent changes of the measured potential are difficult to assess because a variety of processes are bound to follow the establishment of the initial proton gradient,

e.g. secondary fluxes of various ions with accompanying creation of diffusion potentials, and ATP formation. We suspect that in the wake of such events the permeability of the inner enclosing chloroplast membrane is reversibly altered, causing a transformation of the condition of the chloroplast. This would, as discussed before, account for the light dependence of phase 2 and for the fact that the phase 1 – phase 2 induction is not observed with weak background light. From earlier studies with *Nitella* cells it was learned, for example, that considerable changes in the permeability of the plasmalemma are induced by actinic light[15]. For a better understanding of phase 2, its possible sensitivity to selective membrane modifying agents will be investigated.

In their pioneering work on light-induced potential changes in chloroplasts, Bulychev *et al.*[12] have related the fast responses to the secondary ion fluxes at the thylakoid membrane. However, the light dependence, and the temperature insensitivity of phase 1, as we have found in this present work cannot be reconciled on this interpretation.

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