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PHOTOINDUCTION KINETICS OF ELECTRICAL POTENTIAL IN A SINGLE CHLOROPLAST AS STUDIED WITH MICRO-ELECTRODE TECHNIQUE

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SUMMARY

1. Using single chloroplasts of *Peperomia metallica* the kinetics of light-induced potential changes were studied. Three kinetic components (the initial fast rise, the decay in the light and the decay in the dark) were found to be characterized by time constants 4, 220 and 60 ms, respectively at light intensity 5000 lx and temperature 18 °C. After flash excitation the potential kept on rising for about 10 ms. Cooling of the medium down to 5 °C had no effect on the duration of potential rise after the flash.

2. Variations in the medium temperature in the range 2–23 °C had little effect on photoresponse magnitude but resulted in significant acceleration of decay in the light.

3. Addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea ($5 \cdot 10^{-6}$ M) resulted in suppression of the magnitude of the photoresponse but was not accompanied by any change in the rate of initial rise of potential. 3-(3,4-Dichlorophenyl)-1,1-dimethylurea-inhibited photoresponse could be restored and even enhanced by subsequent addition of *N*-methylphenazonium methosulfate (10^{-4} M). *N*-Methylphenazonium methosulfate essentially influenced the time course and light-intensity curves of photoresponse.

4. The chloroplast photoresponses were of different time-courses when elicited by red (640 nm) or far red (712 nm) light. This fact as well as an enhancement effect of combined illumination by two intermittent light beams indicate on the interaction of two photosynthetic pigment systems when the photoelectric response was formed.

5. An imposed electrical field resulted in stimulation or suppression of chloroplast photoresponse depending on the polarity of the field. No indications for the existance of "reversal potential" for photoelectric response were obtained.

6. A kinetic scheme of photoresponse formation is proposed, which includes two sequential photochemical reactions of photosynthesis.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PMS, *N*-methylphenazonium methosulfate; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

INTRODUCTION

A great deal of attention has recently been given to the functional relationship between photosynthesis, transmembrane proton transfer, conformational changes in chloroplast membranes and generation of electrical potential [1–6].

The study of these relationships is of particular importance in connection with hypotheses suggesting a key role of electrical transmembrane potential in the primary events of energy conservation during photosynthesis [1, 2]. However, verification of these hypotheses was complicated by the lack of direct methods for measurement of membrane potential in chloroplasts. Various tests used, such as changes of optical absorbance [7], redistribution of synthetic permeant ions [4], effects of delayed fluorescence [8], may be regarded as indirect methods of detecting electrical potential changes. Therefore it seemed valuable to detect and study transmembrane potential through a direct recording with micro-electrode technique. Using leaf sections of *Peperomia metallica*, whose mesophyll cells contain giant chloroplasts, we succeeded in recording light-induced changes of chloroplast potential, measured with respect to the intracellular medium [9, 10]. We investigated temperature dependence of photoelectric response, action spectrum, the enhancement effect under the action of two light beams, and the effect of inhibitors and cofactors of electron transport. The results obtained led to the conclusion that photoelectric response was closely connected with photosynthesis [9, 10]. The occurence of fast photoinduced changes was confirmed by Vredenberg et al. [11, 12]. These authors observed the reduction of the effect induced by strong white light when it was given in addition to far red background illumination. They also described changes of electrical potential under flash illumination.

In our laboratory [10, 13, 14] photoinduced changes of potential were also found in separate isolated chloroplasts placed in artificial medium.

In the present work the measurements were conducted both on chloroplasts in situ and in vitro with the aim of studying the kinetics of fast photoinduced changes of chloroplast potential, the effect of imposed electrical potential and other factors on the photoresponse. On the basis of these experiments we have proposed a kinetic scheme of the processes involved, in accordance with the commonly accepted scheme of two photochemical reactions of photosynthesis. Some preliminary results of this work have been published elsewhere [14].

METHODS

Experiments were carried out on chloroplasts of *Peperomia metallica*. Growth conditions and the experimental procedure with chloroplasts in situ have been described [9]. Leaf sections used in these experiments were placed in White's solution [15] and the measuring micro-electrode was inserted into the chloroplast. The other (reference) micro-electrode was placed in the cytoplasm of the cell.

In in vitro experiments, measurements were conducted on separate chloroplasts placed in an artificial medium. The medium contained 25 g/l Ficoll, 100 mg/l serum albumin, 0.25 M sucrose and 0.02 M tris-(hydroxymethyl)-aminomethane · HCl buffer (pH 7.5). For isolation of chloroplasts the upper epidermis was first stripped off. Then a few drops of the artificial medium were placed on the exposed region and mesophyll layer was cut several times with a razor blade. The cell free extract was collected and transferred into the measuring chamber. The chloroplast was drawn onto the fire-polished tip of a glass pipette (tip diameter about 15 μm) by suction from a syringe [10].

Membrane potential of isolated chloroplasts was measured with glass micro-electrodes having tip diameter of less than 1 μm and resistance of 20–70 Mohm when filled with 2.5 M KCl. The reference electrode was a calomel electrode connected to the medium via agar-KCl bridge. The micro-electrode was connected to the electrometer with input resistance of 10^{10} ohm and output was displayed on both an oscilloscope and a pen-recorder. The time-resolution of the device was approximately 1 ms. In some experiments the membrane potential of the chloroplast was set at a certain level by passing impulses of current through the recording micro-electrode. The current was stabilized by load resistance of $2 \cdot 10^9$ ohm.

Photoreaction was excited with a tungsten lamp. The duration of the stimuli was controlled by an electromagnetic shutter. Light intensity at the level of chloroplast was about 4000–5000 lx and could be reduced by interposing neutral density filters. Stimulus timing was monitored by a photodiode. Monochromatic illumination was obtained by placing interference filters (half band width 12 nm) in the light beam. In some experiments the light source was a flashtube (energy of the flash 120 J, full width at half height 2 ms).

The temperature of the medium in the chamber was changed with the use of heating or cooling units and was controlled by a thermistor with accuracy of 1 °C.

RESULTS

General description of the photoresponse

The time course of the photoelectric response of chloroplasts in situ elicited by exposure to light for various durations is shown in Fig. 1. The total electric response to continuous illumination and subsequent darkening consists of three distinct components: (1) an initial rapid rise of membrane potential, (2) a decay of membrane potential in the light, (3) a decay of membrane potential in the dark.

The time course of membrane potential change from dark steady state to the light steady state which occurs upon illumination can be expressed by the sum of two exponentials:

$$\Delta E = -A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} + B$$

where A_1, A_2 and B are positive constants (under given conditions), and τ_1, τ_2 are the time constants for the components 1 (rise of potential) and 2 (decay in the light), respectively.

The time course of the dark decay of potential after relatively short light exposures (less than 1 s) can be described by a single exponential

$$\Delta E = A_3 e^{-t/\tau_3}$$

where τ_3 is the time constant for decay in the dark.

At light intensity of 5000 lx and temperature 18 °C, time constants τ_1, τ_2 and τ_3 average (means±standard error) 3.7±1.9 ms ($n = 9$), 225±63 ms ($n = 12$) and 62±17 ms ($n = 9$), respectively. For exposures of less than 1 s, the value of τ_3 does

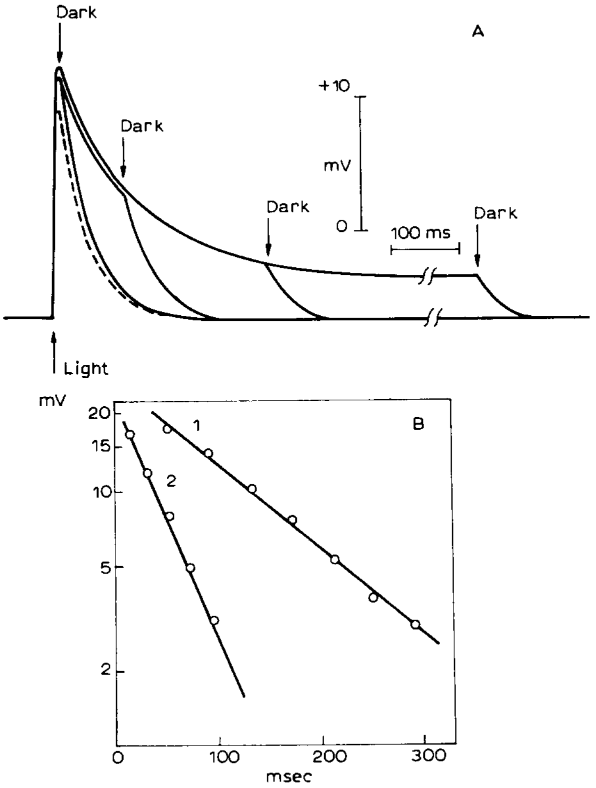

Fig. 1. Photoelectric responses of chloroplast in situ induced by light exposures of different durations (white light, 5000 lx). (a) The kinetic curves; dotted line denotes change of potential induced by 2 ms saturating flash; (b) Analysis of light decay (1) and dark decay (2) of potentials in semi-logarithmic plots.

not depend on the illumination time. After prolonged light exposures (⩾ 3 s) the process of dark decay exhibits a biphasic time course with extremum. Light pulses with duration of more than 10 ms induce photoresponses of equal magnitude. A more intense flash of 2 ms duration gave a potential increase which was little less than the maximum increase obtained in continuous light.

Using flash excitation no period of latency is observed. If a lag period exists, it is less than 1 ms. Membrane potential changes significantly within 1 ms. The maximal rate of potential rise under flash illumination is found to be 20 V/s.

Under flash excitation the rise time of potential varied for different chloroplasts, and even during the experiments on the same chloroplast. Usually the potential kept on rising for about 10–15 ms after the flash excitation (Fig. 2). However in some cases the rise in potential was completed within 1–2 ms. Only the latter type of potential change was observed by Vredenberg and Tonk [12].

Cooling the medium down to 5 °C has no effect on the duration of membrane potential rise after the flash as well as on the rate of initial rise during illumination. The rise of potential after switching off the light was also observed under excita-

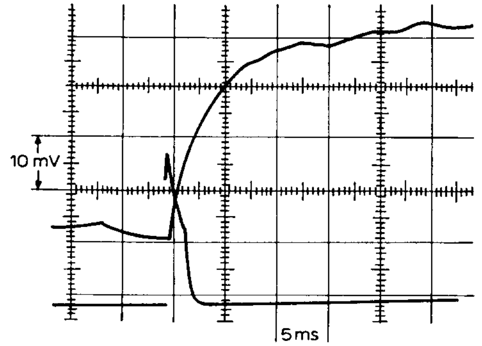

Fig. 2. The stage of increase in potential after flash excitation; the bottom trace displays the shape of the flash; temperature 5 °C.

tion with separate saturating square wave flashes of 2 ms duration that were obtained by the combination of an electromechanical shutter and a rotating sector.

Thus photoinduction of electrical potential includes both fast (10^{-3}–10^{-2} s) and slow (10^{-1}–1 s) stages. These experiments with flash excitation indicate the involvement of a dark, temperature-insensitive processes in the photoresponse generation.

In isolated chloroplasts the photoresponse kinetics correspond to those of chloroplasts in situ. In the same conditions of light intensity and temperature, the time-constants for the three components of the response are $\tau_1 = 2$–10 ms, $\tau_2 = 100$–300 ms, $\tau_3 = 50$–100 ms.

The photoresponse of isolated chloroplasts is influenced by the concentration of salts in the medium. Addition of KCl up to 10^{-2} M (initial concentration of K^+ in suspension determined by means of K^+-sensitive valinomycin electrode is about 10^{-3} M) results in 1.5–2 fold increase in magnitude of photoresponse. In the medium containing 10^{-3} M K^+ the photoresponse magnitude averaged 12.4±3.9 mV ($n = 12$) and after addition of KCl up to 10^{-2} K^+ the magnitude reached 23.1±7.0 mV ($n = 8$).

Dark adaptation

The magnitude and time course of the photoresponse both in vitro and in situ depend upon the duration of the preceeding dark interval [9, 11]. When two light pulses were separated by a short dark interval, the magnitude of the response to the second light beam was considerably diminished with respect to that induced by the first light beam (Fig. 3). This effect is dependent on the duration of the light exposure: with the shorter illumination times, shorter dark intervals are required to observe a reduction of the second photoresponse.

The dependence of the photoresponse magnitude on the duration of the dark adaptation indicates that formation of the photoresponse requires some product (or state) which is consumed in the light and restored in the dark (cf. Vredenberg et al. [11]). The time course of restoration of this product does not coincide with the time course of potential decay in the dark.

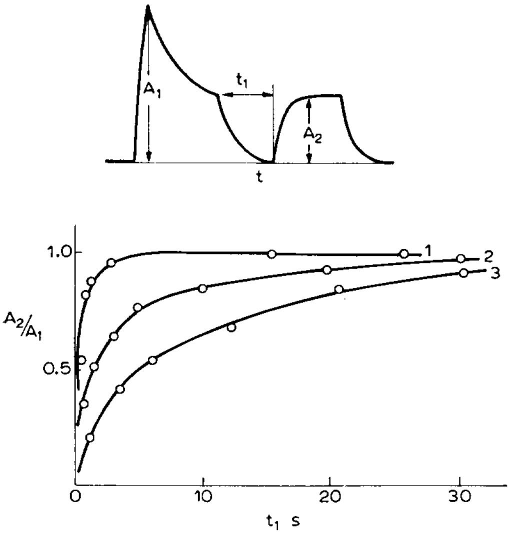

Fig. 3. The dependence of photoresponse magnitude on the duration of preceding dark interval; curves 1, 2, 3 were obtained for light pulses of 40 ms, 1 s and 3 s duration, respectively.

Effect of light intensity.

The kinetics of the photoresponse depend upon the incident light intensity. The reduction in light intensity brings about the decrease in maximal rate of potential rise and a shift of the peak position along the time axis toward longer times (Fig. 4). At low and intermediate light intensities the reciprocal of τ_1 ($K = 1/\tau_1$) increases in proportion to the light intensity. The decrease in light intensity causes the increase of τ_1 and τ_2. The value of τ_3 is almost independent of light intensity.

The analysis of light intensity curves plotted for peak value and steady state

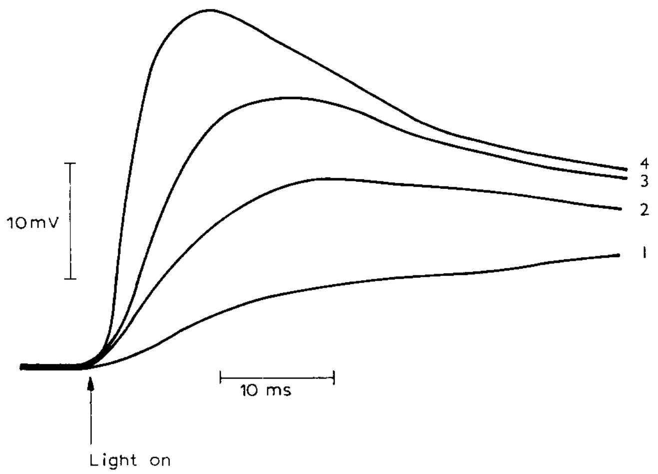

Fig. 4. Photoresponses of one isolated chloroplast under various light intensities: 1–340 lx, 2–600 lx, 3–1700 lx, 4–5000 lx.

value of the photoresponse indicates that light saturation for the steady state is reached at intensities almost an order of magnitude smaller than saturating intensity for the peak value. The light intensity curves and the time courses of the photoresponse at various light intensities measured on chloroplasts in vitro and in situ were similar.

Effect of temperature

The effect of temperature on the photoresponse was investigated with chloroplasts in situ. Changes in the temperature in the range 2–25 °C are without effect on the magnitude of photoinduced changes [9, 10] as well as on the rate of initial rise of potential. However, changes in the temperature have a marked influence on the component of decay in the light. Increasing the temperature of the medium is accompanied by increase of τ_2 (slowing down of decay in the light) and a slight decrease of τ_3 (acceleration of dark decay). The ratio τ_2/τ_3 at 2–5 °C is nearly 1 and reaches 7–8 at temperatures of 25 °C (Fig. 5).

Apparently, fast stages of photoinduction have low temperature sensitivity whereas slow stages are mediated by temperature-dependent processes.

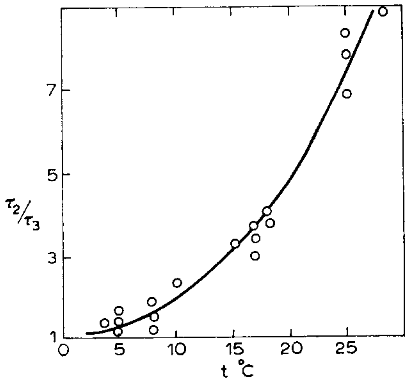

Fig. 5. The effect of temperature on the ratio τ_2/τ_3. τ_2 time-constant of decay in the light. τ_3 time-constant of dark decay.

Enhancement effect

Under illumination of chloroplast in situ with red and far red light beams the enhancement effect for photoelectric response was observed [9] which was similar to the well known Emerson effect for photosynthesis [16]. The magnitude of the response induced by far red (712 nm) light increased 3–5 times after exposure of chloroplast to red (640 nm or 680 nm) or white light. The photoresponse to 712 nm light gradually decreases under subsequent intermittent illumination and then returns to the initial (before action of red light) magnitude. The rate of this return process depends upon the duration of red light illumination. From Fig. 6 it is clear that after 3 s red illumination 7–8 flashes of 712 nm light are required before steady photoresponses are obtained and after 0.5 s red illumination only 2–3 flashes are required for the achievement of steady responses.

The extent of enhancement increases with higher intensity of red light. It is also influenced by the duration of the dark interval separating two light pulses. Dark

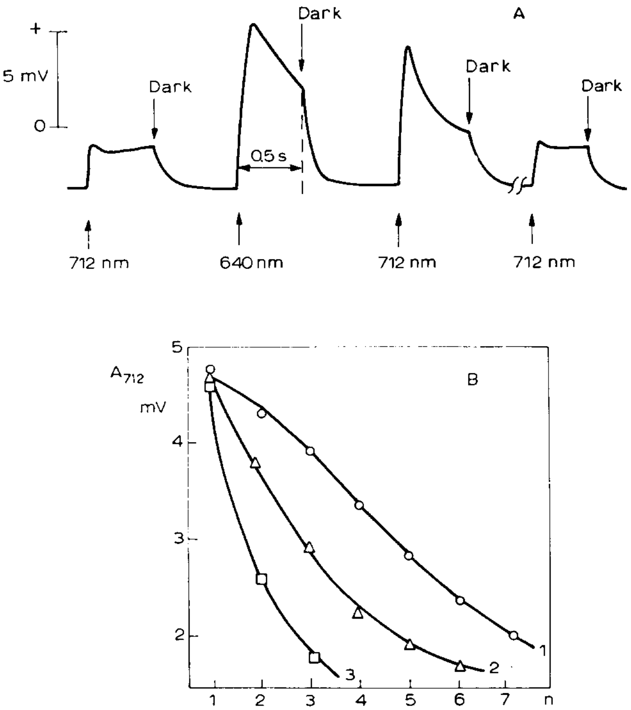

Fig. 6. The enhancement of in situ chloroplast photoelectric response induced by 712 nm light after preceding illumination with 640 nm light. (A) The record of experiment with arrows denoting the time at which the light was switched on and off. (B) Temporal changes in the magnitude of electrical response induced by 712 nm light after exposures to red (640 nm) beams of various duration; 1, 2, 3 are experiments with duration of red light 3 s, 1 s and 0.5 s, respectively; the dark interval between flashes was 5 s; n = number of flashes.

intervals as short as 1 s (and presumably even shorter) are sufficient for enhancement, however the enhancement effect is not observed under simultaneous action of both red and far red beams. The maximal enhancement is achieved when far red light is switched on after 10–30 s after preillumination with red or white light. It is important to note that the enhancement effect can be observed at room temperature and also at low (5 °C) temperatures.

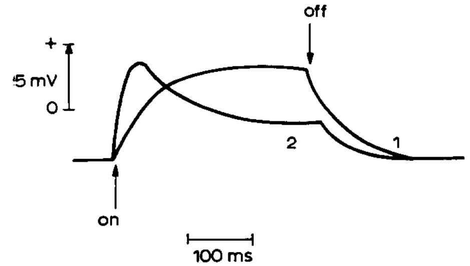

Fig. 7. The difference in time course of chloroplast photoresponses induced by light of 640 nm (1) and 712 nm (2); interference filters with half band width 12 nm.

Experiments with alternate action of red (640 nm) and far red (712 nm) light pulses adjusted to yield equal magnitudes of photoresponse show that the responses differ greatly in time course, particularly τ_1 (Fig. 7). The values of τ_3 were identical in both cases. It can also be seen in Fig. 7 that under red illumination the rise of potential proceeds at a relatively low rate (increase in τ_1) and the stage of decay in the light is poorly exhibited.

The occurrence of the enhancement effect and differences in kinetics of photoresponse under action of various wavelengths indicate on the cooperative participation of Photosystems 1 and 2 of photosynthesis in the formation of photoelectric response. From these results we can assume that two photosystems do not contribute equally to the generation of electrical potential as was suggested by Schliephake et al. [17] and Fowler and Kok [18].

Action spectrum

Action spectrum of chloroplast potential changes measured with the use of interference filters in the range 410–725 nm corresponds to absorption spectrum of intact leaves [10]. The shape of the action spectrum indicates the involvement of chlorophyll *a*, chlorophyll *b* and carotenoides in sensitizing of membrane potential. The far red region of the action spectrum differs from that of the absorbance spectrum and the drop in quantum yield of photoelectric reaction is exhibited which is similar to red drop in quantum efficiency of photosynthesis. It should be noted that the extent of red drop depends on the experimental procedure (i.e. the order of filter change, the number of flashes at each wavelength). The differences in the shape of action spectra can be explained by influence of enhancement effect described above.

Effects of DCMU and PMS

In experiments performed both on chloroplasts in situ and in vitro it has been shown that DCMU, a potent inhibitor of photochemical System 2, at concentration of $5 \cdot 10^{-6}$ M almost completely inhibits photoinduced changes of chloroplast potential. The decrease in the extent of the photoresponse was not accompanied by any change in the rate of initial rise in potential (Fig. 8). Subsequent addition of cofactor of System 1 PMS up to concentration of 10^{-4} M results not only in restoration but a

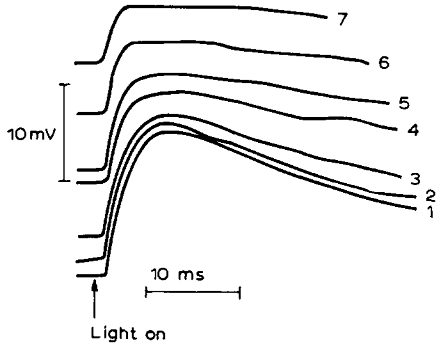

Fig. 8. The time course of photoresponse after addition of DCMU ($5 \cdot 10^{-6}$ M) to in situ chloroplast; 1 is before addition, 2–7 are 0.5 min, 1 min, 1.5 min, 2 min, 2.5 min, and 3 min after addition of DCMU.

substantial increase in photoelectric response. The similar stimulation of photoresponse magnitude of up to 3.5 times with respect to the initial value can be also observed both on isolated chloroplasts and those within the cell after addition of PMS to the medium without DCMU.

In the presence of 10^{-4} M PMS without DCMU the kinetics of the response is greatly altered. The rate of decay in the dark increases several times and τ_3 became about 10 ms instead of 60 ms in normal conditions. After addition of PMS to isolated chloroplasts the time course of photoresponse became complicated because of the appearance of additional slow components. A second, gradually increasing, maximum of the kinetic curves often appears and is probably due to the diffusion of PMS into the chloroplast (Fig. 9). Time courses with two maxima after addition of PMS were also observed on chloroplasts in situ. The complicated kinetics in the presence of PMS without DCMU is probably explained by interaction of cyclic electron flow, activated by PMS and noncyclic electron flow dependent on natural electron carriers.

Another important feature of PMS action is a substantial change in the shape of light-intensity curve. Addition of PMS results in decrease in steepness of the linear portion of light-intensity curve, but at the same time it causes the shift of saturation to the range of much higher light intensities. For this reason the magnitude of the

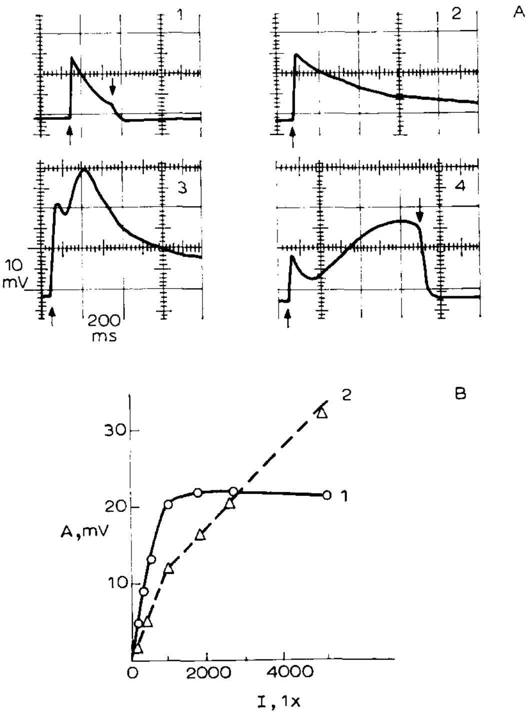

Fig. 9. The effect of PMS on chloroplast photoelectric response. (A) The time-courses of the photoresponse in isolated chloroplast after addition of PMS (10^{-4} M); 1 is before addition and 2, 3, 4 are 0.5 min, 2 min and 5 min after addition, respectively. (B) Light-intensity curves of one in situ chloroplast before (1) and after addition of 10^{-4} M PMS (2). Upward and downward pointing arrows mark the beginning and end, respectively, of the illumination period.

photoresponse diminishes by a factor of 2–3 at low light intensities and at high intensities an enhancement of the photoresponse is observed.

The effects of PMS on the photoinduced response are not determined by the absorbance of PMS itself in the short-wave spectral region. Insertion of a red filter ($\lambda > 600$ nm) in the saturating beam of white light does not cause any changes in magnitude or time course of the photoresponse.

The addition of an uncoupler of photophosphorylation FCCP to the suspension of isolated chloroplasts inhibited light-induced changes by 80 %; however this inhibition was obtained only at concentrations of 10^{-5} M, when FCCP may act nonspecifically by supressing electron transport.

Effect of imposed electrical field

In these experiments chloroplast photoelectric response was measured as a function of an artificially imposed membrane potential. The displacement of membrane potential was produced by an electric current (maximal current $3 \cdot 10^{-9}$ A) flowing through the micro-electrode inserted into isolated chloroplast. The chloroplast membrane was polarized both by rectangular current pulses and linearly increasing current (the rate of current increase 10 nA/s). The same linearly increasing current pulses were used for determination of voltage-current relationships of chloroplasts. A bridge circuit was sometimes employed in the experiments involving the use of current pulses.

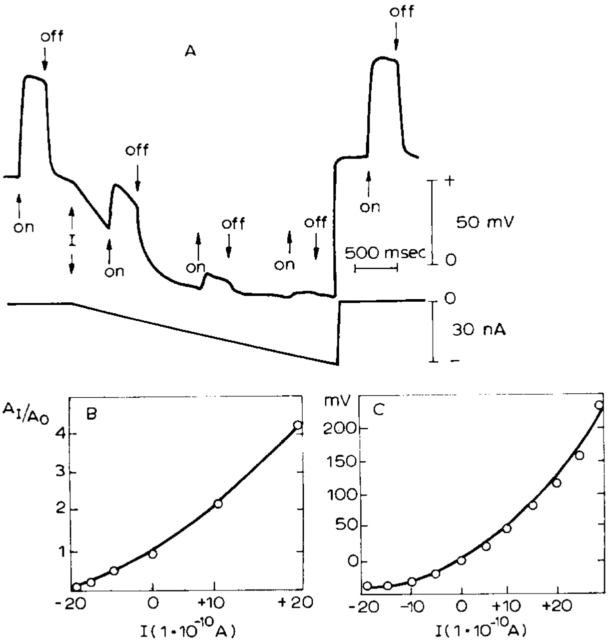

Fig. 10. The effect of electrical polarization on the photoelectrical response in isolated chloroplast. (A) Reversible suppression of the chloroplast photoelectric reaction under an imposed electrical field, with the bottom curve representing the change of electric current through chloroplast, the rate of current increase was 10 nA/s. (B) The dependence of the magnitude of the photoresponse on the extent and direction of current flowing through chloroplast. (C) Voltage – current relationship of isolated chloroplast.

The displacement of dark level of membrane potential towards the positive i.e. in direction coinciding with the direction of light-induced changes, brings about a several fold increase in magnitude of photoresponse, which occasionally reached 130 mV. The displacement of membrane potential to the other (negative) direction results in strong suppression and often complete disappearance of the photoresponse. (Fig. 10). The effect of current on the light-induced changes is fully reversible. From these observations it seems unlikely that light-induced changes of potential can be simply explained by changes in relative ionic permeabilities of the membrane. If the photoresponse was determined by changes in passive permeabilities of the membrane then the reversal of light-induced changes should be obtained in above experiments. Such a reversal of photoresponse was observed on a bimolecular lipid membrane containing chloroplast pigments [19], but was never noticed in our experiments on chloroplasts. It can be assumed that the potential formation is caused by an electrogenic (not passive) mechanism involving light-induced transport of ions (perhaps H^+) across the membrane.

Input electrical resistance of isolated chloroplasts calculated from the slope of voltage-current relationships in normal conditions was found to be 30–50 Mohm. During gradual displacement of the potential to the positive side the input resistance increases several times (increase in the slope of voltage-current relationship) and during the displacement of potential to the opposite side the resistance of chloroplast considerably decreases. The photocurrent flowing through the channels of passive conductance is suggested to bring about a shift in potential, which is proportional to the membrane resistance. It may be assumed therefore from the nonlinearity of the voltage-current relationship that the effects of electrical polarization on the photoresponse are connected with changes in membrane resistance.

While examining voltage-current relationships in chloroplasts it was found that sometimes gradual increase in current flowing through the chloroplast resulted in appearance of oscillatory changes of membrane potential which rose in magnitude with the increase of current. (Fig. 11). We have not yet identified what are these oscillations are caused by nor have we characterized the conditions in which they appear. However, these oscillations indicate the possibility of regenerative changes of chloroplast membrane potential.

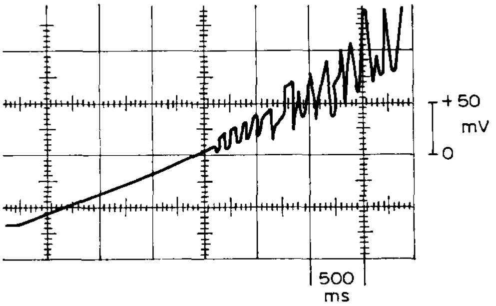

Fig. 11. Oscillations of membrane potential under passing of linearly increasing current through the chloroplast.

On the sign of photoresponse

Fast photoelectric response of chloroplast was described as a shift of potential to the positive [9–11]. In some cases however, experiments both on isolated chloroplasts and chloroplasts in situ gave a fast photoelectric response of the opposite sign. The occasional differences in the sign of the photoresponse can not be attributed to the different dimensions of microelectrode tips. Moreover photoelectric reactions of both signs can be observed during experiments on one isolated chloroplast (Fig. 12). This inversion in the sign of the response is probably connected to the displacement of the microelectrode tip within the limits of chloroplast. This suggestion is supported by the observation that the photoresponse sign can sometimes be reversed by deeper insertion of micro-electrode into the chloroplast. Vredenberg and Tonk [11] assume that photoinduced potential changes occur at the enclosing chloroplast membrane. However we noticed occasionally that after disruption of enclosing membrane photoresponses of isolated chloroplast became diminished but did not disappear completely. These facts illustrate the complexity of the process under study and lead us to conclude that fast photoinduced changes occur not at the outer envelope but at the inner chloroplast membranes. This assumption is supported by a certain similarity between photoelectrical response measured with microelectrodes and electrochromic changes of absorption at 515 nm, which have been interpreted as an indicator of the electrical potential across the thylakoid membranes [2, 20, 21].

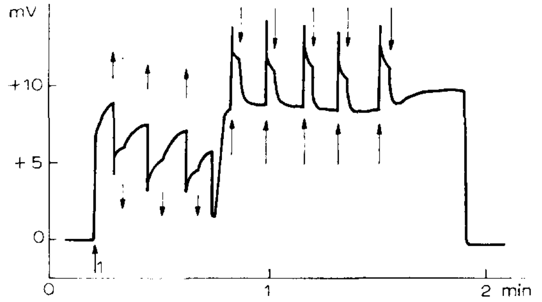

Fig. 12. Spontaneous reversion of photoresponse sign during the experiment on isolated chloroplast. Upward and downward pointing arrows mark the beginning and end, respectively, of the illumination period; 1, the moment of microelectrode insertion.

DISCUSSION

The simplest scheme suitable for explanation of the kinetic data consists of series of first order processes:

$$A \xrightarrow{I\sigma_{II}} B \xrightarrow{K_1} C \xrightarrow{I\sigma_I} P \xrightarrow{K_2} X(\sim E) \xrightarrow{K_3}$$

This scheme is based upon the concept of two sequential photochemical reactions of photosynthesis. The rate constants for two light stage sensitivities by pigment Systems 1 and 2 are denoted by $I\sigma_I$ and $I\sigma_{II}$ where I is light intensity, and σ is the effective section of photoreactions. Experiments with two monochromatic light beams separated in time indicate the existence of dark reaction with the rate-constant k_1. The rise of potential after the flash indicates that a dark step with constant k_2 is also involved. Finally, the product X of this reaction is directly responsible for the generation of potential (E). The last process ($X \xrightarrow{K_3}$) reflects the decay of membrane potential to the initial dark level due to diffusion of ions through the membrane. According to this scheme Photosystem 1 is directly responsible for the formation of potential, whereas Photosystem II provides the substrate for the functioning of System I. This assumption is supported by experiments with DCMU and PMS and action of far red light (712 nm) in exciting Photosystem I.

Under conditions of predominant excitation of Photosystem II (by 640 nm light), the rate-limiting reaction would be the reaction with constants $I\sigma_{\mathrm{I}}$ and the kinetic scheme can be simplified:

$$\mathrm{C} \xrightarrow{I\sigma_{\mathrm{I}}} \mathrm{X}(\sim \mathrm{E}) \xrightarrow{K_3}$$

If $I_{\sigma_{\mathrm{I}}}$ is rather small then the concentration of C may be considered to be in excess and the rate of the reaction C → X would be constant. In this case photo-induced changes of potential must be determined by constant of dark process $k_3 = 1/\tau_3$,

$$\mathrm{E} = \frac{I\sigma_1}{K_3}\,(1-\mathrm{e}^{-K_3 t})$$

The exponential rise in potential with characteristic time τ_1 approximating to that of dark decay ($\tau_1 \approx \frac{1}{2} t_3$) is actually apparent under the action of 640 nm light (see Fig. 7).

If Photosystem I is excited separately by far red light (712 nm) and the intensity of the far red light is high, then the kinetics of photoinduction must be determined by the utilization of substrate C. In this situation the pool of C is not refilled because Photosystem II is inactive.

For this case the scheme may be represented as:

$$\mathrm{C} \xrightarrow{I\sigma_{\mathrm{I}}} \mathrm{P} \xrightarrow{K_2} \mathrm{X}(\sim \mathrm{E}) \xrightarrow{K_3}$$

Under sufficiently strong illumination $I\sigma_{\mathrm{I}} \gg K_2$ the course of potential change should follow the change in concentration of the intermediate product X.

$$\mathrm{E} \simeq \frac{K_2}{K_3 - K_2}\,(\mathrm{e}^{-K_2 t} - \mathrm{e}^{-K_3 t})$$

Curves of approximately such shape were actually observed under action of far red light (see Fig. 7).

According to the above equation the initial rate of increase in potential under the action of far red light is determined by constant k_2 and not by $k_3 = 1/\tau_3$. This assumption is in agreement with the experimental data: the rate of potential rise under the action of 712 nm light was as much as an order of magnitude higher than the rate of dark decay ($\tau_1 \approx \frac{1}{10}\tau_3$).

The assumption of the existence of a stage connecting two photoreactions gives an explanation to the enhancement effect. The concentration of intermediate product C is high when Photosystem II is preferably excited, and when only Photosystem I is excited concentration of C is low. Therefore preliminary illumination with short-wave light should enhance the action of long-wave light (see Fig. 6). The dependence of the effect on the dark period separating two light beams (640 nm and 712 nm) must be determined by the ratio of constants of accumulation (k_1) and the constant of utilization of product C. Preliminary estimation shows that the order of $1/k_1$ is $\lesssim$ 1 s, and that the product C is rather stable in the dark (stored for periods longer than 30 s).

Thus the above scheme permits qualitative interpretation of the kinetic data and, from our point of view, it can be regarded as a working hypothesis explaining the relationship between two main photosynthetic reactions and membrane potential generation in chloroplast.

The question of the mechanism of the processes is more complicated; but some analogies and assumptions can be made. Generally, the fast changes of chloroplast potential support the Mitchell hypothesis about the immediate connection of photosynthetic electron transfer with generation of electrical potential. From our results we can conclude that the activity of Photosystem I is closely coupled with transmembrane electron transfer and charge separation and that the potential generation by Photosystem I depends upon the product of Photosystem II. However, our results do not exclude the possibility of charge separation by Photosystem II.

The time scale correlation of chloroplast membrane potential changes and light-induced H^+ transfer [22] may be regarded as evidence in favour of the chemiosmotic hypothesis. The poor temperature dependence of photoinduction, the sensitivity of response to inhibitors of electron transport and uncouplers of phosphorylation are also evidences supporting the Mitchell hypothesis. Nevertheless the complex character of the photoinduction phenomenon, i.e. appearance of both fast and slow changes of potential [9], the occurrence of fast changes of opposite sign, oscillatory changes and so on, are not explained by the chemiosmotic hypothesis. The important question remains: in what way do the photoinduced potential changes originating in chloroplast result in electric potential changes at the cell membrane.

It is interesting to compare our results with data obtained on bimolecular lipid membranes containing photosynthetic pigments [19, 23, 24]. The time course of chloroplast photoelectric response in many ways reflects the responses measured on bimolecular lipid membranes under illumination [19, 23]. In both cases there is a stage of dark rise in potential after the flash. The most rapid stage (in the microsecond range) of induction observed on model membrane is likely to exist in chloroplast but can't be seen distinctly (see Fig. 2) because of the latency inherent to the method. Nevertheless some important differences of the responses on lipid membranes and chloroplast indicate different mechanisms of generation of potential. Two main differencies are: strict connection of chloroplast photoresponse to photosynthesis and two photosystems, and the lack of inversion of chloroplast photoelectric response in experiments with an externally imposed electric field. A possible explanation for the latter difference is that photoillumination of bimolecular lipid membrane allows a dissipation of a preexisting redox energy [24] whereas illumination of chloroplast membranes result in increase of redox energy gradient across the membrane (passive and active transfer of charges across the membrane).

The kinetic characteristics of dark decay of chloroplast potential (exponential with $\tau_3 = 1/K_3$) is in good agreement with both analogous dark decay measured on chloroplast suspensions by monitoring changes of optical absorption at 515 nm [2] and the decay of potential on bimolecular lipid membrane [19, 23]. In agreement with conclusions made in these works we presume that time constant τ_3 reflects the characteristic RC-time of the membrane, and it appears to be approximately the same for the various systems that have been investigated.

In spite of the complexity of the photoinduction processes in the whole chloroplast, it may be assumed that some of the stages have a physical nature and hence can be modeled in simple systems. As was shown by one of the coauthors [25], dry films made from chloroplasts had properties of molecular semiconductors. The photoconductivity was proposed to be due to crystalline aggregated forms of chlorophyll comprised in Photosystem I. It can be assumed that these aggregates embedded in the thylakoid membranes are in fact the semiconductor electron pores which are responsible for the light-induced charge transfer across the membrane.

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