Biochimica et Biophysica Acta, 766 (1984) 647–652
Elsevier

BBA 41569

DIFFERENT KINETICS OF MEMBRANE POTENTIAL FORMATION IN DARK-ADAPTED AND PREILLUMINATED CHLOROPLASTS

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(Received February 24th, 1984)

Key words: Electron transport; ATPase; Microelectrode; Membrane potential. Photosystem I; (Anthoceros, P. metallica)

The effects of varying dark interval on the kinetics of light-induced formation of the membrane potential were studied on individual chloroplasts of *Anthoceros* with the use of capillary microelectrodes. Illumination of the chloroplast with 1 s light pulse after 3 min dark period induced the photoelectrical response with two peaks of the potential that were located at 20 and 500 ms after the onset of illumination. The position of the second peak was shifted along the time-scale depending on the preceding dark interval. The repeated illumination of the chloroplast with 1 s light pulse after 30 s dark interval induced the electrical response with only one maximum and a monotonous decay of the potential in the light. Distinctions in the electrical responses induced by the first and the second light pulses were eliminated by the addition of 50 μM dicyclohexylcarbodiimide (DCCD). The results show that the photoinduction kinetics of the membrane potential in chloroplasts is affected by functioning of H^+-ATPase. The delayed peak of the membrane potential in the photoinduction kinetics is interpreted as a consequence of the photoactivated electron transport supported by Photosystem I.

Introduction

The time-course of the electrical potential across the chloroplast thylakoid membranes in the first seconds of illumination has been measured by various techniques including microelectrodes [1–4], the electrochromic absorbance change at 515 nm [5,6] and the absorbance change of a potential-sensitive dye, oxonol VI [7,8]. The results obtained by different methods are all consistent with the idea that the initial rapid rise of the electrical potential is followed by a monotonous decay to a low steady level. So far, the absorbance changes of both intrinsic and extrinsic potential-sensitive probes induced by the continuous light were measured only in preparations of isolated thylakoids. The electrical measurements done with in situ *Anthoceros* chloroplasts [3] indicated that the photoinduction kinetics of the membrane potential in intact chloroplast is more complicated than that measured in isolated chloroplasts by various methods. The light-induced formation of the membrane potential in *Anthoceros* chloroplasts is composed of two clearly separated peaks. Two peaks of the potential were also apparent in isolated *Peperomia metallica* chloroplasts under certain conditions [1]. The delayed peak of the electrical potential seems to be typical and physiologically important for intact chloroplasts. However, this peak of the potential has not been well characterized so far and its origin remains obscure.

The results of the present study show that the kinetics of the membrane potential change in the induction period is strongly affected by preillumination conditions and the inhibitor of the

Abbreviations: DCCD, dicyclohexylcarbodiimide; PMS, *N*-methylphenazonium methosulphate; PS I, Photosystem I.

membrane-bound ATPase. Possible mechanisms responsible for the formation of the delayed peak of the electrical potential are discussed.

Materials and Methods

Measurements of the membrane potential were done on single chloroplasts of two species of the liverwort *Anthoceros* and the higher plant *Peperomia metallica*. Plants of *Anthoceros* sp. and *Anthoceros punctatus* were collected in the Batumy's Botanical Garden and in a field near Moscow, respectively. Plants were grown in glass vessels on moistured soil in a dim sunlight. Sections of thalli were placed in the medium containing 10 mM KCl/1.0 mM NaCl/0.5 mM $CaCl_2$. The preparation was kept in the medium for 30 min before the measurements. Because of the increased level of K^+ in the medium, the potential difference across the plasmalemma was low and did not change upon illumination (cf. Ref. 9). Potentials were measured with glass microcapillary electrodes filled with 1 M choline chloride solution as described before [3]. The composition of the medium and the preparation procedure for obtaining isolated chloroplasts of *P. metallica* were described elsewhere [1,4]. Membrane potential changes were recorded from the screen of a storage oscilloscope. Positions of the microelectrode and the chloroplast were adjusted under weak white light ($10\ J \cdot m^{-2} \cdot s^{-1}$).

After the insertion of the microelectrode, the chloroplast was illuminated with 1 s light pulse of saturating intensity (approx. $5 \cdot 10^2\ J \cdot m^{-2} \cdot s^{-1}$), and then it was kept in darkness for 3 min. Following this period of dark adaptation, two consecutive 1 s light pulses of saturating intensity were applied. These light pulses, referred to as the first and the second light pulse, were separated by a variable dark period ranging from 5 s up to 4 min. Afterwards, the cycles of 3 min dark adaptation followed by a sequence of two light stimuli were repeated. The duration of light pulses was controlled with a home-made electromechanical shutter (opening time 3 ms). Each experiment with one single chloroplast lasted from 10 up to 30 min. This period was sufficiently long as to allow reproducible measurements of photoelectric responses from dark-adapted and preilluminated chloroplasts.

Results

Light-induced changes of the membrane potential measured in chloroplast of two species of *Anthoceros* were identical both in magnitude and shape, and exhibited similar dependencies on preillumination.

In Fig. 1 are shown typical changes of the membrane potential that were induced by 1 s light pulses in an individual *A. punctatus* chloroplast after various times of darkness. The prominent feature is that light pulses applied after long (2–4 min) or very short (5–10 s) dark exposures induced multiphasic changes composed of two clearly separated peaks. The light pulses admitted in 20–30 s after the first illumination induced usually the photoresponse with only one peak and a monotonous decay of the potential.

Regular modifications in the time-course of the membrane potential upon increasing the period of a dark exposure could be distinguished. The extent of the first peak rose on increasing the dark period within the range 5–30 s (Fig. 1a, b and c) in a manner completely similar to that described for *P. metallica* chloroplasts [1]. The first peak was independent on the time of preliminary dark adapta-

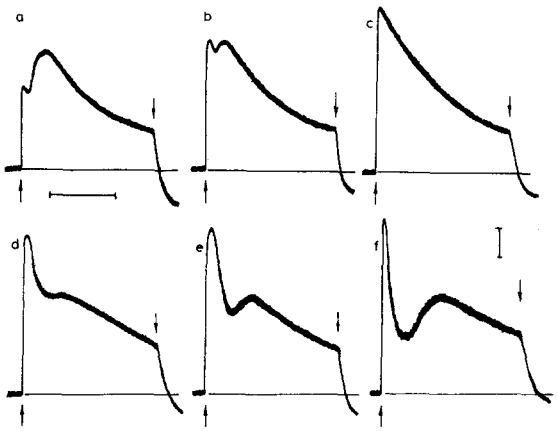

Fig. 1. Effect of the preceding dark interval on the kinetics of the light-induced membrane potential in *Anthoceros* chloroplast. Figures along the curves indicate the duration of the dark period (in seconds) after preliminary 1 s illumination. Upward and downward arrows in this and other figures indicate the light on and light off points, respectively. Horizontal lines denote the dark (zero) level of the potentials. (a)–(f) Potential changes induced by 1 s light pulses after various times of darkness.

tion in the range form 30 s up to 4 min. The result presented in Fig. 1 show that the position of the second maximum of the potential shifted along the time-scale depending on the preceding dark interval.

Since the extent of the first peak and the location of the second peak are variable under different preillumination conditions, it seems likely that these two peaks might in some cases overlap in such a way as to produce the photoresponse with one apparent maximum and a monotonous decay phase (Fig. 1c). Such an interpretation predicts the existence of two electrogenic processes which are partially independent and are responsible for the formation of the fast and the delayed peaks of the membrane potential in the photoinduction period.

In Fig. 2 are shown the light-induced changes of the membrane potential measured in the presence of 10 μM antimycin A. The photoelectrical responses measured immediately after the microelectrode insertion into the chloroplast were similar to that measured in the control without the inhibitor and consisted of two peaks. Shortly afterwards (within 1–3 min after the chloroplast impalement), the delayed peak of the potential disappeared whereas the first peak of the potential remained unchanged. This is in contrast to a fairly good stability of the photoresponse kinetics in the absence of the inhibitor. Under control conditions, the photoelectrical responses with two maxima were usually reproduced many-fold in the course of one experiment lasting up to 20–30 min. The experiments with antimycin A present an additional evidence for the existence of two electrogenic process in the photoinduction period that differ in their dependence on preillumination and their sensitivity to inhibitors.

Our routine measuring procedure consisted of recording the superimposed traces of light-induced potential changes in dark-adapted and preilluminated chloroplasts. The advantage of this procedure is that it permits more accurate detection of distinctions between the photoinduction of the membrane potential in dark-adapted and preilluminated chloroplasts. Fig. 3a shows changes of the membrane potential induced by two consecutive light pulses separated with a dark interval of 20 s. In the initial 200 ms of illumination, there was a rapid drop of the membrane potential in dark-adapted chloroplasts relative to the membrane potential in preilluminated plastids. This rapid drop (a potential dip) was followed by a secondary rise of the potential up to values exceeding or coinciding with the respective values of the potential in preilluminated chloroplasts. The potential traces intersect at about 0.5 s after the onset of illumination. The same intersection of

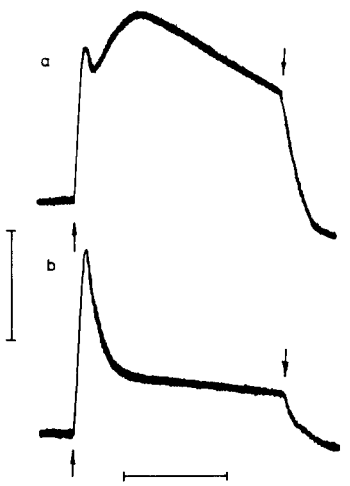

Fig. 2. Alteration of the chloroplast photoelectrical response in the presence of 10 μM antimycin A. (a) Light-induced change of the membrane potential measured immediately after the insertion of the microelectrode; (b) the photoelectrical response of the same chloroplast under similar preillumination conditions measured 3 min after the chloroplast impalement.

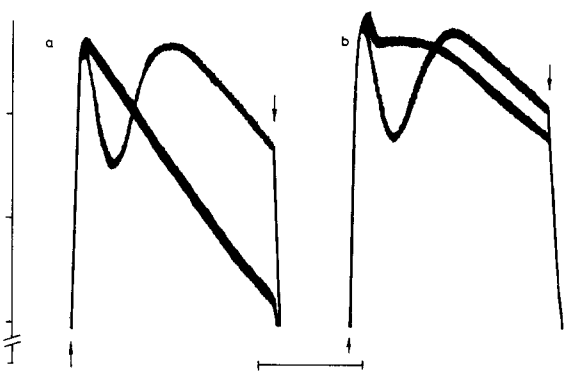

Fig. 3. Changes of the membrane potential induced by the first light pulse after 3 min dark adaptation (1) and by the second illumination after a short period of darkness (2). (a) and (b) are the records of the upper parts of the potential traces measured in the same chloroplast; the length of dark interval separating two consecutive light pulses was 20 s (a) and 30 s (b).

potential traces was observed when two light pulses were separated with a shorter dark interval (5–10 s).

After addition to the medium of 50 μM dicyclohexylcarbodiimide (DCCD), an inhibitor of the chloroplast ATPase, the changes of the membrane potential induced in dark-adapted and preilluminated chloroplasts became identical and proceeded with only one maximum and a monotonous decay to a steady level (Fig. 4). In contrast to antimycin A, DCCD did not cause the acceleration of the potential decay in the light. The phase of the potential decay in the light proceeded in DCCD-treated chloroplasts as slowly as that one in preilluminated chloroplasts under control conditions. The photoresponse magnitude was not essentially altered by the addition of DCCD but sometimes a slight increase in the peak value of the membrane potential was noticed.

Ammonium chloride is known to uncouple photophosphorylation and to stimulate ATPase activity in chloroplasts [10]. In the presence of 1 mM NH_4Cl, the photoinduction kinetics with two peaks of the potential as well as its dependence on preillumination were clearly exhibited. When isolated *P. metallica* chloroplasts were suspended in the medium containing 1 mM NH_4Cl, the first light pulse applied after 3 min dark exposure induced the electrical response with two maxima. The next light pulse separated by a dark interval of a few seconds elicited the response with only one maximum (Remish, D. and Bulychev, A.A., unpublished data), which is similar to the observation on *Anthoceros* chloroplasts.

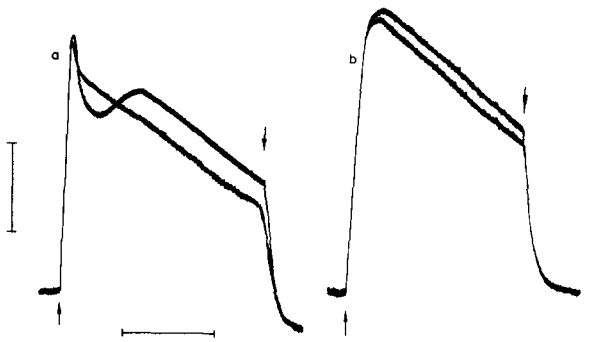

Fig. 4. Light-induced changes of the membrane potential in dark-adapted (1) and preilluminated (2) chloroplast under control conditions (a) and in the presence of 50 μM DCCD (b). The length of dark adaptation was 3 min; the second light pulse was applied in 20 s after the first one.

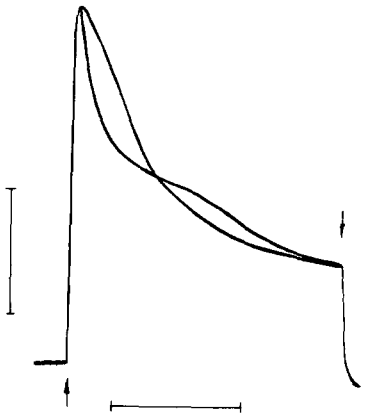

Fig. 5. Changes of the membrane potential induced by two consecutive light pulses in the presence of 50 μM PMS. Photoresponses 1 and 2 were induced by the first light pulse after 3 min of darkness and by the second illumination applied in 20 s after the first one.

Addition to the medium of the artificial electron carrier, *N*-methylphenazonium methosulphate (PMS), at a concentration of 50 μM resulted in a 2- to 3-fold increase in the peak value of the light-induced membrane potential. The peak value of the membrane potential in *Anthoceros* chloroplasts was usually of about 100 mV in the presence of PMS and reached sometimes 150 mV (Fig. 5). The difference between the potential traces measured in the first and the second light pulses in the presence of PMS was not as apparent as in the control, since this difference was small with respect to the photoresponse magnitude, although it was still large in absolute values.

Discussion

The present results show clear-cut distinctions in the kinetics of the membrane potential formation in dark-adapted and preilluminated chloroplasts as well as the elimination of these distinctions by DCCD. The effects of preillumination and DCCD could be tentatively attributed to an activation and inhibition, respectively, of chloroplast ATPase (cf. Ref. 11). In intact chloroplasts, the coupling factor is converted into an active state in a light-triggered manner and transforms slowly back to inactive state in the dark [10–13]. A 1 s preillumination was shown to be sufficient to accelerate the decay of the flash-induced ab-

sorbance change at 515 nm, correlated with the activity of membrane-bound ATPase [11]. It is therefore conceivable that the photoelectric response with a transient minimum and a secondary rise of the potential, that is typical for dark-adapted chloroplasts and is abolished by DCCD (Figs. 1, 3 and 4), is related to a functional transition of the chloroplast coupling factor. The time of recovery of 'dark-adapted state' in our experiments (of about 3 min) is comparable with the decay time of the activated state of ATPase in intact plastids [14].

The complicated time-course of the membrane potential in the induction period might be caused by at least two factors: (1) a non-monotonous change in the rate of charge separations across the thylakoid membrane and (2) the ionic conductance change of the membrane. The activation of the chloroplast ATPase is presumably associated with an opening of H^+-conduction channel in the hydrophobic part of the enzyme and a resulting increase of the membrane conductance [15] (but see Refs. 5 and 16). The increased electrical leak through the open channel of ATPase would account for the occurrence of the transient minimum of the potential in response to first illumination. The elimination of the potential dip by DCCD, which is known to prevent the light-induced increase in the membrane conductance [3], is consistent with this idea. The delayed rise of the potential could reasonably be explained as a consequence of acceleration of electron transport coupled with a translocation of protons across the membrane. The alternative of a secondary decrease of the membrane conductance after a transient rise is rather improbable.

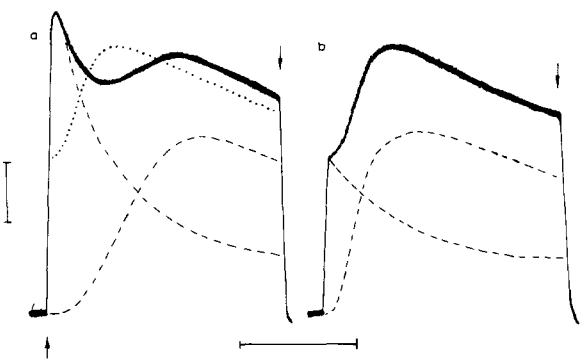

Fig. 6. Schematic representation of the light-induced changes of the membrane potential as a sum of the fast (I) and the delayed (II) electrogenic components . (a) The electrical response of the chloroplast induced by the first light pulse after 3 min dark adaptation; (b) the electrical response induced by the second illumination in 5 s after the first one; this response is also shown as a dotted line on the left side of the figure.

The photoactivation of electron flow in first seconds of illumination has also been concluded from studies of chlorophyll fluorescence in *Bryopsis* chloroplasts [17]. It has been assumed that the photoactivation is due to a reduction of some redox component in the acceptor side of Photosystem I (PS I). Moreover, the PS-I-dependent electron transport is presumably involved in the activation of ATPase through the reduction of thioredoxin [14,18]. It seems likely that the photoactivation of the electron transport mediated by PS I is the main cause for the delayed rise of the membrane potential. Photoactivation of cyclic electron transport is evidenced by the effect of antimycin A (Fig. 2), an inhibitor of cyclic electron flow and of a slow electrochromic absorbance change [19,20]. However, the involvement of a photoactivated electron flow to oxygen is not excluded [17].

It thus appears that the first and the second peaks of the potential are related to different electrogenic processes which are partially independent. The existence of different electrogenic reactions giving rise to the fast and the slowly generated electrical fields was formerly shown by measurements of fast and slow absorbance changes of carotenoids at 515 nm [5,19,21,22]. It should be noted, however, that the results obtained with single-turnover flashes and 1 s light pulses are not easily comparable. So far, no evidence has been presented for a correlation between slow electrogenic reactions measured with short flashes and long light pulses.

Fig. 6 presents a schematic decomposition of the time-course of the membrane potential into two separate electrogenic components. For simplicity, the negative diffusion potential, which is relatively small at the end of the 1 s illumination, is omitted here. Only extreme types of the potential kinetics are presented that are observed after very short (5 s) or long (3 min) dark periods. Intermediate forms of the photoresponse kinetics including the kinetics with a single potential peak

might also be explained in terms of two superimposed components. Within the framework of the above interpretation, diverse kinetics of the membrane potential formation as well as typical overcrossing of the potential traces shown in Figs. 3 and 4a, result mainly from the shift of the delayed component along the time-scale, depending on preillumination conditions.

According to our interpretation, the electron transport responsible for the creation of the second component is related to the state of coupling factor. The photoactivation of that electron flow is presumably delayed in dark-adapted chloroplasts, but occurs with a shorter delay after preliminary illumination. The interaction of the ATPase with electron transport through the oxidation-reduction reactions at the level of the acceptor side of PS I has been discussed [14].

With the concept of two superimposed electrogenic components, the complicated time-course of the potential with a transient minimum finds its explanation without the need of invoking changes of membrane conductance for protons (cf. Ref. 16). However, it seems more probable that the complicated kinetics of the membrane potential after a long dark adaptation is determined both by the photoactivation of electron transport and by the change in H^+-conductance of the thylakoid membrane.

Acknowledgements

I wish to thank Drs. V.R. Filin and M.Yu. Davitadze (Batumi) for help in collecting plant material and Dr. G.A. Kurella for his interest and support.

References

1 Bulychev, A.A., Andrianov, V.K., Kurella, G.A. and Litvin, F.F. (1976) Biochim. Biophys. Acta 430, 336–351
2 Vredenberg, W.J. (1976) in The Intact Chloroplast (Barber, J., ed.), pp. 53–88, Elsevier, Amsterdam
3 Bulychev, A.A., Andrianov, V.K. and Kurella, G.A. (1980) Biochim. Biophys. Acta 590, 300–308
4 Remish, D., Bulychev, A.A. and Kurella, G.A. (1981) J. Exp. Bot. 32, 979–987
5 Schapendonk, A.H.C.M. (1980) Doctoral Thesis, Technical University of Agriculture, Wageningen, The Netherlands
6 Hope, A.B., Ranson, D. and Dixon, P.G. (1982) Aust. J. Plant Physiol. 9, 385–397
7 Schuurmans, J.J., Casey, R.P. and Kraayenhof, R. (1978) FEBS Lett. 94, 405–409
8 Admon, A., Shahak, Y. and Avron, M. (1982) Biochim. Biophys. Acta 681, 405–411
9 Bulychev, A.A. and Turovetsky, V.B. (1983) J. Exp. Bot. 34, 1181–1188
10 Mills, J.D., Mitchell, P. and Schürmann, P. (1980) FEBS Lett. 112, 173–177
11 Morita, S., Itoh, S. and Nishimura, M. (1982) Biochim. Biophys. Acta 679, 125–130
12 Mills, J.D. and Hind, G. (1979) Biochim. Biophys. Acta 547, 455–462
13 Inoue, Y., Kobayashi, Y., Shibata, K. and Heber, U. (1978) Biochim. Biophys. Acta 504, 142–152
14 Shahak, Y. (1982) Plant Physiol. 70, 87–91
15 Witt, H.T. (1979) Biochim. Biophys. Acta 505, 355–427
16 Schreiber, U. and Rienits, K.G. (1982) Biochim. Biophys. Acta 682, 115–123
17 Satoh, K. (1982) Plant Physiol. 70,, 1413–1416
18 McKinney, D.W., Buchanan, B.B. and Wolosiuk, R.A. (1978) Phytochemistry 17, 794–795
19 Crowther, D., Mills, D. and Hind, G. (1979) FEBS Lett. 98, 386–390
20 Garab, G. and Farineau, J. (1983) Biochem. Biophys. Res. Commun. 111, 619–623
21 Vredenberg, W.J. (1981) Physiol. Plant. 53, 598–602
22 Peters, F.A.L.J., Van Spanning, R. and Kraayenhof, R. (1983) Biochim. Biophys. Acta 724, 159–165