ON THE COUPLING OF ELECTRICAL EVENTS AT THE MEMBRANES OF ENERGIZED CHLOROPLASTS IN INTACT PLANT LEAF CELLS

W. J. VREDENBERG and W. J. M. TONK
Plant Physiological Research Center, P.O. Box 52, Wageningen, The Netherlands

Received 5 March 1974

1. Introduction

It has been shown that the potential of the enclosing membrane of chloroplasts in plant leaf cells rapidly alters upon illumination with photosynthetically active light [1–3]. Three phases have been distinguished. A rapid depolarisation of up to 40 mV (phase 1) is followed by a slower hyperpolarisation (phase 2) towards a steady state (phase 3), which in general is slightly above the dark potential [3,4]. The rapidity of the phase 1 change [3], its insensitivity to the electron transport inhibitor DCMU and its association with the absorption change of P 515 [4], have led us to suggest that this change is associated with an ion binding reaction at the negative sites on the stroma-facing side of the thylakoid membrane. These sites have been evidenced to be generated in the primary charge separating photochemical acts within the membrane-bound photosynthetic reaction centers in the thylakoid membrane [5].

This report deals with a kinetic analysis of the phase 1 potential change in saturating short light flashes, measured in the absence and presence of weak continuous background light, in which the number of chargeable binding sites will be altered. The results reveal that the kinetics and extent of the phase 1 potential change are quantitatively dependent on the actual number of negative binding sites at the outer surface of the thylakoid membrane. A coupling mechanism is discussed.

2. Materials and methods

Measurements were performed on individual chloroplasts in mesophyll cells of leaf sections of the terrestial plant Peperomia metallica. Growth conditions, preparation of specimen and details of the measuring device have been described [3,4]. Flash illumination of the chloroplasts occurred by a light beam from an electronic camera flash (rise time 5 μsec, 2 msec width at half intensity), or from a flash tube (G.E. FT230, rise time 2 μsec, 50 μsec width at half intensity). Background illumination occurred by a second beam from a 24 V d.c., 250 W lamphouse assembly. Light of each beam reached the specimen via a common collecting light guide. The fast light-induced potential changes across the chloroplast enclosing membrane, measured with conventional fine-tipped microcapillaries inserted into a single chloroplast, were monitored on an oscilloscope. The time resolution of the measuring system usually was set at 30 μsec. Experiments were carried out at room temperature.

3. Results and interpretation

Fig. 1 shows the potential response $V(t)$ of the chloroplast membrane upon a saturating light flash (rise time 2 μsec, half width 50 μsec). The phase 1 depolarization V_{max} is completed within 1 msec, and the decay in the dark towards the dark potential

 North-Holland Publishing Company – Amsterdam

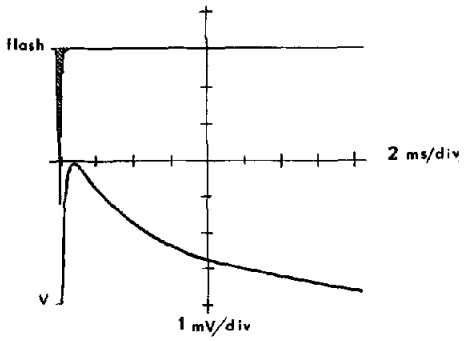


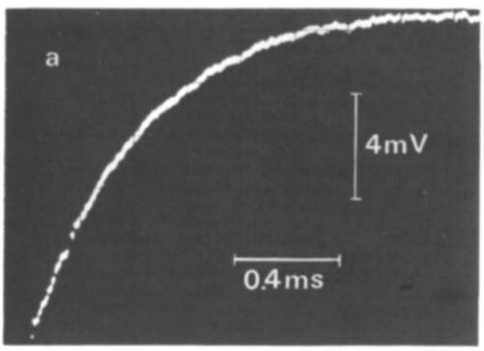
Fig. 1. Potential response V (lower curve) of the chloroplast enclosing membrane upon a saturating light flash (upper curve). The dark potential E_0 was -110 mV.

takes about 15 msec. It has been found for a variety of chloroplasts in different cells that the time in which the phase 1 change is completed after a saturating flash is between 0.5 and 1.5 msec. The decay time was found between 5 and 50 msec.

Fig. 2 shows the kinetics of the phase 1 potential change upon a saturating light flash (rise time 5 μsec, half width 2 msec) in the absence (a) and presence of continuous 717 nm background illumination of 6.5 (b), 13 (c) and 29 (d) kergs/(cm^2·sec) intensity, respectively. It is important to note that the background light did not cause a change in the dark potential. The log plots of $V_{max}-V(t)$ against time for each of these curves are shown in fig. 3. The straight lines indicate a first order reaction:

$$V(t) = \alpha \cdot V_{max} \cdot (1-\exp(-kt)) \qquad (1)$$

with $k = (2.7 \pm 0.3) \cdot 10^3\ \mathrm{sec}^{-1}$, $t_{1/2} = 0.25 \pm 0.03$ msec



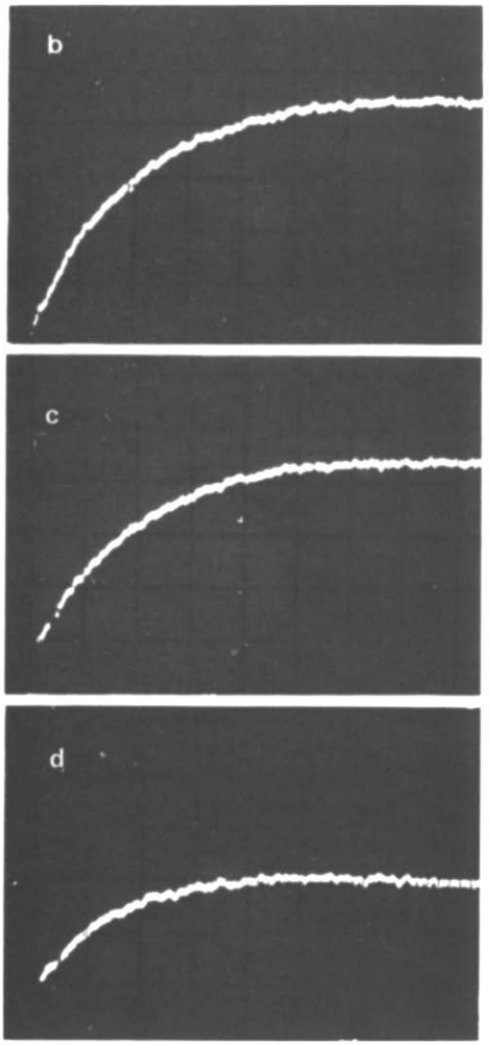


Fig. 2. Kinetics of the flash induced initial depolarization phase (phase 1) of the potential change of the chloroplast membrane in the absence (a) and presence (b, c, d) of 717 nm background light. The intensity of the background light was 6.5 (b), 13 (c) and 29 (d) kergs/cm^2·sec, respectively. The flash was fired 5 s after the onset of background illumination. E_0 was not affected by the background light and was -130 mV.

and $\alpha = 1.0$ (a), 0.59 (b), 0.44 (c) and 0.35 (d), respectively. The small differences in the slope of the lines are unrelated to the background light; such differences were also found for the response in the absence of background light during the course of an experiment. Similar results as shown in figs. 2 and 3

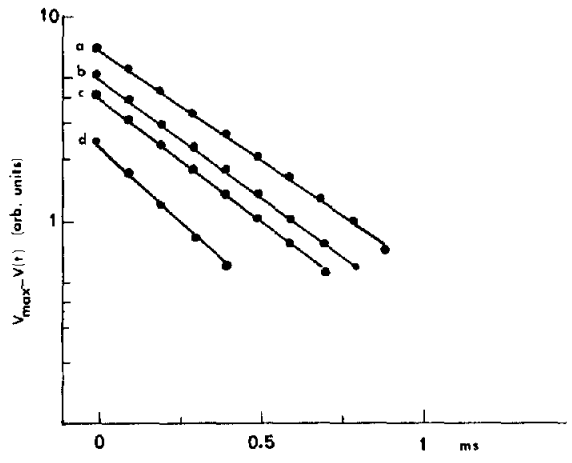


Fig. 3. Log plots $V_{max} - V(t)$ against time (data from fig. 2).

were found with 676 nm background light. These data show that background light has caused a decrease in the extent of the potential change induced by the flash, without affecting the rate constant k of the reaction.

The results can be described in terms of the reaction kinetics of an ion binding process (probably of protons) at the thylakoid membrane. This binding has been suggested to occur at the negative binding sites at the outer surface of the thylakoid membrane, formed in the primary photochemical acts upon flash excitation within 20 nsec [6]. It has been discussed that the binding results in a reduction of catalysts occupied by the sites (i.e. the primary electron acceptors X and Q of system 1 and 2, respectively) with a concomitant adsorption of a negative ionic charge (probably OH^-) at the stromafacing thylakoid surface [7]. Such process may be formalized by the following dark reaction:

$$S^- + H_2O\,(H^+ \cdot OH^-) \rightarrow SH \cdot OH^- \qquad (2)$$

in which S^- and $SH \cdot OH^-$ are the active (electron charged) and bound (reduced and ion charged) states, respectively of the binding sites. It may be assumed that, in analogy with the situation in an homogeneous system, reaction (2) proceeds with first order kinetics ($[H_2O] >> [S^-]$). This would yield $S^-(t) = S^-(0) \cdot \exp(-k_d t)$, in which $S^-(t)$ is the number of active states at time t, and $S^-(0)$ the number of active states initially formed by the flash. The number of bound states at time t may be denoted by $(SH \cdot OH^-)_t$. With $S^-(t) + (SH \cdot OH^-)_t \equiv S^-(0)$ we would get:

$$(SH \cdot OH^-)_t = S^-(0) \cdot (1 - \exp(k_d t)) \qquad (3a)$$

In saturating flashes all available sites (S_0) will be charged, i.e. $S^-(0) = S_0$. In the presence of non-saturating weak background light a fraction $(1-\alpha)$ of the sites will be in the bound (reduced) state. In this case the number of sites charged by the flash will be $S^-(0) = \alpha S_0$ and accordingly:

$$(SH \cdot OH^-)_t = \alpha S_0 \cdot (1 - \exp(-k_d t)) \qquad (3b)$$

The close similarity between the kinetics of the phase 1 potential change measured at the enclosing membrane (eq. 1) and those evidenced for the binding process at the thylakoid membrane (eq. 3a), both in the absence and presence of background light (e.g. fig. 3 and eq. 3b), suggests that the potential change $V(t)$ induced by the flash is proportional to the number of bound states $(SH \cdot OH^-)_t$ formed after the flash. The following explanation, based on physical reasoning, is proposed. Certain assumptions made on the structural level need to be confirmed for chloroplasts of this plant species (e.g. ref. [12]).

The potential (-change) recorded by an electrode in the stroma phase will be due to electric fields induced by activated thylakoids. As separate and closed thylakoids, with the photosynthetic apparatus homogeneously distributed over the membrane, will not contribute to an external electric field, this field is created by lamellae of which the membranes are in continuity with the (black) inner enclosing membrane. Thus the net negative ionic charge ($- \cdot OH^-$) at the stroma-thylakoid interface of these lamellae will cause a charge polarisation at the inner membrane. This would result in a proportional positive ionic charge in the stroma phase adjacent to the inner enclosing membrane, and a net negative ionic charge in the interspace between inner and outer envelope. Therefore it seems likely that the microelectrode, inserted just across the envelope, is probing the positive going potential associated with the induction of positive charges in this layer. The extent of the potential jump of course will be dependent on the distance

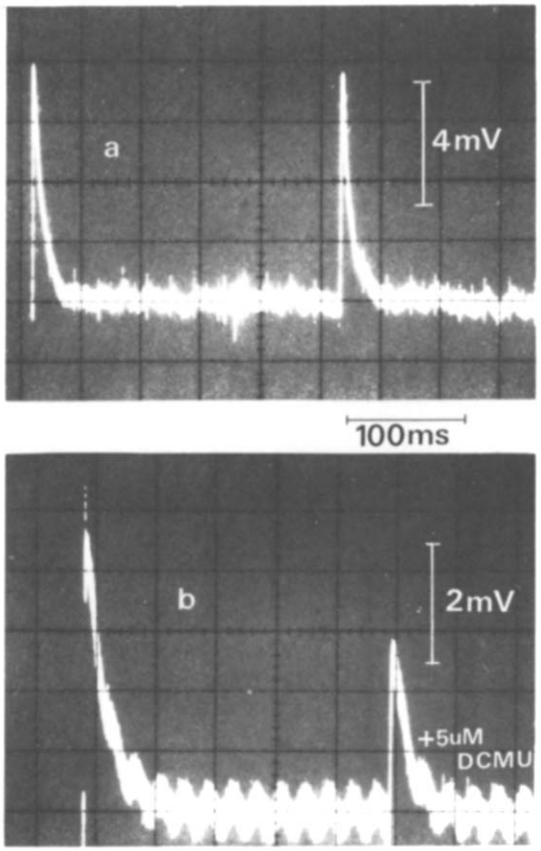


Fig. 4. Potential response of the chloroplast enclosing membrane upon two consecutive saturating flashes in the absence (upper trace) and, for a different chloroplast, in the presence of 5 μM DCMU (lower trace). A third flash, fired 250 msec after the second one, gave in both cases the same response as the second one. E_0 was approx. -130 mV.

over which the electrode has penetrated into the stroma phase. This would explain why the extent of the potential responses is highly variable for different impalements. It is interesting to remark that in very few cases, which were hardly to reproduce, the light-induced potential response was observed to be of opposite polarity, but with exactly the same kinetics (e.g. phases 1, 2 and 3) and characteristics. Such response would be predicted when the electrode is just in the interspace of the enclosing membrane, as discussed before.

Fig. 4 shows the potential response upon two consecutive short flashes (rise time 2 μsec, halfwidth 50 μsec), fired at a dark interval of 250 msec in the absence and presence of 5 μM DCMU. In the presence of the inhibitor the response in the second flash is about half of the response in the first flash. This result is consistent with our interpretation. The sites occupied by the primary acceptor Q of system 2 will be in the reduced state upon the firing of the second flash, because of the DCMU-retarded dark oxidation of $QH\cdot -$, reduced by the first flash [8]. The fact that after the first flash the potential decays in the dark towards the dark potential with the system 2 site remaining reduced, might be indicative for the neutralization of the ionic charge at this site ($-\cdot OH^-$) due to the field-driven ion fluxes across the thylakoid membrane as discussed by Witt [5,6]

Summarizing, the results justify the conclusion that the phase 1 potential change at the chloroplast membrane is quantitatively associated with an ion binding process at the thylakoid membrane surface and due to induced charge polarization at the enclosing membrane. On basis of our interpretation we have to conclude that this binding process occurs with a rate constant $k = 2.7 \cdot 10^3$ sec^{-1}. This conclusion is consistent with the observation [4], that the change in absorption of P 515, indicative for the charging of the thylakoid membrane [9,10], in these leaves proceeds with the same reaction kinetics as those of the phase 1 potential changes, i.e. is completed in about 0.5 to 1.5 msec at saturating flash intensities. However, the rate constant of the binding process is far much less than the one concluded from experiments with stripped chloroplasts [11]. The reason for this discrepancy is unknown as yet. The kinetic data do not contain evidence for the suggestion, proposed before [3,4], that the potential changes are due to changes in the Nernst potential of protons across the chloroplast enclosing membrane.

Acknowledgements

This research was partly supported by the Netherlands Foundation for Biophysics (Stichting voor Biofysica), financed by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.). Thanks are due to Prof. L. N. M. Duysens and Mr. E. Kooi from the Biophysical Laboratory at Leiden, who kindly put flash equipment at our disposal.

References

[1] Bulychev, A. A., Andrianov, V. K., Kurella, G. A. and Litvin, F. F. (1971) Soviet Plant Physiol. (Engl. Transl.) 18, 204.
[2] Bulychev, A. A., Andrianov, V. K., Kurella, G. A. and Litvin, F. F. (1972) Nature 236, 175.
[3] Vredenberg, W. J., Homann, P. H. and Tonk, W. J. M. (1973) Biochim. Biophys. Acta 314, 261.
[4] Vredenberg, W. J. Proceedings Workshop on Membrane Transport in Plants and Plant Organelles, 4–8 February, 1974 Juelich, FRD, Springer Verlag, Heidelberg, New York. in the press.
[5] Witt, H. T. (1971) Q. Rev. Biophys. 4, 365.
[6] Wolff, Ch., Buchwald, H. E., Rüppel, H., Witt, K. and Witt, H. T. (1969) Z. Naturforsch. 23b, 1038.
[7] Grünhagen, H. H. and Witt, H. T. (1970) Z. Naturforsch. 25b, 373.
[8] Duysens, L. N. M. and Sweers, H. E. (1963) in: Studies in Microalgae and Photosynthetic Bacteria, Spec. Issue Plant and Cell Physiol. Tokyo, p. 353.
[9] Junge, W. and Witt, H. T. (1968) Z. Naturforsch. 23b, 244.
[10] Jackson, J. B. and Crofts, A. R. (1969) FEBS Letters 4, 185.
[11] Witt, H. T. and Zickler, A. (1973) FEBS Letters 37, 307.
[12] Neumann, D. (1973) Protoplasma 77, 467.