INDUCTION OF PHOTOSYSTEM II ACTIVITY IN FLASHED LEAVES*

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1. Introduction

The formation of photosynthetically functioning, normal chloroplasts in higher plants by exposure of etiolated leaves to light is well documented: morphologically [9], physiologically [10], and biochemically [4, 15, 16]. A net oxygen production was generally reported after several (2–4) hr of continuous illumination [17]. In recent years, some groups have exposed etiolated leaves to intermittent light; a series of 1 msec flashes alternating with 15 min dark periods were used [6, 11, 10, 3, 2]. In this case, it was found that 300 to 700 flashes (i.e. a total illumination of 0.3 to 0.7 sec) induce the formation of chloroplasts containing "primary thylakoids". These chloroplasts exhibit PMS catalyzed photophosphorylation, but no non-cyclic electron transport [10]. Grana are not observed. Absence of some photosystem II properties was reported [3].

It was suggested [11] that short impulses of white light might induce the functioning of photosystem I only. In fact the development of PS II activity appears to require more than one brief illumination, even in the presence of excess chlorophyll in the leaf [7, 14].

In this paper we report activation of photosystem II in flashed bean leaves when illuminated for the first time with continuous light, by measuring simultaneously oxygen production and fluorescence emission.

2. Material and methods

2.1. *Plant material*

Primary bean leaves (*Phaseolus vulgaris* L. var. Commodore) were used. They were grown under conditions similar to those described by other authors [3, 10]. The plants were grown 7 days in darkness. After that they remained in darkness (etiolated control), or were submitted to a flash regime (1 msec saturating flashes alternating with 15 min dark periods) for 1 up to 7 days. The same electronic flash was used as described earlier [13]. When under the flash regime, they received from 100 to 700 flashes according to the series (x times flashed plants; fig. 1). Green controls were cultivated for 14 days under continuous light from a fluorescent tube (about 6 Klux). Leaf samples were examined under the electron microscope. The leaves looked as described earlier [12]; in particular, flashed leaves never showed grana, neither before, nor after the described experiments.

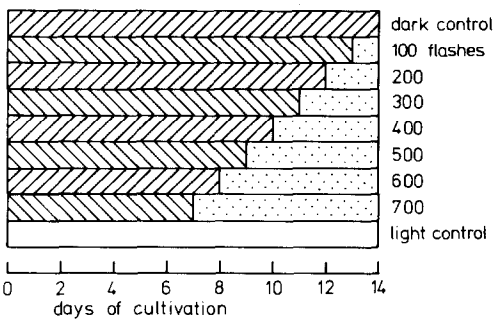

Fig. 1. Scheme of the different cultivations of the plant material during 14 days. ▪ Dark; ▪ intermittent light (1 flash of 1 msec every 15 min of darkness); □ continuous light.

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2.2. *Measurement of oxygen exchanges*

The measurement of the oxygen exchanges occurred as reported elsewhere [1], putting the leaves (discs of a diameter of 8 mm) directly in contact with an electrode. The electrode was a Yellow Spring Clark-type oxygen electrode. The leaf disc was covered with a plexigas ring forming a small assimilation chamber of about 0.3 ml. The side of the chamber opposite to the electrode surface was covered with a teflon membrane. The arrangement of the electrode and the assimilation chamber containing the leaf disc was placed in a well thermostated and air saturated water-bath. The oxygen and CO_2 concentrations were equilibrated by diffusion through the teflon membrane to the assimilation chamber. In order to make different experiments comparable, the reference was always the dark respiration activity measured by the decrease of the oxygen concentration at the electrode surface (taking into consideration the consumption of oxygen by the electrode itself).

2.3. *Light sources for the induction of oxygen-evolving-activity*

We used: a) white light from a white projection lamp "Prado Universal"; b) blue light obtained by passing the light from a Xenon lamp through a blue filter Schott BG 28 (large transmission band between 450–550 nm); c) red monochromatic light from a laser 632.8 nm. The intensity of the light at the level of the leaf was in all cases between 10^4 to 10^5 ergs·cm^{-2}·s^{-1}.

The illuminations were continuous, or chopped with a light-dark period of 4 msec:4 msec.

2.4. *Fluorescence measurements and chlorophyll determination*

A three armed light guide was placed in front of the leaf sample. One arm of the light guide conducted the actinic light to the sample. A second arm made it possible to register the fluorescence emission spectrum at room temp. passing the emitted light through a monochromator. The third arm was used to measure the variation of the room temp. fluorescence at 685 nm as a function of time.

After the experiment the sample was sometimes dipped in liquid nitrogen and the low temperature fluorescence spectrum excited at 436 nm was registered between 600 and 750 nm. Afterwards the sample was

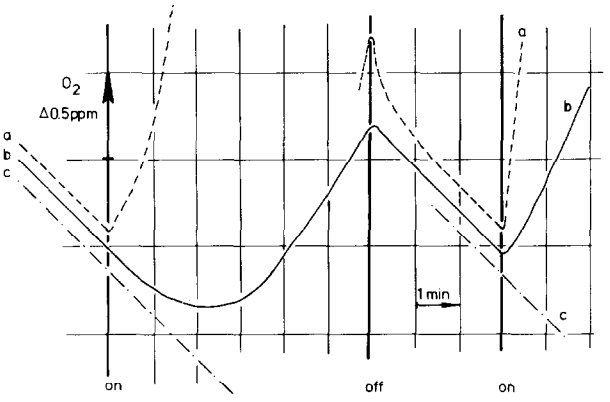

Fig. 2. Traces of the registrations of the oxygen concentration measured on the under side of a green leaf (a), a flashed leaf, which has received 700 flashes, that means 0.7 sec light (b) and an etiolated leaf (c). On: light on; off: light off.

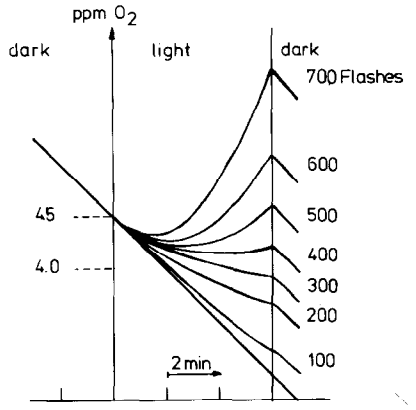

Fig. 3. The induction of oxygen production-activity in flashed leaves which have received 0 to 700 flashes during the cultivation period of 14 days.

warmed up and extracted in 80% acetone at room temp. The absorption of the extract was estimated at 652, 663, 645 nm [5].

3. Results

3.1. *Induction of PS II activity*

The dark respiration of the leaves was measured (green leaves, etiolated leaves, or x times [x = 100, 200, ..., 700 flashes] flashed leaves) for about 10 min in order to equilibrate the system. When the

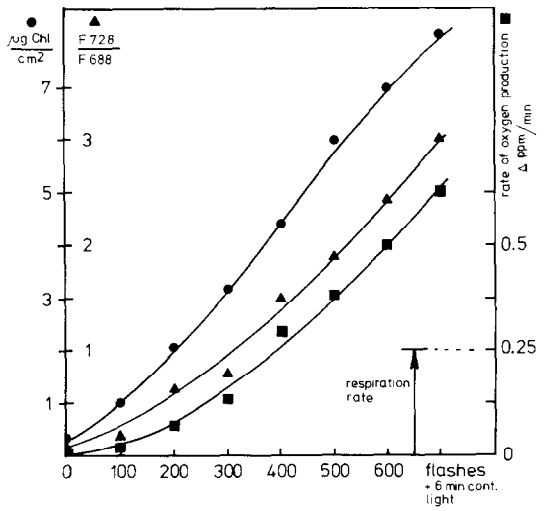

Fig. 4. Comparison of i) chlorophyll content (•), ii) low temperature fluorescence properties (quotient of the emission at −196° by 728 nm/688 nm) (▲) and iii) capacity of photosynthetic activity (measured as oxygen production rate after an illumination with white light 3×10^4 ergs/cm^2·sec for 6 min) in bean leaves grown under a flash regime, having received 0 to 700 flashes during their cultivation period of 14 days (■).

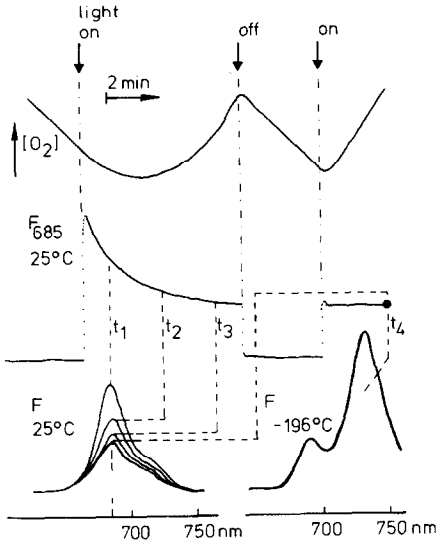

Fig. 5. The induction of PS II activity of a flashed leaf which has received 700 flashes during the cultivation period, measured as oxygen production and decrease in the fluorescence at 685 nm. Bottom right: low temperature fluorescence spectra of the sample at the end of the experiment. The experiment was made by a yellow background illumination of less than 100 ergs · cm^{-2} · sec^{-1}.

signal indicated a concentration of 4.5 ppm oxygen, the light was turned on. By light on (blue, red, or white light) a net oxygen production was seen in green leaves (fig. 2a), while etiolated leaves did not show any oxygen production (fig. 2c). Immediately by light on, the flashed leaves did not show any oxygen production; but an induction phase took place (fig. 2b) and a net oxygen production clearly appeared growing after some 30 to 60 sec.

A maximum rate of O_2 production was usually reached within some 6 min. The induction process took place only one time, i.e. at the first illumination of the flashed leaves by continuous or chopped light. Inserting a dark period for 3 min after induction did not apparently change anything: the rate of oxygen production was maximal after 15–20 sec when turning the light on again. Hence a complete active photosynthetic apparatus was formed during the induction period. The oxygen production remains constant even for longer illumination time than 6 to 8 min and it is in rough relation with the chlorophyll content and the number of flashes received during the cultivation period. For long illumination (hours) the photosynthetic activity increases together with the increase of the chlorophyll content in the leaf up to the photosynthetic activity of a normal green leaf.

3.2. *Relationship between inducibility of photosystem II activity and chlorophyll content*

Series of 14 day old leaves which had received from 100 to 700 flashes were used (fig. 1). The chlorophyll content of the leaves increased with the number of flashes received and the ratio of the intensities of the low temperature fluorescence peaks F_{728}/F_{688} also increased as shown in fig. 4. The increase in the expression F_{728}/F_{688} at low temperature is due mainly to the increase of the chlorophyll content in the leaf with an emission at 728 nm.

An induction of oxygen evolving activity took place in all series when continuous light was turned on (fig. 3). The rate of oxygen production had reached a maximum after some 6 min of light in all series. At the end of the induction the O_2 production was the highest for the highest chlorophyll content of the leaves (figs. 3 and 4).

3.3. *Fluorescence emission during the induction*

Traces of simultaneous registration of oxygen exchange and fluorescence emission at 685 nm during the induction phase are shown in fig. 5. After about 30 sec, a decrease in the fluorescence emitted at 685 nm was observed during the induction. The decrease stopped when the oxygen production reached a constant, maximal rate. We compared the low temperature emission spectra of two pieces of a same flashed leaf, one taken before, and the other after the induction period, but we could not find any obvious qualitative differences between the two pieces.

3.4. *Induction of PS II activity by different illumination conditions*

Qualitatively, the same induction process was observed by illuminating flashed leaves with white, blue or monochromatic red light. The light was given continuously, or as chopped light (with a frequency 150/sec) with the same result. An advantage of using chopped light was that there was no detectable difference between photorespiration and dark-respiration in this case.

4. Discussion and conclusions

The data confirm previous reports following which light given as short flashes (1 msec) every quarter of an hour [12] induces the formation of green chloroplasts, containing primary thylakoids, which do not exhibit any PS II activity. These chloroplasts acquire PS II activity when submitted to continuous light. The full PS II activity was induced within a few minutes, either under continuous or under chopped light (fig. 2). This could indicate that under the flash regime a "capacity" for both PS I and PS II activity is formed, but that, as far as it is concerned, PS II is present in some kind of inactive form. If this is true, the induction would be comparable to a process along which non-active PS II "learns" how to produce oxygen.

The oxygen production is more or less zero at the start of the continuous illumination (by light on); thereafter it climbs up until a constant rate is reached which represents the potential capacity for PS II activity already present in the leaf at the end of its cultivation under the flash regime. The amount of this potential capacity in the leaf is in rough relationship to the chlorophyll content (fig. 4). The exact relationship between the capacity, the number of flashes received and the amount of chlorophyll accumulated in the flash regime remains to be established. As expected, the increase of the rate of oxygen production during the induction process is concomitant to a decrease of the amount of fluorescence emitted at 685 nm (at room temp; fig. 5).

We are thus able to distinguish 3 roles for light: i) light is needed for synthesis and accumulation of chlorophyll as well as for membrane formation; ii) it is needed in order to transform inactive PS II into active PS II, iii) finally, it is needed to maintain the complete photosynthetic apparatus into function.

The induction of PS II activity described in this paper was also seen in flashed barley leaves, or flashed *Lemna Gibba* G 1 and *Lemna Perpursilla* 6746 plants. It may be considered as a general phenomenon.

Acknowledgements

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References

[1] L.R. Blinks and R.K. Skow, Proc. Natl. Acad. Sci. 24 (1938) 420–427.
[2] J.W. Bradbeer, H. Clijsters, A.O. Gyldenholm and H.J.W. Edge, J. Exp. Bot. 21 (1970) 525–533.
[3] E. Dujardin, Y. de Kouchkovsky and C. Sironval, Photosynthetica 4 (1970) 223–227.
[4] F.F. Litvin and O.B. Belyaeva, Photosynthetica 5 (1971) 200–209.
[5] G. Mackinney and D.I. Arnon, in: Plant Photosynthetic Production Manual of Methods, eds. Z. Sestak, J. Catsky and P.G. Jorvis (The Hague, 1971) p. 684.
[6] A. Madsen, Biochem. and Photobiol. (Chlor. Metabol. Symp.) 2 (1963) 93–100.
[7] K.D. Nadler, H.A. Herron and S. Granick, Plant Physiol. 47 suppl. (1971).
[8] K.D. Nadler, H.A. Herron and S. Granick, Plant Physiol. 49 (1972) 388–392.

[9] S. Phung Nhu Hung, A. Lacourly and C. Sarda, Z. Pflanzenphysiol. 62 (1970) 1–16.
[10] S. Phung Nhu Hung, A. Hoarau and A. Moyse, Z. Pflanzenphysiol. 62 (1970) 245–258.
[11] C. Sironval, R. Bronchart, J.M. Michel, M. Brouers and Y. Kuyper, Bull. Soc. Franç. Physiol. Végét. 14 (1968) 195–225.
[12] C. Sironval, J.M. Michel, R. Bronchart and E. Englert-Dujardin, in: Progress in Photosynthesis Research, ed. H. Metzner, On the Primary Thylakoids of Chloroplasts Grown under a Flash Regime, Tübingen 1969, 1, 47–54.
[13] C. Sironval, M. Brouers, J.M. Michel and Y. Kuiper, Photosynthetica 2 (1968) 268–287.
[14] R.J. Strasser, Verh. Schweiz. Naturforsch. 71 (1971) 104–106.
[15] S.W. Thorne, Biochim. Biophys. Acta 226 (1971) 113–127.
[16] S.W. Thorne, Biochim. Biophys. Acta 226 (1971) 128–134.
[17] S. Wieckowski, Photosynthetica 5 (1971) 44–49.