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CORRELATION BETWEEN THE INDUCTION OF OXYGEN EVOLUTION AND OF VARIABLE FLUORESCENCE IN FLASHED BEAN LEAVES

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SUMMARY

Beans grown under intermittent light (1 msec polychromatic intense light flash every 15 min during cultivation in darkness) do not show photosynthetic oxygen production and variable fluorescence emission immediately after the administration of continuous light for the first time. However, a weak light preillumination is enough to sensitize the leaf in such a way that an actinic illumination then provokes quite normal oxygen production behaviour, as well as variable fluorescence emission. Kinetic studies show that both oxygen production criteria (oxygen outburst and induction kinetics of oxygen production) and variable fluorescence emission criteria (position and intensity of the emission peaks) change in the same way as a function of the amount of light given during the preillumination period.

We conclude that the induction of oxygen evolution and the induction of variable fluorescence in flashed leaves are correlated phenomena regulated by a weak light intensity multiquantum mechanism.

INTRODUCTION

It has been reported that when grown under a flash regime (a 1-msec polychromatic flash every 15 min), etiolated been leaves form chloroplasts with primary thylakoids (flashed leaves) [1]. Chloroplasts of such leaves lack Hill activity with H_2O as electron donor [2,3], but show PMS-catalyzed photophosphorylation [2]. When the flashed leaves are continuously illuminated for the first time, the ability to evolve oxygen appears within a 6 to 8 min illumination (induction of the ability for oxygen production) [4,5]. This induction consists of a light-dependent mechanism distinct from the known light reactions of photosynthesis [6]. Flashed leaves exhibit no or very little variable fluorescence [7], but a 6-min period of continuous actinic [8,12] or even weak green light [9] induces the ability for exhibiting variable fluorescence.

The present paper reports facts indicating that in flashed bean leaves the induction of both the capacity for oxygen evolution and for exhibiting a variable fluorescence seem correlated and similarly light-dependent.

MATERIAL AND METHODS

Plants of *Phaseolus vulgaris* were grown for 7 days in darkness, and then for 7 days under a flash regime (1 msec polychromatic white flashes every 15 min in darkness). Primary flashed leaves were harvested from the 14-day-old plants.

The experimental conditions were the same as described previously [5,10], *e.g.* a disk taken from a primary flashed leaf (8 mm diameter) was placed directly in the dark on an oxygen-Clark-type electrode and covered with a small cuvette and a teflon membrane. A light pipe with two arms and one common end to the leaf was used: (*a*) for exciting with photosynthetic actinic light and (*b*) for conducting the emitted fluorescence from the leaf to a photomultiplier.

We showed previously that the induction of oxygen production by flashed leaves needs continuous or rapidly chopped light [5] and that this light may be of a very low intensity [6]. It was thus possible to induce the production capacity of oxygen even in the absence of any photosynthetically active light [9]. We divided each experiment into 2 periods: (*i*) a preillumination period of 6 min during which 6 low-intensity light pulses of variable durations were given (preillumination light). The effect of this preillumination was measured afterwards during (*ii*) a test period during which photosynthetically active light (test light) was given. During this period oxygen production and fluorescence emission were simultaneously recorded.

The test light was a broad-band blue light from a Xenon lamp filtered through a 10 cm saturated $CuSO_4$ solution at 25° (intensity, $I = 4 \cdot 10^4$ erg $cm^{-2} sec^{-1}$ on the leaf). The same light was used for preillumination but at a 20-fold smaller intensity $I_p = 2 \cdot 10^3$ erg$\cdot cm^{-2}$ sec^{-1}.

The preillumination light I_p was always given as 6 pulses during the 6 min preillumination period. The length of these pulses varied from 1 to 60 sec. One single pulse was given at the start of each minute during the preillumination period.

During the test period, the light was chopped in 4 msec dark : 4 msec light cycles. It was possible in this way to equalize the photorespiration to the dark respiration [10].

Oxygen gas exchange and fluorescence were measured simultaneously and continuously as described elsewhere [5,10,11]. The fluorescence was measured through an interference filter at 680 nm (Baird Atomic Inc. Cambridge B10) with a photomultiplier type S20 EMY 5558 B. The temperature was 25°.

(1) Oxygen measurements

When the test light of $I = 4 \cdot 10^4$ erg·cm^{-2} sec^{-1} was given continuously to flashed bean leaves, and induction of oxygen evolution was observed (curve 0, Fig. 1). When the leaves first received a small amount of weak light with an intensity $I_p = 1/20\ I$, given at intervals during a preillumination period of 6 min, and were thereafter transferred in continuous test light, I, an oxygen outburst ("jet") was observed while the rate of oxygen evolution rose to a steady-state level V_{SS} (curves 1 to 60 in Fig. 1). The diffusion of oxygen through the leaf and through the teflon membrane of the electrode explains the fact that the outburst was recorded with a 10- to 20-sec delay after the transfer to the test light. Thus two kinds of events were distinguished when flashed leaves were first preilluminated with $I_p = 1/20\ I$ and thereafter transferred to I: (*i*) a certain amount J of oxygen was released during an outburst; (*ii*) the rate of oxygen production rose up to a constant steady-state level V_{SS}.

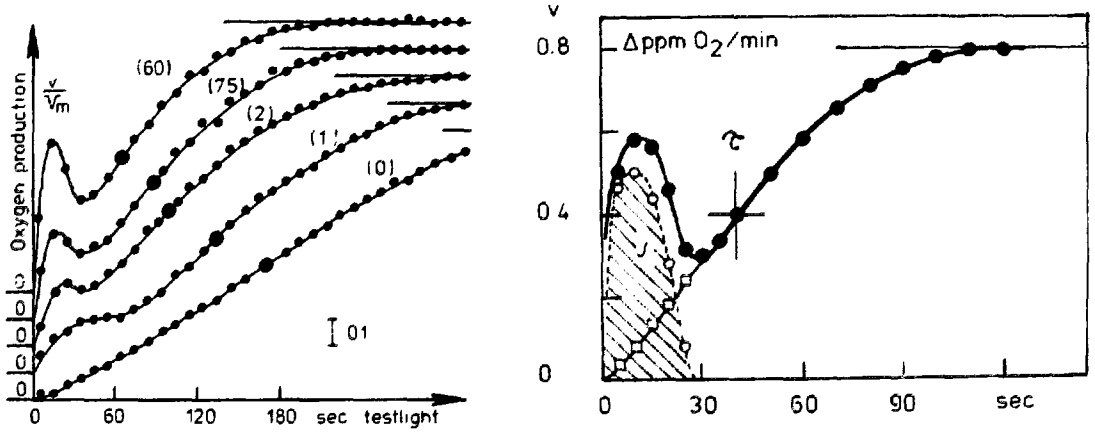

Fig.1. Relative oxygen production rate during the first test light illumination of flashed bean leaves. The number by each curve indicates the amount of preilluminating light (in sec per min) given at the beginning of each min during the preillumination period of 6 min.

Fig.2. Distinction of the oxygen outburst J from the induction kinetics (characterized here by τ) during the test light illumination. •, registered oxygen production rate; ○, estimated oxygen outburst; □, approximation of the induction kinetics below 30 sec of test light illumination.

We thus distinguished empirically (Fig. 2): (*1*) A time τ necessary for reaching half the steady-state rate of oxygen production V_{SS}. This time characterizes the induction under the test light I. (2) A value J for the amount of oxygen released during the outburst. J was approximated as the difference between the integrals of the experimental curve and of the induction curve:

$$J = \int_{t_0}^{t_1} v^{exp} \cdot dt - \int_{t_0}^{t_1} v^{ind} \cdot dt \qquad (1)$$

in which v^{exp} is the actual rate of oxygen production and v^{ind} the calculated rate of oxygen production when isolating the induction from the outburst. The oxygen outburst J, determines the shape of the experimental curve in the first 30 sec under the test light I. Assuming that, during that time, the change of the rate due to induction was more or less zero order (see curve 0, Fig. 1), we have:

$$v^{ind}_{(\tau)} \simeq \frac{V_{SS}}{2\tau} \cdot t \qquad (2)$$

Introducing (2) in (1) with $t_0 = 0$ and $t_1 = 30$ sec

$$J \simeq \int_0^{30\ sec} v^{exp} \cdot dt - 225\ (sec^2) \cdot \frac{V_{SS}}{\tau} \qquad (3)$$

In fig. 4, J and τ are plotted versus the cumulated duration of the pre-illumination with $I_p = 1/20\ I$.

(2) Fluorescence emission measurements

The intensity of the fluorescence emission at 680 nm was recorded simultaneously with oxygen production (Fig. 3). The fluorescence intensity did not vary or varied smoothly when the test light $I = 4 \cdot 10^4$ erg·cm^{-2} sec^{-1} was given for the first time to flashed bean leaves. A preillumination of these leaves was able to induce a state which exhibited clear variations of the fluorescence emitted at the onset of the test light.

The facts may be summarized as follows: (*i*) In the absence of any pre-illumination, the fluorescence of flashed leaves exposed to the test light I exhibited first a very sharp emission peak F_1 (whose height varied from one sample to another), diminished thereafter to a constant level for some 30 sec and reached a second intensity maximum F_3 after 55 sec. (*ii*) The maximum F_3 was observed earlier and it appeared more evident when a preillumination period was given to flashed leaves. (*iii*) While after the first sharp emission peak F_1 at the onset of the test light, flashed leaves did not show any variable fluorescence during the following 30 sec of illumination, they clearly showed an emission outburst, F_2, 5 to 10 sec after the onset of the test light when preilluminated. The intensity J^* of F_2 increased by increasing the amount of preillumination light. (*iv*) After peak F_3, the intensity of the fluorescence oscillated for at least 3 min before finally reaching the steady-state level F_{SS}. Oscillations were not observed in all the experimental series.

We defined empirically a time τ^* at which the decreasing fluorescence emission was half way between the intensity of the emission at F_3 and the minimal emission intensity before reaching F_{SS} in the test light I, in such a way that:

$$F_{(\tau^*)} = \tfrac{1}{2}\,(F_3^{max} + F^{min}) \qquad (5)$$

On the other hand, we wrote the intensity at the maximum emission F_2 relatively to F_{SS} as:

$$J^* = \frac{F_2 - F_{SS}}{F_{SS}}$$

τ^* and J^* are plotted in Fig. 4 (bottom) *vs.* the cumulated duration of the preillumination with $I_p = I/20$.

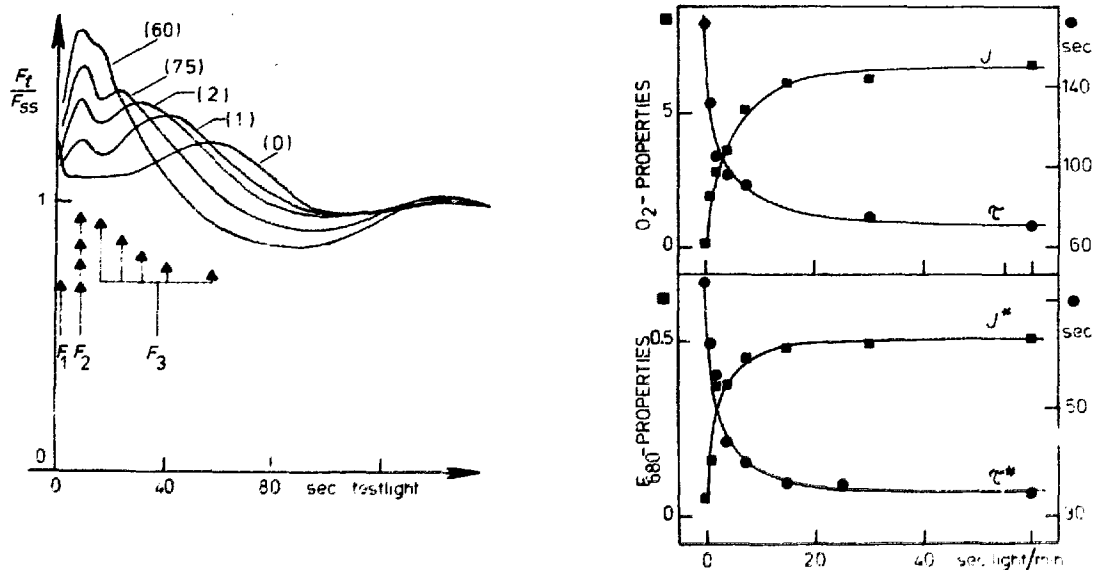

Fig.3. Relative fluorescence emission at 680 nm during the first test light illumination of flashed bean leaves. The number by each curve indicates the amount of preilluminating light (in sec per min) given at the beginning of each min during the preillumination period of 6 min. For the definitions of F_1, F_2, F_3, see text.

Fig.4. Comparison of J and τ with J^* and τ^* in flashed bean leaves. The test light was given after a preillumination period of 6 min during which the leaf received 0 to 60 sec I_p light per min (abscissa).

DISCUSSION AND CONCLUSION

In earlier papers we showed that when given continuously, even a weak light induced an ability for oxygen production in flashed leaves as well as an ability for exhibiting variable fluorescence [9]. In the experiments reported here, the amount of weak light $I_p = 1/20\ I$ was varied during a constant 6-min preillumination of the flashed leaves. The effect of these preilluminations was estimated by registering the fluorescence emission and oxygen production activities of leaf pieces during an identical test period for all kinds of pre-

illuminations. In this way we were able to follow the development in the leaf of the ability for both oxygen evolution and variable fluorescence.

Two kinds of data were distinguished in both cases under the test light I: (*i*) An amount J of outburst oxygen released within 30 sec from the start of the test light and an induction time τ needed for reaching under the test light an oxygen evolution rate equal to half the steady-state rate. (*ii*) A fluorescence outburst J^* and a time τ^* representing the time necessary for getting a fluorescence intensity half way between F_3 and the minimal fluorescence intensity from F_3 to F_{SS}.

J and τ were seen behaving in the same way as J^* and τ^* when plotted against the cumulated duration of preillumination within the 6-min preillumination period (Fig. 4). Indeed by increasing the amount of preillumination light, the amount of J and J^* both increased while the values for τ and τ^* shifted similarly to shorter values. It was, moreover, seen in separate experiments that a fast repetitive flash treatment given at the end of the preillumination period (before the test light) supressed both the outbursts J and J^* and shifted both τ and τ^* to shorter times [9]. This also shows a correlation.

We emphasize two points: (*i*) All measurements were done with fresh pieces of leaves (just harvested). When the experiments were performed some hours or some days after gathering the leaves, very slow and weak induction phenomena were observed [12]. (*ii*) Peaks F_3 of the variable fluorescence were separated only when a very small leaf surface was illuminated (in our case circles of 4 mm diameter in the center of the 8 mm diameter leaf piece [9]. The use of new types of light pipes with several arms and one common end made it possible (construction by Schott and Gen, Mainz, Germany) [11].

We conclude that: (*i*) The inductions of oxygen production and variable fluorescence appear to be correlated phenomena. (*ii*) The ability for oxygen production and the ability for the emission of a variable fluorescence are probably induced in flashed bean leaves by an identical light-dependent mechanism. (*iii*) This induction mechanism may be sensitized by light pulses of weak intensity.

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