# EVIDENCE FOR INDUCTION OF PHOTOSYSTEM II ACTIVITIES IN PRIMARY THYLAKOIDS WHEN ILLUMINATED WITH CONTINUOUS LIGHT FOR A SHORT TIME

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## 1. Introduction

"Flashed etiolated bean leaves" are defined in this paper as etiolated leaves having received a certain number (from 175 to 1000) of polychromatic flashes of one msec duration every 15 min. It has been previously shown that the chloroplasts of such leaves contain "primary thylakoids" and are devoid of grana [1]. They apparently lack photosystem II as is suggested by the following: i) flashed leaves accumulate high amounts of chlorophyll a [2]; ii) they do not exhibit light induced 475 nm and 515 nm absorbance changes [3]; iii) they do not emit oxygen in the light [4]. However, measurements of the kinetics of the light induced absorbance change around 700 nm clearly showed a distinct absorbance decrease on irradiation of isolated primary thylakoids [Grunehaguen and Dujardin, unpublished results]. On the other hand, it was found that some characteristic features related to photosystem II activity appeared in flashed leaves when they received continuous white light for 48 hr [3].

In this paper we compare the shape of the initial rise of the fluorescence emitted by flashed leaves at 685 nm at room temp. when excited with 436 nm light (called below "measuring light") to the shape of the rise observed when flashed leaves had been illuminated with a continuous illumination (called below "inducing light") before fluorescence excitation with the 436 nm light. It was found that submitting flashed leaves to a continuous pre-illumination for a time duration as short as 30 sec changes the shape of the initial rise of the fluorescence emission.

## 2. Material and methods

### 2.1. *Plant material*

Seedlings of *Phaseolus vulgaris*, variety Commodore, were grown in complete darkness. Sixteen days after sowing, the primary leaves were cut and submitted to a certain number of flashes of polychromatic, white light (from 175 to 1000 flashes = flashed leaves) as described previously [5].

### 2.2. *Experimental schedules*

All experiments were done at room temp. ($\simeq$ + 23°). Two types of experiments were performed:

*Experimental procedure* 1) Flashed leaves were placed for a given time ($x$ min) in a Petri dish on a layer of moistened cotton at a distance of 15 cm from a fluorescent "inducing" source ("inducing, white light"). The leaves were kept thereafter in darkness for 1 to 60 min; after that time, they were placed in the fluorometer for recording the time-course of the fluorescence emission. Control flashed leaves remained (for $x$ min + 1 to 60 min) in darkness before the fluorescence emission was recorded.

*Experimental procedure* 2) One flashed leaf was placed in a sample holder in the fluorometer; a moistened cotton plug was placed under the leaf; the time-course of the fluorescence emission at 685 nm was first recorded ("flashed leaf emission"); the leaf was thereafter irradiated in the same physical position inside the fluorometer by means of an inducing blue, red or white light; when the pre-selected duration of the inducing illumination had elapsed ($x$ min), the

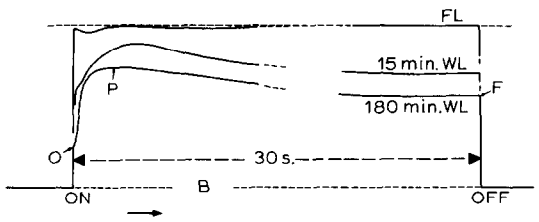


Fig. 1. Time course of the fluorescence emission at 685 nm measured at room temperature of a flashed leaf (control = FL) and of two flashed leaves pre-illuminated with continuous white light: 15 min WL = the flashed leaf was pre-illuminated for 15 min; 180 min WL = the flashed leaf was pre-illuminated for 180 min. All flashed leaves received 175 flashes. The two pre-illuminated leaves were kept in darkness for 60 min between the end of the continuous, white illumination and the record of the fluorescence emission. The control remained 240 min in darkness after flash no. 175.

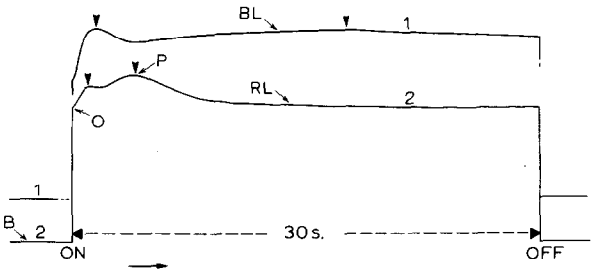


Fig. 2. Time course of the fluorescence emission at 685 nm measured at room temperature of a flashed leaf (500 flashes) pre-illuminated for 30 sec with intense blue light (BL) and of a flashed leaf (900 flashes) pre-illuminated for 60 sec with intense red light (RL). The leaves were kept in darkness for 60 sec between the end of the pre-illumination and the registration of the time course of the fluorescence emission.

leaves remained in darkness for times ranging from 1 to 60 min, and the time-course of the fluorescence was recorded for a second time. Control flashed leaves were handled as under (1) here above.

The inducing (intense) blue light was provided by a high pressure mercury discharge lamp fitted with a filter which isolated the 436 nm line; the intensity was $3.6 \times 10^4$ ergs $\cdot$ cm$^{-2}$ $\cdot$ sec$^{-1}$ at the level of the leaves. The inducing (intense) red light was obtained from a tungsten lamp equipped with a Schott RG 630 filter; the intensity was $1.71 \times 10^5$ ergs $\cdot$ cm$^{-2}$ $\cdot$ sec$^{-1}$ at the level of the leaves. The inducing white light came from a fluorescent source: a 40 W Philips "cool white" giving about $2.2 \times 10^4$ ergs $\cdot$ cm$^{-2}$ $\cdot$ sec$^{-1}$ at the level of the leaves.

### 2.3. *Measurement of the fluorescence time-course*

The apparatus for the measurement of the kinetics of the fluorescence emission was a modified version of the Zeiss spectrofluorimeter equipped with a EMI 9558 B photomultiplier. The voltage across the load resistor of the photomultiplier was fed directly into a potentiometric recorder with short response time (300 msec travel time for a chart width of 250 nm). The intensity of the 436 nm light used for the excitation of the fluorescence ("measuring light") was $3.6 \times 10^3$ ergs $\cdot$ sec$^{-1}$ $\cdot$ cm$^{-2}$.

## 3. Results

### 3.1. *Occurrence of a variable yield fluorescence in flashed leaves pre-illuminated with continuous light*

The time-course of the fluorescence emitted by flashed control leaves always appeared as follows: on turning the exciting light on, the emission rose instantaneously to a maximum level which remained constant thereafter. Sometimes a little decrease of the emission below the initial maximum was observed within the seconds following the onset of the light; or a feeble transient was seen (fig. 1). But a variable fluorescence similar to that found in normal green leaves was never recorded. The kinetics of the fluorescence emitted by flashed leaves which had been pre-illuminated continuously by an inducing white light for 48 hr showed clearly a variable part, in agreement with previous results [3].

The duration of the inducing pre-illumination was reduced in subsequent experiments. Typical kinetics of the fluorescence emitted by flashed leaves when pre-illuminated by a continuous inducing light for 15 and 180 min are reproduced in fig. 1. It was found that continuous blue or red light, as well as white light, were able to induce a variable part in the kinetics of the fluorescence. It was also found that the variable part appeared in flashed leaves even after an inducing pre-illumination period as short as 30 sec, provided the intensity of the inducing light was high enough.

Table 1
The ratio [(P–O)/(O–B)] as a function of the duration of an inducing, continuous pre-illumination (average of three measurements).

| Duration of the inducing pre-illumination (min) | [(P–O)/(O–B)] |
|---|---|
| 0.5* | 0.35 |
| 5.0+ | 0.51 |
| 30.0+ | 0.58 |
| 60.0+ | 0.61 |
| 240.0+ | 0.75 |

* The induction of the variable fluorescence was with intense blue light; the experiment was performed as in fig. 2.
+ Induction with white light; 5 to 240 min of inducing light + 60 min darkness. The leaves received 175 flashes before the inducing, continuous illumination; the ratio [(P–O)/(O–B)] was calculated from the registration of the variable fluorescence observed when irradiating with measuring blue light at the end of the 60 min dark period.

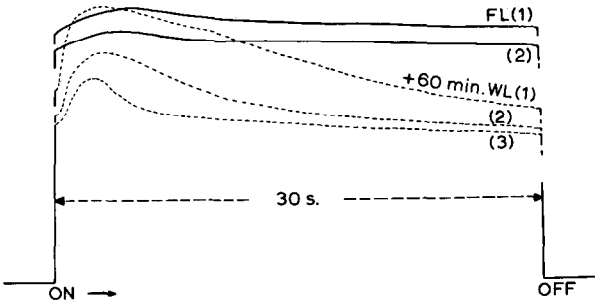


Fig. 3. Time course of the fluorescence emission at 685 nm of a flashed leaf (1000 flashes) before (solid lines FL (1) and (2)) and after a continuous illumination with white light for 60 min (dashed lines WL (1), (2) and (3)). The time schedule was as follows: the fluorescence of the leaf was measured immediately after flash no 1000 (FL 1); the leaf was then kept in darkness during 60 sec and the fluorescence measured again (FL 2). After that second measurement the leaf received inducing white light for 60 min followed by a 60 min dark period. Thereafter the fluorescence emission was registered again three times (+ 60 min WL 1, 2 and 3). The duration of each registration was 30 sec, the leaf was kept in darkness for 60 sec between two consecutive registrations in one series.

For instance, 30 sec of intense blue light (436 nm; $3 \times 6 \times 10^4$ ergs $\cdot$ cm$^{-2}$ $\cdot$ sec$^{-1}$ at the leaf surface), or red light ($\lambda > 630$ nm; $1.71 \times 10^5$ ergs $\cdot$ cm$^{-2}$ $\cdot$ sec$^{-1}$ at the leaf surface) were both effective for inducing clear variations of the fluorescence (fig. 2). We were unable to observe an effect of the duration of the dark period following the continuous inducing illumination on the time course of the variable fluorescence (the dark period ranging from 1 to 60 min).

### 3.2. *Changes in the shape of the variable fluorescence when the duration of the inducing pre-illumination was increased*

After a thirty second pre-illumination with intense blue or red light, the variable part of the fluorescence from flashed leaves has shapes similar to those seen in fig. 2. Levels O and P were clearly distinguishable.

Increasing the duration of the pre-illumination up to some minutes increased the difference between the O and P levels. For instance, when pre-illuminating with inducing white light for times ranging from 5 to 60 min, the ratio of the magnitude of the variation from level O to level P, (P–O) to the magnitude of the initial variation from the base line B to level O, (O–B), was between 0.5 and 0.6, (P–O)/(O–B) = 0.5 to 0.6. An estimation of how the ratio changed when lengthening the duration of the inducing pre-illumination from some min to 60 min was difficult; the magnitude of the change was within the experimental errors due to the variability of the leaf response (from leaf to leaf). However an increase of the (P–O)/(O–B) ratio was observed when pre-illuminating for more than 2 to 4 hr with inductive, white light (table 1). After a 24 hr white pre-illumination the ratio was higher than 2 as normally found in green leaves.

### 3.3. *Origin of the variable part of the fluorescence*

The intensity of the constant fluorescence from a flashed leaf was compared with that of the fluorescence of the same leaf after having been submitted to an inducing pre-illumination for a given time (experimental schedule no. 2 in Methods). A typical result is seen in fig. 3. Before the inducing pre-illumination, the fluorescence of the flashed leaf showed a feeble variable part. After the pre-illumination of the leaf (white, continuous light for 60 min) the fluorescence showed clear variations. These variations resulted obviously from a quenching of the fluorescence initially emitted by the flashed leaf.

## 4. Conclusions

The reported experiments show that the time-course of the fluorescence emitted by flashed leaves does not exhibit any variable part. This is in agreement with previous results and with all the data supporting the conclusion that primary thylakoids of flashed leaves are devoid of photosystem II activity [1, 3, 4]. It is established on the other hand that irradiation of flashed leaves with continuous light induces the appearance of a variable fluorescence.

But since – as shown here – a continuous pre-illumination as short as 30 sec was sufficient to induce some variable part of the fluorescence kinetics, it seems difficult to explain the induction by admitting something like the building of new membranes. On the other hand, since the variable part of the fluorescence clearly came from a quenching of the initial fluorescence of flashed leaves without modifying the maximum level, it is suggested that the changes resulting from the induction are not connected with a change in the amount of chlorophyll in the leaves. It is therefore probable that the induction consists of the activation of some mechanism whose essential constituents already pre-exist in an inactive form in the primary thylakoids of flashed leaves. It should be noted however that the appearance of the variable part of the fluorescence depended on the intensity of the light. The 436 nm measuring light was apparently unable to promote the variable fluorescence by itself within 30 sec (fig. 3; compare FL 1 with FL 2) while the 436 nm inducing light with an intensity ten times higher promoted it within this time (fig. 2; 30 sec BL).

The occurrence of a variable fluorescence is generally accepted as a good criterion for the functioning of the photosystem II. If we accept this we are brought to the suggestion that the continuous illumination of flashed leaves triggers into working condition that part of the photosynthetic apparatus related to photosystem II activity.

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