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INDUCTION KINETICS OF DELAYED LIGHT EMISSION IN SPINACH CHLOROPLASTS

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SUMMARY

Induction curves of the delayed light emission in spinach chloroplasts were studied by measuring the decay kinetics after each flash of light. This study differs from previous measurements of the induction curves where only the intensities at one set time after each flash of light were recorded. From the decay kinetics after each flash of light, the induction curves of the delayed light emission measured 2 ms after a flash of light were separated into two components: one component due to the last flash only and one component due to all previous flashes before the last one. On comparing the delayed light induction curves of the two components with the fluorescence induction curves in chloroplasts treated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea and in chloroplasts treated with hydroxylamine and 3-(3,4-dichlorophenyl)-1,1-dimethylurea, the component due to the last flash only is found to be dependent on the concentration of open reaction centers and the component due to all previous flashes except the last is dependent on the concentration of closed reaction centers. This implies that the yield of the fast decaying component of the delayed light emission is dependent on the concentration of open reaction centers and the yield of the slow decaying component is dependent on the concentration of closed reaction centers.

INTRODUCTION

The causes of delayed light emission in green plants are closely associated with the energy trapping and storage processes in the Photosystem II reaction center. Mutants of green algae that lack Photosystem II reaction centers have negligible delayed light emission [1]. Since the state of the reaction center changes with time following illumination after a long dark period, a study of the related change in the delayed light emission should give information of the relationship of the delayed light emission to the state of the reaction center. For this reason, induction curves of the delayed light emission have been extensively studied [2–17]. Induction curves are generally obtained by illuminating the plant cells after a long dark period with a

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

repetitive train of light flashes until the delayed light emission has reached a steady state level. Most induction curves are measured with a phosphorescope [3–5, 8–10, 12–17]. The measured intensity of the delayed light in such an induction curve is the integrated intensity under a decay curve between two closely spaced time points which are in between two irradiation pulses. The fact that only the integrated intensity is recorded leads to complications. The first complication of the phosphorescope method is a result of the high repetition rate of the exciting light pulses. The delayed light emission due to a pulse of light may not have completely decayed before being excited by another pulse. The resultant delayed light emission in the induction curve is then not just the intensity due to the last light flash but is a complicated sum of the delayed light emission due to all previous flashes. The second complication of the phosphorescope method is that the delayed light emission is not measured immediately but at some times after a light flash. Thus the change in the intensity as measured by a phosphorescope then may be due both to a change in the initial yield of the delayed light emission and to a change in the decay kinetics. Interpreting an induction curve obtained from a phosphorescope as due only to a change in the total yield of the delayed light emission may lead to erroneous conclusions. The induction curve measured by a phosphorescope is a highly complex signal and should be interpreted with great care.

The problem of the contribution of all previous flashes except the last was eliminated by Ruby [11]. He used a measuring light flash that is spaced far enough from an activating light flash so that the delayed light intensity due to the activating flash was negligible compare with the intensity due to the measuring flash. He studied in detail the induction kinetics of the delayed light measured 1 msec after a measuring flash in *Chlorella*. In this report, we solve the problem of the contribution of all previous flashes except the last by actually measuring the decay kinetics of the delayed light emission after each flash of light and finding out the contribution due to previous flashes. The delayed light emission induction curves were compared with the fluorescence induction curves to obtain information concerning the relationship between delayed light emission to the state of the reaction center.

MATERIALS AND METHODS

Chloroplasts were isolated from spinach by the method outlined by Miles and Jagendorf [18]. After isolation the chloroplasts were resuspended in 0.16 M sucrose and 0.004 M Tris buffer (pH 7.8). The chloroplasts samples used have an absorbance of 0.5 at 680 nm. All measurements were done at room temperature.

A block diagram of the instrument used for delayed light emission measurements is shown in Fig. 1. The excitation source was a Flash-Tac stroboscope with two Corning filters CS 4-96 and CS 3-73. The light pulse duration was approx. 40 μs. The repetition rate was determined by a mechanical chopper (PAR Model 125) operating at 40 rev./s. The delayed light emission was detected by an EMI 9558 QB photomultiplier with a Corning CS2-64 filter placed in front of it. The photomultiplier signal was recorded on a Tektronic 564 B storage oscilloscope.

The instrument is designed with a triggering system such that the delayed light decay kinetics could be measured after a certain number of flashes. A beam of light from a He/Ne laser (Spectra Physics Model 133) passes through a slit in the

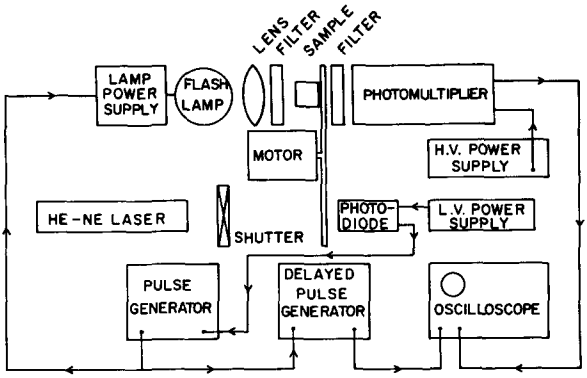

Fig. 1. Schematic diagram of the measuring apparatus.

mechanical chopper to activate an electrical signal in a photodiode. This signal triggers a pulse generator (General Radio Co. Type 1318A) which in turn triggers the flash lamp and a delayed pulse generator (Hewlett Packard Model 8005A). The pulse generator is adjusted such that the flash occurs 1.5 ms before the photomultiplier is unblocked by the mechanical chopper. The delayed pulse generator is adjusted so that it will allow a certain number of pulses to go through before triggering the oscilloscope. The number of light pulses that can go through is controlled by a mechanical shutter placed in front of the He/Ne laser.

To illustrate how the triggering system works, the method used in measuring the delayed light emission decay kinetics due to the 21st pulse of light will be outlined. After a dark period of 5 min, the shutter was opened for 0.5 s to trigger 20 pulses of light. The delayed pulse generator was adjusted such that it will trigger the oscilloscope after 19 pulses, so that the decay kinetics of the delayed light emission from the 20th pulse of light was recorded. After another dark period, the shutter was opened to trigger more than 20 pulses of light. Without adjusting the delayed pulse generator, the delayed light emissions from the 20th and 21st pulses of light were recorded. The difference between the two recordings is the delayed light emission kinetics due only to the 21st pulse of light.

The fluorescence induction curves were measured with the same instrument. The only change made is the output of the delayed pulse generator which is now connected to the lamp power supply instead of the oscilloscope. The delayed pulse generator is adjusted so that the lamp is triggered when the photomultiplier is unblocked by the mechanical chopper.

RESULTS AND DISCUSSION

Fig. 2 shows the relative intensities and the decay kinetics of the delayed light emission of spinach chloroplasts following various numbers of light flashes spaced 25 ms apart. Fig. 3 shows the results with spinach chloroplasts with 10^{-5} M 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) added. On examining Figs 2 and 3, we see that the delayed light has not decayed to zero intensity before being excited by another flash of light. The measured delayed light intensity then is composed of two

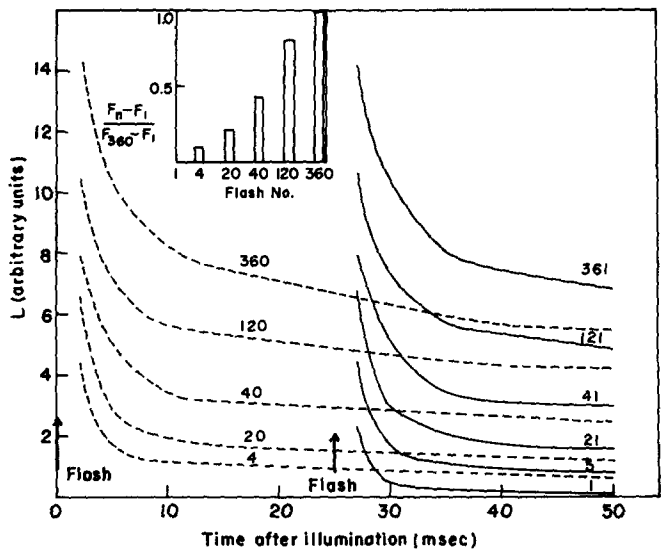

Fig. 2. The intensity of the delayed light emission (L) as a function of time following illumination by various numbers of light flashes spaced 25 ms apart for spinach chloroplasts. The numbers of light flashes are indicated in the figure. Insert: the difference between the fluorescence intensity of the nth flash and the 1st flash normalized to the intensity of the 360th flash minus the 1st flash as a function of the flash number.

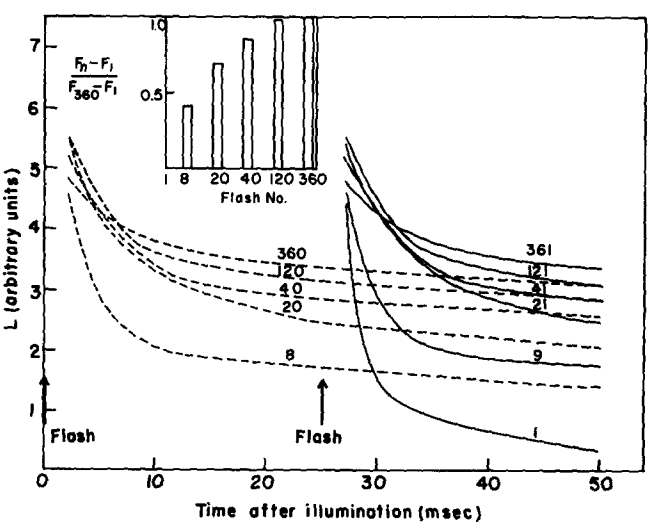

Fig. 3. L as a function of time following illumination by various numbers of light flashes spaced 25 ms apart for chloroplasts with 10^{-5} M DCMU added. The numbers of light flashes are indicated in the figure. Insert: the difference between the fluorescence intensity of the nth flash and the 1st flash normalized to the intensity of the 360th flash minus the 1st flash as a function of the flash number.

components: one due to the delayed light produced by the last flash of light and one due to that produced by previous flashes except the last. To find the contribution due to the nth flash only, we subtract the intensity of the delayed light emission after $n-1$ flashes from the intensity after n flashes. Using the results shown in Fig. 2, the induction curve for chloroplasts as measured 2 ms after the light flash can be drawn and is shown in Fig. 4. The component of the induction curve that is due to the nth flash only is shown by the dotted curve. The dashed curve shows the component that is due to all $n-1$ flashes. Similarly, Fig. 5 shows the induction curve for chloroplasts with DCMU added as measured 2 ms after the light flash, separated into two similar components. As shown in Figs 4 and 5 the induction curve of the two components are quite different from the measured induction curve. A change in either component can lead to a change in the measured induction curve. Hence an induction curve measured at one set time after a light flash and at one repetition rate [2–10, 12–17] as with a phosphorescope does not indicate which component is changing.

The component of the induction curve that is due to all $n-1$ flashes is due to the slow decaying component of the delayed light. It has been shown that this slow decaying component may be due to the recombination of the primary oxidized photoproduct Z^+ and the primary reduced photoproduct Q^- [19, 20]. Hence as the concentration of Q^- and Z^+ increases, the intensity of the delayed light would also increase. The induction curve for the production of Q^- in chloroplasts is approximately represented by the fluorescence induction curve [21]. One would expect then that the fluorescence induction curve would be similar to the induction curve of the delayed light due to the recombination of Q^- and Z^+. As shown in Figs 4 and 5, for both normal chloroplasts and chloroplasts with DCMU added, the fluorescence

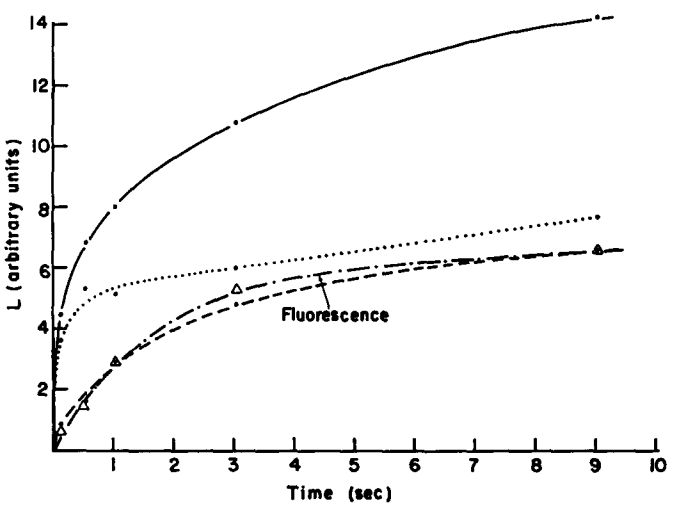

Fig. 4. Induction curve for chloroplasts. Top solid line is for the delayed light emission measured 2 ms after a light flash. Dashed line is for the component of the delayed light emission that is due to all previous flashes except the last. Dotted line is for the component of the delayed light emission that is due to the last flash only. Triangles denote the fluorescence induction curve. The fluorescence intensity of the 360th flash is normalized to the intensity of the delayed light emission of the dashed curve at the 360th flash. All data used are from Fig. 2.

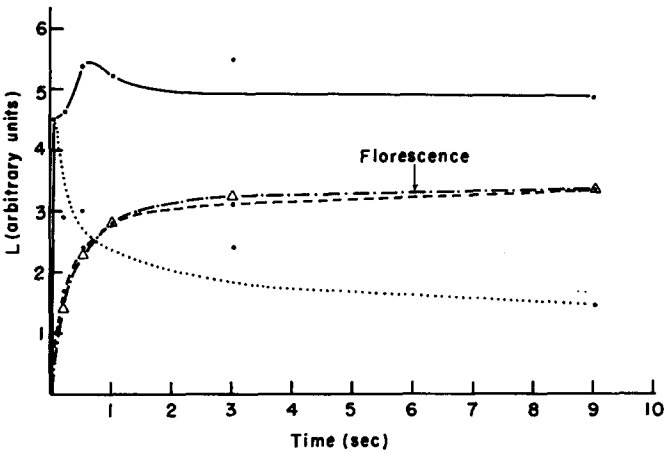

Fig. 5. Induction curve for chloroplasts with 10^{-5} M DCMU added. Top solid line is for the delayed light emission measured 2 ms after a light flash. Dashed line is for the component of the delayed light emission that is due to all previous flashes except the last. Dotted line is for the component of the delayed light emission that is due to the last flash only. Triangles denote the fluorescence induction curve. The fluorescence intensity of the 360th flash is normalized to the intensity of the delayed light emission of the dashed curve at the 360th flash. All data used are from Fig. 3.

induction curve has about the same rise time as the induction curve of the delayed light that is due to all $n-1$ flashes. The increase with time of the slow decaying component of the delayed light emission may then be due to the increasing concentration of Q^- and Z^+ with increasing time of illumination.

The component of the induction curve that is due to the nth flash only is the induction curve of the fast decaying component of the delayed light. On examining the data of chloroplasts with DCMU added in Fig. 5, one sees that this component decreases with increasing time of illumination. This has also been observed in *Chlorella* by Ruby [11]. To show more clearly how the induction curve due to the fast component of the delayed light decreases with time, we add hydroxylamine to chloroplasts treated with DCMU. The addition of hydroxylamine to DCMU-treated chloroplasts has been shown to eliminate the slow component of the delayed light [19, 20] so that the measured induction curve would be the induction curve for the fast decaying component only. Fig. 6 shows the decay kinetics of the delayed light emission following different numbers of light flashes of chloroplasts with 10^{-5} M DCMU and 10^{-3} M hydroxylamine added. Note that the decay kinetics change only slightly following the 1st flash to the 360th flash. Hence the decrease with time in the induction curve of the delayed light measured 2 ms after a light flash (Fig. 7) is due more to a decrease in yield than a change in the decay kinetics. Furthermore, Zankel [22] has shown that the delayed light emission measured 40 μs after a light pulse for the first flash is larger than for all subsequent flashes. On comparing the fluorescence induction curve, obtained by subtracting the mesasured fluorescence yield of each flash from the steady-state fluorescence yield, to the induction curve of the delayed light emission, one can see that they are very similar (Fig. 7). The calculated fluorescence induction curve represents approximately the induction curve for the concentration of open reaction centers. Hence the yield of the fast decaying component of the delayed light

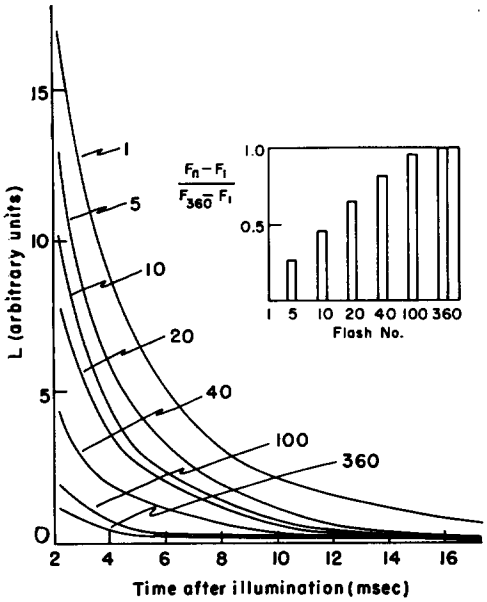

Fig. 6. L as a function of time following illumination by various numbers of light flashes spaced 25 ms apart for chloroplasts with 10^{-5} M DCMU and 10^{-3} M hydroxylamine added. The numbers of light flashes are indicated in the figure. Insert: the difference between the fluorescence intensity of the *n*th flash and the 1st flash normalized to the intensity of the 360th flash minus the 1st flash as a function of the flash number.

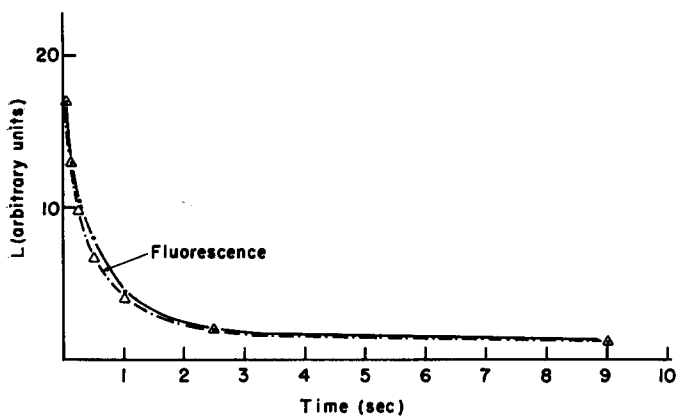

Fig. 7. Induction curve for chloroplasts with 10^{-5} M DCMU and 10^{-3} M hydroxylamine added. The dots are for the delayed light emission measured 2 ms after a light flash. The triangles are for the difference in the intensity of the fluorescence measured after *n* light flashes and the fluorescence intensity of the 360th flash. The difference in the fluorescence intensity of the 1st flash and the 360th flash is normalized to the difference in the intensity of the delayed light emission after the 1st flash and after the 360th flash. All date are from Fig. 6.

is proportional to the concentration of open reaction centers. Since algae that lacked reaction centers have negligible delayed light [1], illuminating reaction centers that are closed should also produce negligible delayed light. It is not surprising then that the intensity of the delayed light emission that is not due to the reconbimation of Q^- and Z^+ should be proportional to the concentration of open traps.

In chloroplasts with DCMU added, after absorbing one photon, the reaction center cannot absorb another photon until the back reaction between Q^- and Z^+ has occurred. This implies that if the reaction center can exist as a single, double or triple positively charged entity [23, 24], only the single charged entity would exist for chloroplasts with DCMU added. In normal chloroplasts where Q^- can undergo reaction with the next component in the electron transport chain, the reaction center can have single, double or triple positively charged Z's. Zankel [22] have shown that the intensity of the delayed light with double or triple charged Z is greater than that with a single charged Z. Hence it is not surprising that the component of the induction curve that is due to the fast decaying component for normal chloroplasts should increase with time as more and more Z^{2+} and Z^{3+} are generated. This increase would mask the decrease due to the decrease in the concentration of open reaction centers. Also these higher charged species would be more affected by membrane potential [14] and pH gradient [17] and would cause greater changes in the environment surrounding them, which in turn will affect the intensity of the delayed light emission. The induction curve of the normal chloroplasts is highly complex because it is a function of many variables which are also changing in time. It is unlikely that without detailed analysis of the decay kinetics after each flash of light that the induction curve could be used as an indicator of electrical potential [14] or pH gradient [17] or the concentration of high energy intermediates [12, 13].

In summary, delayed light induction curves measured with a phosphorescope should be interpreted with great care. This is due partially to the fact that the delayed light emission measured is composed of two components: one component due to the last flash only and one component due to all previous flashes before the last one. By measuring the induction curve of the delayed light by monitoring the decay kinetics after each exciting flash of light in a simpler system of chloroplasts with DCMU added, we find that the component due to the last flash only is dependent on the concentration of open reaction centers and the component due to all previous flashes before the last one is dependent on the concentration of closed reaction centers.

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