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DELAYED LUMINESCENCE YIELD KINETICS IN FLASH ILLUMINATED GREEN PLANTS

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SUMMARY

Oscillations of a delayed light emission have been studied in *Chlorella* cells, pea leaves and isolated pea chloroplasts illuminated by series of short saturating flashes. The oscillation is determined by changes of S-state numbers of the O_2-evolving system and by pH lowering inside the thylakoids after a flash. The rate of deactivation of the S-states drastically decreases upon temperature lowering to +4°C, and its pH-dependence has a maximum at pH 6. In isolated chloroplasts, transitions between individual S-states are inhibited at some certain temperatures in the range from −17°C to −40°C.

INTRODUCTION

Under illumination by a series of short saturating flashes, the intensity of the delayed light emitted by *Chlorella* cells or green plant leaves and isolated chloroplasts oscillates with a period of four, a behavior similar to that of O_2-yield [1—4]. It has been suggested that delayed light emission (DLE) yield increases with a S-state number of the O_2-evolving system.

One would expect the DLE oscillation pattern to be affected by the release of H^+ into the intrathylakoid space after the flash [5], because the DLE yield is enhanced by the light-induced proton concentration gradient across the thylakoid membrane [6].

In the present work we have measured the yield of the DLE excited by flash as a function of the time interval after preillumination by series of flashes in an attempt to try to observe separately the effects of alternations in the S-state number and of acidification of thylakoid interior.

Abbreviation: DLE, delayed light emission.

MATERIALS AND METHODS

Cultures of *Chlorella pyrenoidosa* were grown at 10 000 lx in the Tamiya medium [7]. Chloroplasts were prepared by the method of Arnon [8] from leaves of pea seedlings grown 10—12 days at 10 000 lx. Prior to experiments, chloroplasts were diluted in reaction medium containing 5×10^{-5} M phosphate buffer, 0.4 M sucrose and 5×10^{-3} M $MgCl_2$. pH of the medium was adjusted by varying proportions of phosphate buffer components. DLE was measured in a conventional phosphoroscope with a standard time interval of 1.25 ms between illumination and measurements. Excitation was given by xenon flashes which were synchronized with the opening of the phosphoroscope window. Standard interval between flashes was 1 s. The electric energy of a flash was approx. 3 J, the half-width 8 μs. Flashes were filtered through a cut-off filter ($\lambda \geqslant 650$ nm).

For DLE measurements at a temperature range from +50°C to −170°C, a thin-layer cuvette attached to the container with liquid nitrogen by means of brass holder was used. Sample temperature was adjusted by a heater held in a holder. The temperature of the sample was monitored by a Cu-constantan thermocouple.

RESULTS

DLE oscillations

After illuminating with a series of short saturating flashes the millisecond component of the DLE from *Chlorella* cells, pea leaves and isolated pea chloroplasts appears to oscillate with a period of 4 flashes, in a way the oxygen yield does [9], except that the basic level of oscillations rises monotonously with flash position in a series (Fig. 1). Oscillations are greater in intact leaves and algae.

The oscillation pattern depends on the time interval between the flash and the measurement (t_d). As it can be seen from Fig. 1, the normalized intensity of the DLE excited by the 2nd flash (L_2/L_1) does not vary while L_3/L_1 and L_4/L_1 decrease, with characteristic time of 2.5 ms.

DLE yield dark relaxation kinetics

The amplitude of DLE yield oscillations decreases, as one increases the time interval between the flashes. We have measured normalized yields of the DLE excited by the 2nd, 3rd and 4th flashes, as a function of the dark interval in samples preilluminated by one (1, Δt, 2), two (1, 2, Δt, 3) and three (1, 2, 3, Δt, 4) flashes (Fig. 2). The relaxation of DLE yield in preilluminated chloroplasts appears to be biphasic in character, indicating that two processes are involved in flash-induced DLE changes. The half-time of a slow phase (~200 s) is nearly the same as the relaxation time of the O_2-evolving system [2,4]. The rapid phase has a half-time of approx. 5—10 s.

Changes in DLE yield (L_n/L_1), slow phase amplitude (L_n^s/L_1) and ratio

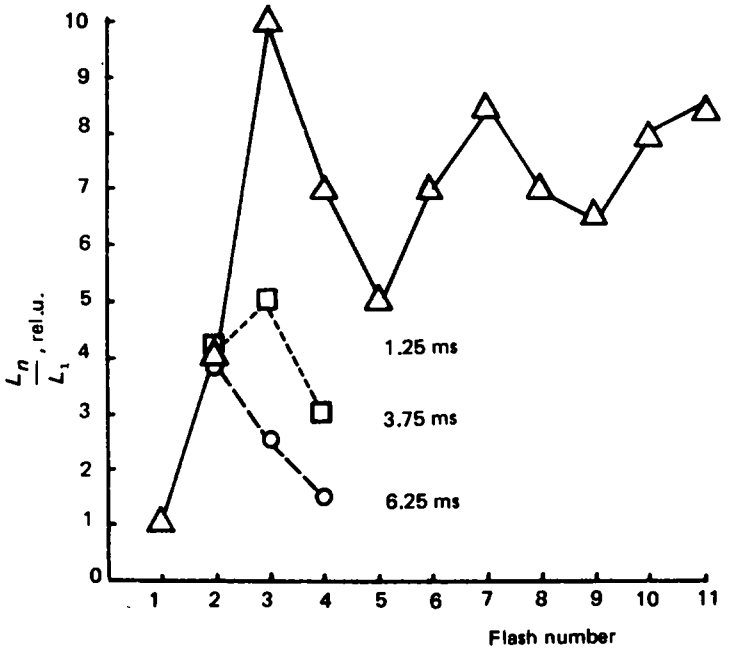

Fig. 1. Oscillations of DLE intensity in *Chlorella* cells measured after different time intervals between flash and measurement. L_n/L_1 is the intensity of the DLE excited by the n-th flash, as normalized to that after the first flash. Time intervals between flash and measurement are given at the curves.

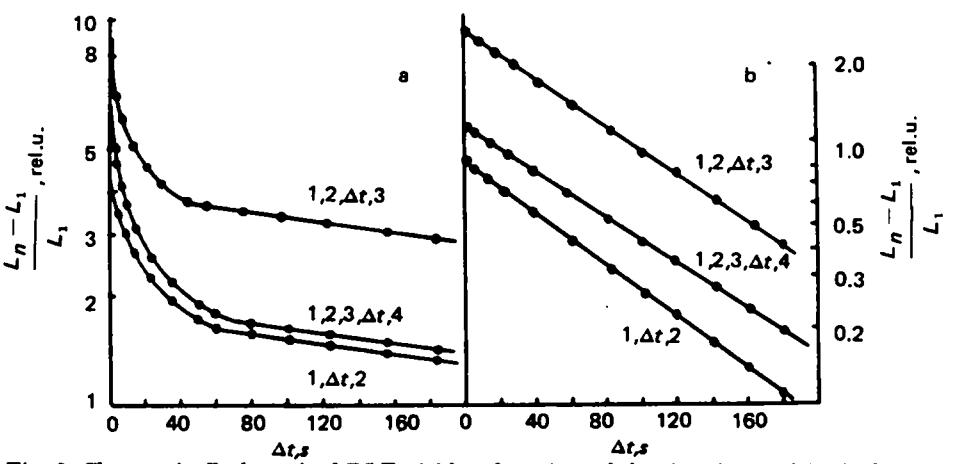

Fig. 2. Changes in flash excited DLE yield as function of the time interval (Δt) after preillumination of chloroplasts by one (1, Δt, 2), two (1, 2, Δt, 3) or three (1, 2, 3, Δt, 4) flashes. *a*-chloroplasts were suspended in a reaction medium (pH 7.8), as described in Materials and Methods. *b*-chloroplasts were hypotonically shocked in distillated water (pH 5.8).

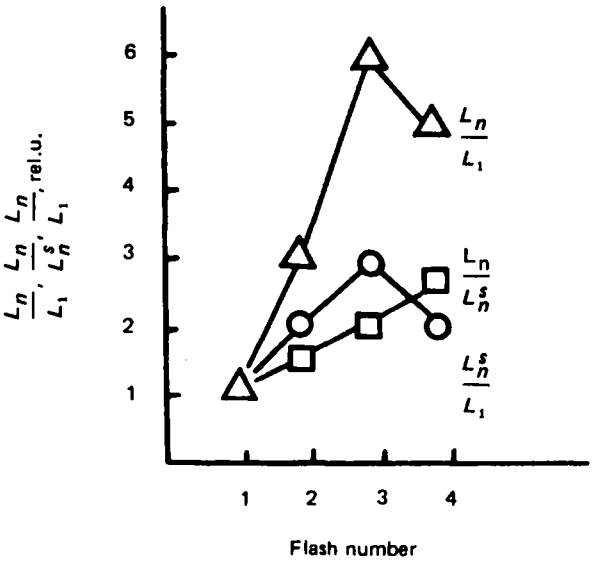

Fig. 3. Flash number dependency of the overall DLE yield of chloroplasts, as normalized to that after the first flash (L_n/L_1); normalized amplitude of the slow phase of the dark relaxation of the DLE yield after $n-1$ preilluminating flashes (L_n^s/L_1) and ratio L_n/L_n^s. The reaction medium has a pH of 7.8.

L_n/L_n^s plotted as a function of flash position in a flash succession are shown in Fig. 3. It can be seen that the amplitude of the slow phase undergoes considerable oscillations, while L_n/L_n^s increases monotonously with flash number. A similar dependency of L_n^s/L_1 and L_n/L_n^s was found for *Chlorella* cells and pea leaves. In both cases the oscillations of the slow phase L_n^s/L_1 are more pronounced, then those of L_n/L_1.

After the disruption of thylakoids by hypotonic shock in distillated water, the rapid phase disappears and DLE oscillations become greater (Fig. 2b). The acceleration of the slow relaxation phase after hypotonic shock is due to pH lowering resulting from suspending chloroplasts in distillated water (pH 5.8) as will be seen below.

pH-dependence of the DLE dark relaxation.

The slow relaxation kinetics are influenced by pH of the medium (Fig. 4). They increased sharply, as the pH was lowered from 8.0 to 6.0 and decreased at lower pH.

The rate of rapid phase is independent of pH, but its amplitude is reduced with pH lowering.

Temperature dependence of oscillations

The DLE temperature dependences for isolated pea chloroplasts excited by the first, second, third and fourth flashes are shown in Fig. 5. The DLE

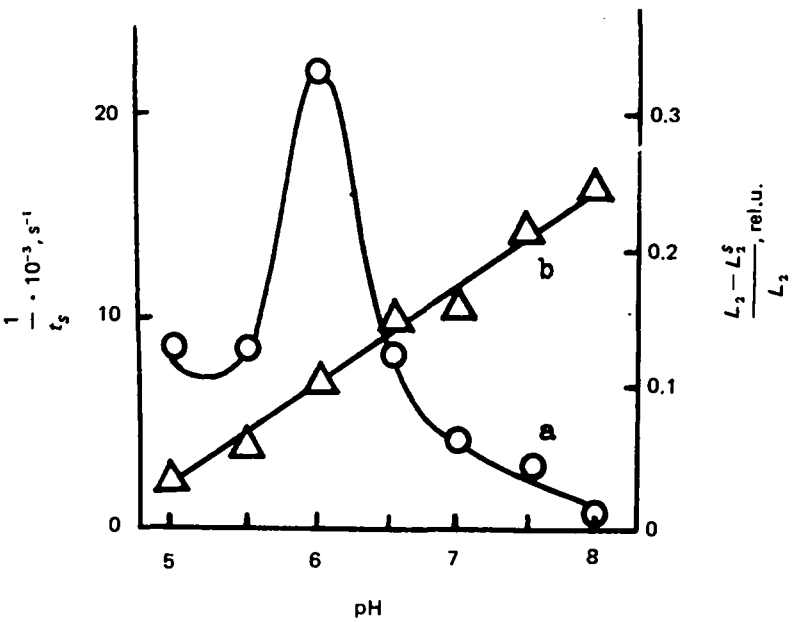

Fig. 4. pH-dependence of DLE yield dark relaxation in chloroplasts preilluminated by one flash: (a) The rate of the slow dark relaxation phase ($1/t_s$); and (b) the contribution of the rapid phase $(L_2\text{-}L_2^s)/L_2$.

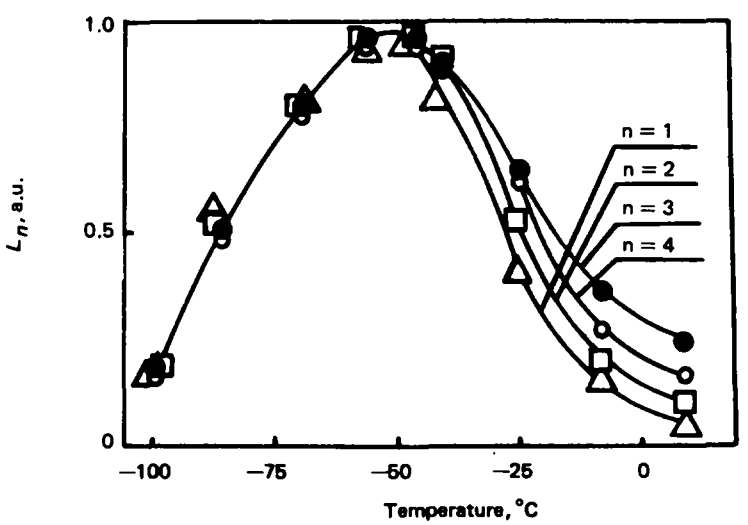

Fig. 5. Temperature dependence of the DLE intensity in chloroplasts after excitation by the 1, 2, 3 and 4th flash. The reaction medium has a pH of 7.8.

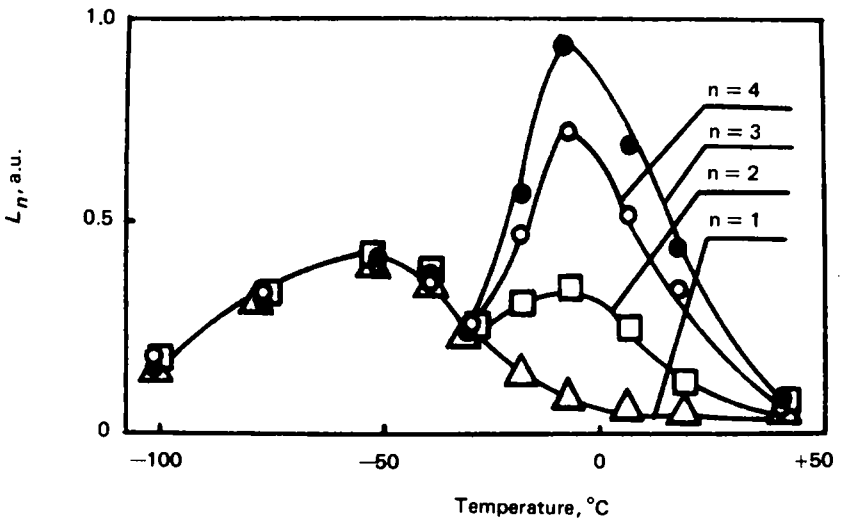

Fig. 6. Temperature dependence of the DLE intensity in whole pea leaves after excitation by the 1, 2, 3 and 4th flash.

intensity increases, as the temperature is lowered to −50°C and then decreases at temperatures from −50°C to −120°C.

The amplitude of DLE yield oscillations decreases from 0°C to −40°C. With temperature lowering, DLE yields are leveled, and for various flashes this occurs at their own temperatures. This may be due to an inhibition of

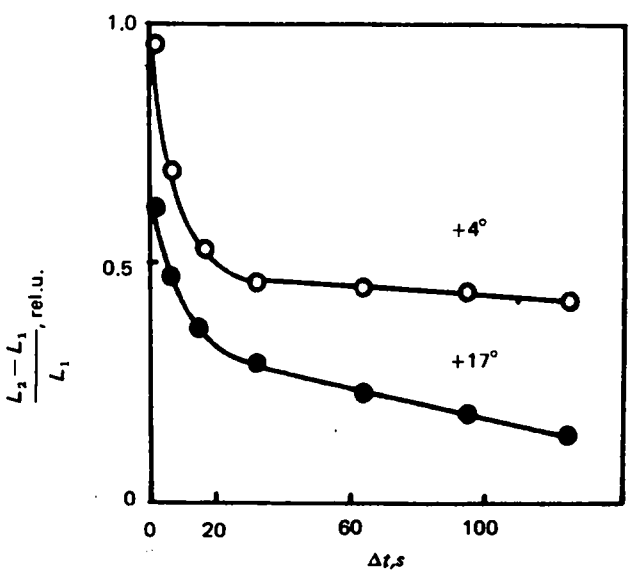

Fig. 7. Dark relaxation kinetics of DLE yield changes after preillumination of whole pea leaves by single flash at different temperatures.

transitions between individual S-states of the O_2-evolving system at different temperatures, as suggested Inoue and Shibata from the results on oscillations of thermoluminescence [10].

In whole leaves, the temperature dependence of DLE excited by a series of short flashes shows two maxima, at −4°C and −50°C (Fig. 6). The first maximum lacks for DLE excited by the first flash and for the DLE of DCMU-treated leaves.

The dark relaxation kinetics of the DLE yield ($L_2/L_1 - 1$) in leaves and in chloroplasts show two phases with half-times of 3—5 s and 60 s (Fig. 7). After temperature lowering to +4°C, the half-time of the slow phase increases, and the amplitude slightly lowered. The amplitude of the rapid phase shows a 2-fold increase.

DISCUSSION

Oscillations of DLE yield after illumination of chloroplasts by a series of short saturating flashes reflect transitions between S-states within the O_2-evolving system. The pattern of oscillations changes with increasing the dark time interval between excitation and measurement [11]. The characteristic time of this change was estimated by us as 2.5 ms. This is near to the value obtained by Zankel [3] from decay kinetics of DLE excited by second and third flashes. Assuming that the number of S-states is not altered 1 ms after the flash (minimal time interval between excitation and measurement in our experiments), i.e. that delayed light is emitted from the centers with S-states unchanged after the flash, one may ascribe a time of 2.5 ms to the $S_n \rightarrow S_{n+1}$ transition.

If in the O_2-evolving system the transition occurs in less than 1 ms, change in oscillations pattern reflects the $S_4 \rightarrow S_0$ transition. At present one cannot say which situation is taking place here.

Positive charges accumulated under actinic flashes in the O_2-evolving system relax in the dark and the DLE yield is reduced. The kinetics of the dark relaxation of the DLE yield have two phases. The rapid phase has a half-time of 5—10 s. The elimination of this phase in osmotically-shocked chloroplasts and the monotonous increase of its amplitude with flash number suggests that this phase is due to the efflux of protons released after each flash [5] into the intrathylakoid space by the O_2-evolving system.

The slow dark relaxation phase apparently reflects the deactivation of the O_2-evolving system. The half-time of this phase increases with pH in the range from 6.0 to 8.0.

In whole leaves the DLE oscillations have the maximal amplitude at 0°C. Apparently, this is due to increase of the rapid phase amplitude with temperature lowering (Fig. 7). The reason for more pronounced stimulation of DLE by protons pumped into thylakoid interior by single flash at lower temperatures is not clear.

The increase in the DLE intensity at temperatures from 0°C to −50°C,

which has previously been observed [12], is apparently caused by a slowing of electron transfer from the primary acceptor of photosystem II.

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