Binary Oscillation of Delayed Luminescence: Evidence of the Participation of Q_B^- in the Charge Recombination

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The flash-induced oscillation of a slowly decaying component of delayed luminescence (half time ~ 42 s) was investigated in spinach chloroplasts for various redox states of the secondary acceptor pool (Q_B). In preilluminated chloroplasts (30 s light followed by 5 min dark) the slow component exhibited a period-4 oscillation as a function of flash number. Upon oxidation of a major part of the Q_B pool by dark-adaptation or by ferricyanide treatment of chloroplasts, the period-4 oscillation was converted into a period-2 oscillation, providing direct experimental evidence of the participation of Q_B^- in the charge recombination reaction. The measured oscillatory patterns could be simulated in model calculations by assuming that the slow component originates from charge recombination of the redox couples $S_2Q_B^-$ and $S_3Q_B^-$.

Introduction

It has been widely accepted that delayed luminescence is generated by charge recombination between positively charged donors and negatively charged acceptors of the photosystems [1–4]. The various phases of delayed luminescence detected in the μsec to min time range oscillate with a periodicity of four, indicating that the reservoirs of positive charges are the S states of the water-splitting system [5–8]. The negatively charged counterparts of the S states undergoing charge recombination have not yet been unambiguously identified. It has been suggested that in the time range 100 ms to several seconds delayed luminescence excited by flashes prior to DCMU addition originates from $S_2Q_A^-$ and $S_3Q_A^-$ recombination [7, 9]. Since period-2 oscillation of delayed luminescence emission could not be detected in the absence of DCMU, it was concluded that, in untreated chloroplasts, Q_B^- is not the main source of electrons involved in the generation of slow delayed luminescence [7].

However, it has recently been observed by Rutherford and Inoue [8] that the period-4 oscillation pattern of a slow phase of delayed luminescence (decaying in the seconds to minutes time range) depends greatly on the redox state of the Q_B pool. The oscillatory maxima appearing at the 2nd and 6th flashes were shifted to the 1st and 5th flashes during the dark-adaptation of chloroplasts [8]. A similar change was observed in the flash pattern of the main thermoluminescence band (B band at about +30 °C) when the ratio Q_B/Q_B^- was increased either by dark-adaptation or by ferricyanide treatment of chloroplasts [10–13]. The measured oscillatory patterns could be simulated in model calculations by assuming that the redox states $S_2Q_B^-$ and $S_3Q_B^-$ are involved in the charge recombination [10–14]. Since delayed luminescence and thermoluminescence are generated by the same recombination mechanism [8, 15], it was inferred that the slow component of delayed luminescence corresponds to the B thermoluminescence band and also arises from $S_2Q_B^-$ and $S_3Q_B^-$ recombination [8].

Accepting that Q_B^- indeed contributes to the charge recombination, one can expect the appearance of period-2 oscillation of the delayed luminescence intensity in a series of flashes in chloroplasts having a largely oxidized Q_B pool. In order to acquire this decisive experimental evidence on the slow phase of delayed luminescence, the flash-induced oscillation of luminescence intensity was investigated in long-term dark-adapted and in ferricyanide-treated chloroplast. In agreement with thermoluminescence results [11, 12], the slow phase of delayed luminescence (half-time about 42 s) exhibited binary oscillations.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanolsulfonic acid; PS, photosystem; Q_A, primary quinone electron acceptor of photosystem II; Q_B, secondary electron acceptor of photosystem II.

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Materials and Methods

Chloroplasts were prepared from spinach as previously described [16] and suspended in a medium containing 0.4 M sorbitol, 10 mM NaCl, 1 mM $MnCl_2$, 5 mM $MgCl_2$, 2 mM EDTA and 50 mM HEPES (pH 7.5) to give a concentration of 30 μg Chl/ml. Delayed luminescence was excited in a 1 cm cell with xenon flashes (Stroboslave, model 1539, General Radio, Concord, Mass. 01742, USA) spaced 80 ms apart. Observation of the emitted delayed light was initiated 5 s after the final flash by the opening of an Uniblitz shutter (model 22L4AOX5, Vincent Associates, Rochester, New York 14607, USA). In single flash experiments the observation started 80 ms after the flash.

DCMU treatment of chloroplasts was performed immediately after the flash by mixing the sample with 20 μM DCMU in the dark during the 5 s period prior to the shutter opening. Modifications of the redox state of the secondary acceptor pool was carried out by ferricyanide treatment according to [17]. Chloroplasts were incubated for 1 h at room temperature, in the dark, in the presence of 50 μM ferricyanide. Following centrifugation, the pellet was washed twice and resuspended in the suspension buffer. Delayed luminescence was detected by a photomultiplier (EMI 9558 B) situated at right angles to the actinic light. The output of the photomultiplier was amplified by a differential amplifier and the signal was stored either in a multichannel analyser (model ICA 70, Central Res. Inst. Phys., Budapest, Hungary) or in a storage oscilloscope (model PM 3310, Philips, Eindhoven, The Netherlands). The decay curves were plotted on an X-Y recorder.

The rate of oxygen evolution was measured at saturating light intensity by using a Clark electrode in a temperature-controlled cell at +25 °C. The assay medium contained 0.1 M sorbitol, 10 mM K_2HPO_4, 20 mM NaCl, 4 mM $MgCl_2$, 2 mM EDTA, 250 μM *p*-benzoquinone, 1 mM methylamine and 50 mM HEPES at pH 7.5, together with chloroplasts carrying 50 μg chlorophyll in a final volume of 3 ml.

The oscillation of delayed luminescence in sequence of flashes was simulated by model calculation as described in Ref. 11.

Results and Discussion

It has been reported that delayed luminescence excited by flashes consists of a quickly and a slowly decaying phase in the seconds to minutes time scale [8, 9]. Accordingly, Fig. 1 shows that in untreated chloroplasts the decay of delayed luminescence excited by a single flash at 25 °C is biphasic and can be resolved into two exponential components with half-lives of 2.9 s and 41.6 s (Fig. 1, solid line). The addition of DCMU before flash excitation eliminated the slow component, with a concomitant increase in the intensity of the fast one (Fig. 1, dashed line). The half-life of the fast component proved to be the same (2.9 s) as that measured in untreated chloroplasts. Thus, the measured half-lives are in good agreement with those obtained previously for the Q ($t_{1/2} \sim 3$ s) and B ($t_{1/2} \sim 48$ s) thermoluminescence bands (appearing in the glow curve at about +10 and 30 °C, respectively) [10, 11]. These observations indicate that the fast and slow delayed luminescence components correspond to the Q and B thermoluminescence bands, respectively.

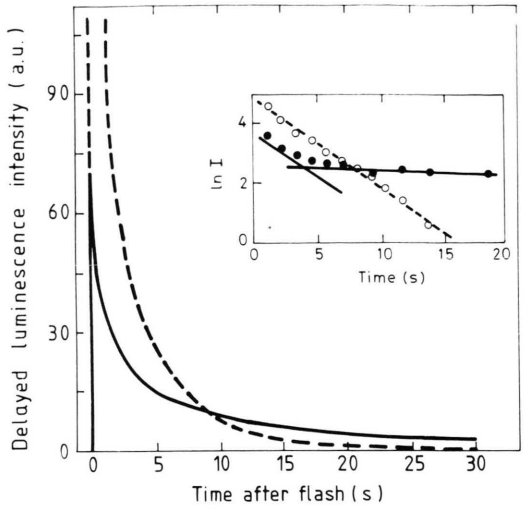

Fig. 1. Delayed luminescence of untreated (solid line) and 20 μM DCMU-treated (dashed line) spinach chloroplasts as a function of time after a single flash. Measurement of delayed luminescence started 80 ms after the flash. The insert shows the resolution of decay kinetics into exponentials.

When excited by a series of flashes, the intensity of delayed luminescence depended greatly on the excitation flash number and on the dark-adaptation time of the chloroplasts. In chloroplasts preilluminated with continuous light for 30 s and kept in the dark for 5 min at 25 °C prior to flash excitation (Q_B:Q_B^- = 50:50), the delayed light intensity, measured at 30 s after the final excitation flash, oscillated with a

periodicity of 4, with maxima at flash numbers 2 and 6 (Fig. 2A and 3A). After a 2 h dark-adaptation of chloroplasts, the oscillatory maxima were shifted to the 1st and 5th flashes (Fig. 3B). As the dark-adaptation time was increased further (4 h), the period-4 oscillation was converted into a period-2 oscillation, with maxima at uneven flash numbers (Figs. 2B and 3C, solid line).

The changes in the period-4 oscillatory pattern of delayed light during the dark-adaptation of chloroplasts has already been reported by Rutherford and Inoue [8]. However, they did not observe binary oscillation. The binary oscillation of delayed light could also be induced in chloroplasts treated with ferricyanide according to the procedure of Robinson and Crofts [17] (Fig. 3C, dashed line). Since the dark-adaptation and ferricyanide-washing of chloroplasts influence the redox state of the Q_B pool [17, 18], the changes in the oscillation patterns, and especially the period-2 oscillation, can be interpreted as a phenomenon reflecting the changes in the amount of Q_B^- in the sequence of flashes. The thermoluminescence results strongly support this assumption. The main thermoluminescence band appearing at about +30 °C and attributed to $S_2Q_B^-$ and $S_3Q_B^-$ recombination [10, 11] exhibits similar oscillations during the dark-adaptation of chloroplasts [11, 12] to those of the slowly decaying phase of delayed luminescence.

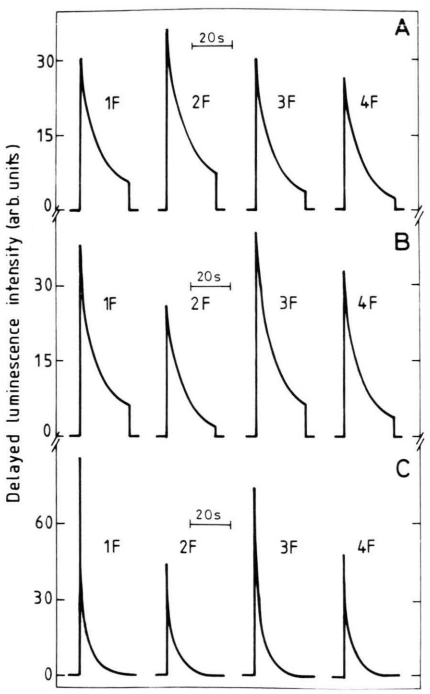

Fig. 2. Dependence of delayed luminescence intensity on the number of excitation flashes and on the dark-adaptation period of chloroplasts. A: Chloroplasts were preilluminated with continuous light for 30 s and kept in the dark for 5 min before flash excitation. B: Chloroplasts were dark-adapted for 4 h at room temperature before flash excitation. C: Flash excitation of dark-adapted (4 h) chloroplasts was followed by mixing with 20 μM DCMU.

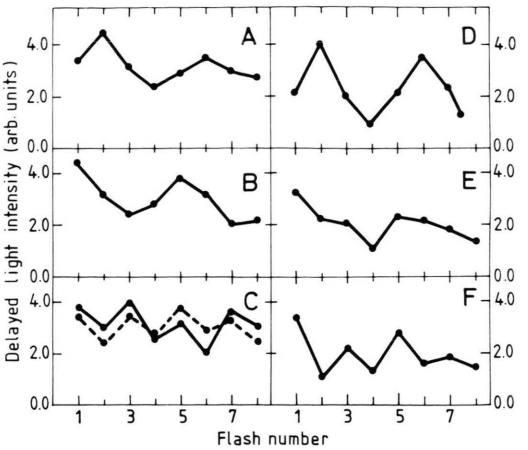

Fig. 3. Oscillation of delayed luminescence intensity at 30 s as a function of flash number. A: Chloroplasts were preilluminated with continuous light for 30 s, followed by 5 min dark-adaptation. B: The same as A, except that chloroplasts were stored for 2 h in the dark at room temperature before flash excitation. C: Chloroplasts were stored for 4 h in the dark before flash excitation (solid line). The dashed line represents chloroplasts incubated in the presence of 50 μM ferricyanide. D, E and F: Computer-simulated oscillations assuming that, after each flash, the intensity of delayed luminescence is determined by the sum of the centers present in the states $S_2Q_B^-$ and $S_3Q_B^-$. Miss and double hit parameters are 8% and 4%, respectively. The emission yield in the $S_2Q_B^-$ state is half that emitted in the $S_3Q_B^-$ state. D: The Q_B pool is 50% oxidized and the distribution of the possible redox states of the reaction centers is S_0Q_B:$S_0Q_B^-$:S_1Q_B:$S_1Q_B^-$ = 12.5:12.5:37.5:37.5. E: The Q_B pool is 80% oxidized and S_0Q_B:$S_0Q_B^-$:S_1Q_B:$S_1Q_B^-$ = 20:5:60:15. F: The Q_B pool is completely oxidized and S_0Q_B:$S_0Q_B^-$:S_1Q_B:$S_1Q_B^-$ = 25:0:75:0.

On the basis of the similarities of the delayed luminescence and thermoluminescence oscillations, model calculations were carried out with the assumption that the intensity of the slow component of delayed luminescence is determined by the numbers of centers present after each flash in the redox states $S_2Q_B^-$ and $S_3Q_B^-$. The luminescence intensity arising from $S_2Q_B^-$ was considered to be half that arising from $S_3Q_B^-$ [13, 19]. The dark distribution of S states was

taken as $S_0:S_1:S_2:S_3 = 25:75:0:0$. The efficiency of flashes in the S state transitions was considered by assuming probabilities $\alpha = 0.08$ for misses and $\beta = 0.04$ for double hits. The changes in the oscillatory patterns during the dark-adaptation of chloroplasts were taken into account by varying the ratio Q_B/Q_B^-. It was assumed that after preillumination of chloroplasts the Q_B pool was in a steady-state distribution ($Q_B/Q_B^- = 50:50$), which gradually relaxed into the completely oxidized state ($Q_B/Q_B^- = 100:0$) during the 4 h dark-adaptation of the chloroplasts. With the above assumptions, the measured oscillatory patterns could be simulated satisfactorily (compare Figs. 3A to C with Figs. 3D to F). The successful simulation of the binary oscillation of delayed luminescence extends the earlier work of Rutherford and Inoue [8] and confirms their suggestion that the slow phase of delayed luminescence can be accounted for by charge recombination of the redox couples $S_2Q_B^-$ and $S_3Q_B^-$.

Addition of DCMU after a series of flashes eliminated the slow component and intensified the fast one (Fig. 2C). In dark-adapted chloroplast (4 h) excited by flashes before DCMU addition, the fast phase of delayed luminescence oscillated with a periodicity of 2 (Fig. 2C). A similar period-2 oscillation was observed in chloroplasts incubated in the presence of *p*-benzoquinone before flash excitation and DCMU addition [7]. The phenomenon is probably caused by a back-transfer of electrons from Q_B^- to Q_A, induced by DCMU addition. As a result of this process, the concentration of Q_A^- reflects the amount of Q_B^- present before DCMU addition, and consequently a period-2 oscillation appears in the intensity of the fast phase of delayed luminescence. On the basis of a computer simulation of the oscillatory pattern (not shown), we attribute this delayed luminescence component to $S_2Q_A^-$ and $S_3Q_A^-$ recombination, in agreement with a previous suggestion [7]. The fast delayed luminescence component apparently corresponds to the Q thermoluminescence band, which also arises from $S_2Q_A^-$ and $S_3Q_A^-$ recombination, and displays binary oscillation in the dark-adapted chloroplasts preilluminated with flashes prior to DCMU addition [11, 20].

Since the chloroplasts used in our delayed luminescence measurements were stored at room temperature, a check was made as to whether or not the changes in the oscillatory pattern were caused by a gradual inactivation of the water-splitting system.

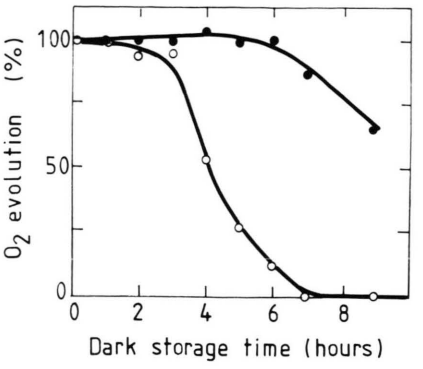

Fig. 4. Oxygen evolution rate as a function of incubation time of chloroplasts. Chloroplasts were stored at 25 °C either in room light (○–○) or in the dark (●–●). The electron transport was measured from water to *p*-benzoquinone at saturating light intensity. The absolute rate of oxygen evolution before dark incubation of chloroplasts was 98 μmol $O_2 \cdot (\text{mg Chl})^{-1} \cdot h^{-1}$.

The rate of electron transport measured from water to *p*-benzoquinone was compared in chloroplasts stored at room temperature either in room light or in the dark (Fig. 4). While chloroplasts stored in room light lost 50% of their oxygen-evolving capacity in 4 h (the maximum duration of dark-adaptation used in our delayed luminescence experiments), no detectable decrease could be measured in the rate of oxygen evolution of chloroplasts kept in the dark for the same time. Since the chloroplasts used in our delayed luminescence experiments were stored at room temperature in the dark, it can be excluded that the changes observed in the oscillatory patterns are due to any kind of degradation of the oxygen-evolving system.

To summarize the results, we can say that the binary oscillations of delayed luminescence, observed here for the first time in dark-adapted and ferricyanide-treated chloroplasts, provide firm experimental evidence that Q_B^- participates in the generation of delayed luminescence in the seconds to minutes time region. Model calculations permit the conclusion that the slowly decaying component of delayed luminescence arises from charge recombination of the redox couples $S_2Q_B^-$ and $S_3Q_B^-$.

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[1] J. Lavorel, in: Bioenergetics of Photosynthesis, pp. 223–317 (Govindjee, ed.), Academic Press, New York 1975.
[2] S. Malkin, in: Primary Processes of Photosynthesis, **Vol. 2,** Topics in Photosynthesis, pp. 351–431 (J. Barber, ed.), Elsevier, Amsterdam 1977.
[3] J. Amesz and H. J. van Gorkom, Annu. Rev. Plant Physiol. **29,** 47–66 (1978).
[4] Govindjee and P. Jursinic, Photochem. Photobiol. Revs. **4,** 125–205 (1979).
[5] K. L. Zankel, Biochim. Biophys. Acta **245,** 373–385 (1971).
[6] G. Barbieri, R. Delosme, and P. Joliot, Photochem. Photobiol. **12,** 197–206 (1970).
[7] J. Lavergne and A.-L. Etienne, Biochim. Biophys. Acta **593,** 136–148 (1980).
[8] A. W. Rutherford and Y. Inoue, FEBS Lett. **165,** 163–170 (1984).
[9] J. Lavergne and A.-L. Etienne, in: Proceedings 6th International Congress on Photosynthesis, pp. 939–948 (C. Sybesma, ed.), 1984.
[10] A. W. Rutherford, A. R. Crofts, and Y. Inoue, Biochim. Biophys. Acta **682,** 452–465 (1982).
[11] S. Demeter and I. Vass, Biochim. Biophys. Acta **764,** 24–32 (1984).
[12] S. Demeter, I. Vass, and Zs. Rózsa, in: Proceedings 6th International Congress on Photosynthesis, pp. 265–268 (C. Sybesma, ed.), 1984.
[13] A. W. Rutherford, G. Renger, H. Koike, and Y. Inoue, Biochim. Biophys. Acta **767,** 548–556 (1984).
[14] Y. Inoue, in: The Oxygen Evolving System of Photosynthesis, pp. 439–450 (Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger, and K. Satoh, eds.), Academic Press, Japan, Tokyo 1983.
[15] T. S. Desai, V. G. Tatake, and P. V. Sane, Biochim. Biophys. Acta **681,** 383–387 (1982).
[16] S. Demeter, Zs. Rózsa, I. Vass, and A. Sallai, Biochim. Biophys. Acta (in the press).
[17] H. H. Robinson and A. R. Crofts, FEBS Let. **153,** 221–226 (1983).
[18] F.-A. Wollman, Biochim. Biophys. Acta **503,** 263–273 (1978).
[19] S. Demeter, Zs. Rózsa, I. Vass, and É. Hideg, Biochim. Biophys. Acta (in the press).
[20] S. Demeter, FEBS Lett. **144,** 97–100 (1982).