Oscillation of delayed luminescence from PS II: recombination of $S_2Q_B^-$ and $S_3Q_B^-$

A.W. Rutherford* and Y. Inoue

Solar Energy Research Group, RIKEN, Hirosawa, Wako-shi, Saitama 351, Japan and **Service Biophysique, Dept. Biologie, CEN-Saclay, Gif-sur-Yvette Cedex, France*

Received 18 October 1983

Luminescence decaying in the seconds to minutes time scale was studied in spinach chloroplasts and the following results were obtained: (1) After a series of flashes a slow phase which decays in the tens of seconds to minutes time scale was observed to oscillate with a pattern characteristic of $S_2Q_B^-$ and $S_3Q_B^-$ recombination. This phase was lost upon Tris-treatment or upon the addition of DCMU. (2) After every flash a small faster phase of luminescence decaying in the seconds time scale was also present. This phase progressively increased with increasing numbers of flashes but when methyl viologen was present no such progressive increase of this phase occurred. In the presence of DCMU this seconds time scale luminescence was greatly increased. This phase of luminescence is attributed to $S_2Q_A^-$ recombination. (3) Tris-treatment resulted in the appearance of an even faster phase of luminescence which may be due to $Z^+Q_B^-$ recombination. These results demonstrate a close correlation of the kinetics of luminescence decay with thermoluminescence emission temperature.

Thermoluminescence *Oxygen evolution* *Photosynthesis* *S-state deactivation* *Secondary quinone acceptor*

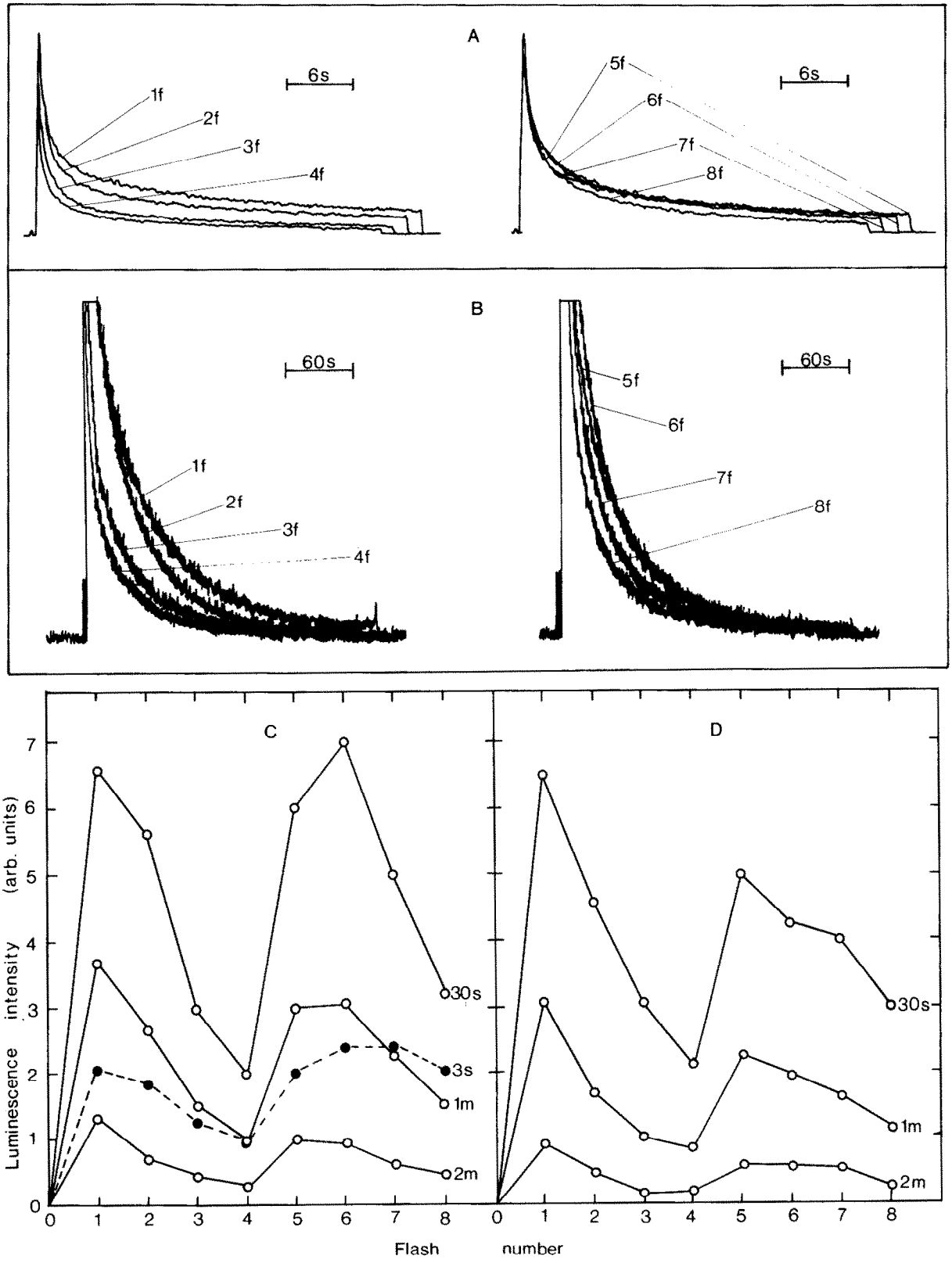
1. INTRODUCTION

Most of the delayed luminescence originating from chlorophyll in plants is due to recombination of positive charges on photo-oxidized donors with negative charges on photoreduced acceptors in Photosystem II (PS II) (reviews [1,2]). The various kinetic phases of delayed luminescence reflect the stability of the photoinduced charge pairs, faster decay kinetics being observed from recombination of less stable and therefore usually more primary charge pairs. In untreated dark-adapted chloroplasts excitation by a single flash results in the formation of a pair of charges which are still closely associated with the reaction centre but are stable in the tens of seconds time scale. The positive charge is located on the S states of the O_2-evolving enzyme which has the capacity for storing 4 positive charges before O_2 is evolved [3,4], while the negative charge is located on the secondary quinone acceptor, Q_B, which has a capacity for storing 2 electrons before donating to the plastoquinone pool, PQ [5,6]. However, phases of delayed luminescence from recombination of S state positive charges with Q_B^- electrons have not been reported in chloroplasts. Authors in [7] were unable to detect luminescence associated with this recombination reaction in the hundreds of milliseconds to seconds time scale but they were able to characterise luminescence arising from $S_2Q_A^-$ and $S_3Q_A^-$ recombination under conditions were DCMU was added after flash excitation (Q_A is the primary quinone acceptor). The luminescence resulting from $S_2Q_A^-$ and $S_3Q_A^-$ recombination decayed in the range of 1–2 s.

Thermoluminescence probably reflects the same phenomena as delayed luminescence although, for technical reasons, the recombination of various charge pairs with different stabilities is reflected as emission of light at different temperatures (glow peaks) rather than as kinetic phases [8,9]. The recent identification of the charge pairs responsible

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V.
001457593/84/$3.00


A
6s
1f
2f
3f
4f
5f
6f
7f
8f
B
60s
C
D
Luminescence intensity (arb. units)
30s
3s
1m
2m
Flash number

for specific glow peaks allowed a more specific comparison of thermoluminescence with delayed luminescence to be made [9]. In the presence of DCMU a single flash resulted in the formation of a state which gave rise to a single thermoluminescence band at 0°C. This band was attributed to $S_2Q_A^-$ and it was suggested that this glow peak represented the same light observed as the 1–2 s phase of delayed luminescence [9]. Support for this suggestion was provided independently by thermoluminescence experiments in which DCMU was added after flash excitation although the results were not interpreted in this way at that time [10]. The resulting oscillations of the 0°C glow peak time were found to be similar to those reported earlier for the 1–2 s phase of delayed luminescence [7].

In the absence of DCMU, flash excitation results in the formation of a single thermoluminescence band at around 25°C [9,11]. This band was identified as being the result of recombination of $S_2Q_B^-$ and $S_3Q_B^-$ and it was predicted that this glow peak would be equivalent to a phase of delayed luminescence in the tens of seconds time scale [9]. Extending this work authors in [12] measured the rate of $S_2Q_B^-$ recombination using an elegant fluorescence technique and obtained a value of 25 s. We report here the presence of delayed luminescence in spinach chloroplasts which decays with the predicted kinetics and oscillates with a pattern expected from $S_2Q_B^-$ and $S_3Q_B^-$ recombination.

2. METHODS

Chloroplasts were obtained from market spinach by standard methods [13]. Tris-washing was carried out by resuspending the chloroplasts in 0.8 M Tris (pH 8.2) under room light, followed by incubation at 4°C in darkness for 45 min before being pelleted and resuspended in buffer (15 mM Hepes, 150 mM sorbitol, 5 mM $MgCl_2$, 10 mM NaCl). Concentrated chloroplasts were stored on ice for 1–12 h before being used in experiments. Small volumes of the chloroplasts were diluted (40 μg Chl/ml) into room temperature buffer just before luminescence measurements were made. A fresh sample was used for each kinetic trace except for the experiments shown in fig. 2 (see legend). Delayed luminescence was measured in an apparatus which consisted of a 1 cm sample cuvette containing a 1 ml sample, all of which could be illuminated by a white xenon flash (2.5 μs half-time duration); a manually operated shutter was opened after flash excitation allowing luminescence to be measured using a cooled Hamamatsu photomultiplier R-550 and a Hamamatsu photon counter (C-1230) with a gate time of 0.1 s. Thermoluminescence was measured as in [9,14]. Chlorophyll concentration was 0.8 mg Chl/ml.

3. RESULTS AND DISCUSSION

3.1. *Oscillations of delayed luminescence after a series of flashes*

Delayed luminescence in spinach chloroplasts was recorded in the seconds to tens of seconds time range (fig. 1A) and in the tens of seconds to minutes time range (fig. 1B) after a series of flashes. The amplitude of the luminescence at all time ranges measured after the flash showed a marked dependence upon flash number (fig. 1C). At longer time ranges oscillations with maxima on the 1st, 2nd and 5th, 6th flashes and minima on the 3rd, 4th and 7th, 8th flashes were clearly present. At shorter time ranges the same pattern was present but was distorted by an underlying increase of luminescence with consecutive flashes. This flash

←

Fig. 1. Oscillations of delayed luminescence in the seconds to minutes time scale recorded in spinach chloroplasts. Conditions were as described in section 2. A and B are traces of luminescence intensity recorded as a function of time after a series of flashes (indicated in the figure by f). A was recorded with a gain 10-times smaller and a chart speed 10-times greater than in B. The data from A and B were used in C, where the luminescence intensity at given times (3 s, 30 s, 1 min, 2 min) after the last of a sequence of flashes is plotted as a function of flash number. The data plotted for 3 s in C (broken line), are on a scale 10-times greater than that for the other times. Chloroplasts were dark-adapted for about 2 h at 4°C before experiments were begun. D is as for C but using chloroplasts dark-adapted for about 8 h at 4°C.

dependence pattern is similar to that previously observed when the flash-induced thermoluminescence band at 25°C was measured [9]. This pattern was accounted for by assuming that the luminescence arises from $S_2Q_B^-$ and $S_3Q_B^-$ recombination taking into account that a proportion of Q_B^- is present in dark-adapted chloroplasts ([9], see also [15]). The amount of Q_B^- present before flash excitation has been shown to have a major effect upon the relative intensities of thermoluminescence after the 1st and 2nd (and 5th and 6th) flashes. Increasing the amount of stable Q_B^- present in the dark by preillumination resulted in increased 2nd flash amplitude relative to the 1st [9] while long dark adaptation resulted in increased 1st flash amplitude relative to the 2nd ([10], see also [16]). Fig. 1D shows that similar effects were observed with the oscillation pattern of delayed luminescence. After long dark adaptation the amplitude of luminescence after the 2nd and 6th flash decreased. This effect is interpreted as being due to gradual decrease in the number of centers in which Q_B^- is present in the dark.

After all flashes luminescence decaying in the tens of seconds to minutes time scale was observed. This phase of luminescence decay corresponds well with that predicted for $S_2Q_B^-$ and $S_3Q_B^-$ recombination from previous thermoluminescence studies [9] and with the $t_{1/2}$ value for $S_2Q_B^-$ recombination of 25 s recently measured by a fluorescence technique [12].

It can be seen in fig. 1A that traces recorded after all flashes exhibit a phase of luminescence which decays within a few seconds after the flash. This 1.5 s phase of luminescence may be due to $S_2Q_A^-$ and $S_3Q_A^-$ recombination since luminescence decaying in the same time scale is more clearly seen when DCMU is present ([7] and see below). The presence of this kind of luminescence in the absence of DCMU might reflect the equilibrium, $Q_A^-Q_B \rightleftarrows Q_AQ_B^-$, [12,17] or possibly centres where Q_B is absent.

3.2. *Delayed luminescence after many flashes*

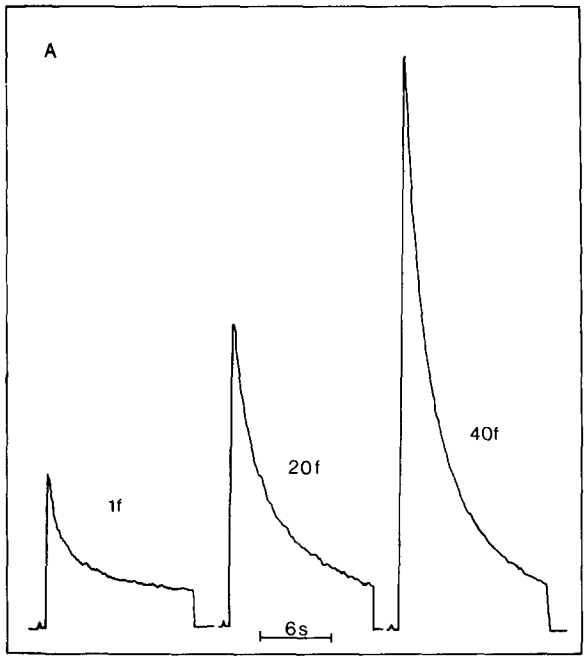
As mentioned above a progressive increase in the amplitude of the luminescence was observed with increasing number of flashes. This effect is demonstrated after 20 and 40 flashes in fig. 2. The effect is largely due to an increase in the phase decaying in the 1–10 s time scale associated with $S_2Q_A^-$ and $S_3Q_A^-$ recombination. The luminescence associated with $S_2Q_B^-$ and $S_3Q_B^-$ recombination was progressively lost as the number of flashes was increased. Gramicidin, an ionophoric uncoupler which dissipates both pH and potential gradients, had no effect upon this gradual increase in short-lived luminescence, however, the addition of methyl viologen which acts as an electron acceptor from PS I completely suppressed this phenomenon. In fact a slight decrease in luminescence is observed in the chloroplasts after 20 and 40 flashes. The presence of gramidicin and methyl viologen had very little effect upon the oscillation pattern except for the removal of the accumulated luminescence effect at short time ranges (not shown). The increase of luminescence in the absence of methyl viologen is interpreted as being due to the progressive reduction of the PQ pool and, due to the equilibria which determine binding of PQ and PQH_2 to the Q_B site [12,17,18], $S_2Q_A^-$ and $S_3Q_A^-$ recombination are increased.

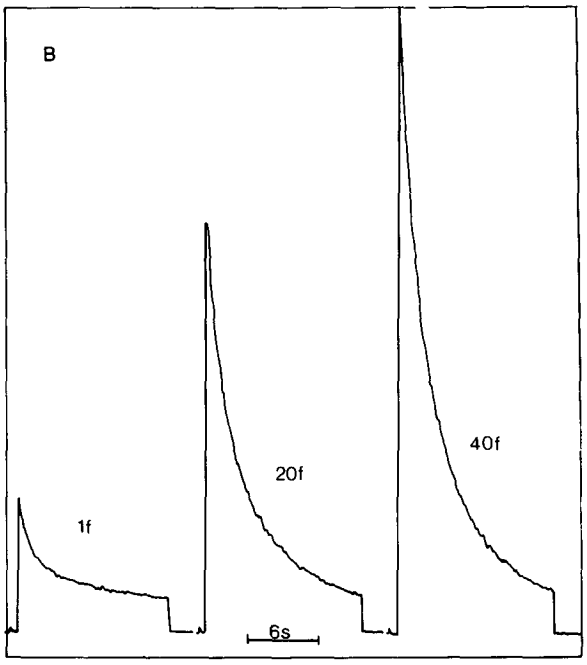
Under conditions where the PQ pool is reduced the presence of luminescence due to $S_2Q_B^{2-}$ and $S_3Q_B^{2-}$ recombination might be also expected, however, estimates of the equilibrium constant for the reaction $Q_A^-Q_B^- \rightleftarrows Q_AQ_B^{2-}$, i.e. $\geqslant 50$ [17], indicate that these recombination reactions might occur only with slow kinetics.

3.3. *The effect of DCMU and Tris upon the delayed luminescence*

The assignment of the slow phase of luminescence to $S_2Q_B^-$ and $S_3Q_B^-$ recombination leads to the prediction that both DCMU addition (an inhibitor which blocks electron transport between Q_A and Q_B) and Tris-treatment (which disconnects the S states from the reaction center) should result in loss of this phase of luminescence. Fig. 3A, B shows the effect of these treatments upon the luminescence. Indeed, luminescence in time scales longer than a few seconds was lost in both cases. Instead luminescence at shorter times was greatly enhanced. In the presence of DCMU most of the luminescence decayed in a fast phase of about 1.5 s although a slightly slower phase was also present. This luminescence is attributed to $S_2Q_A^-$ recombination in accordance with previous work [7].

The luminescence in Tris-treated chloroplasts decays even more quickly than in those treated





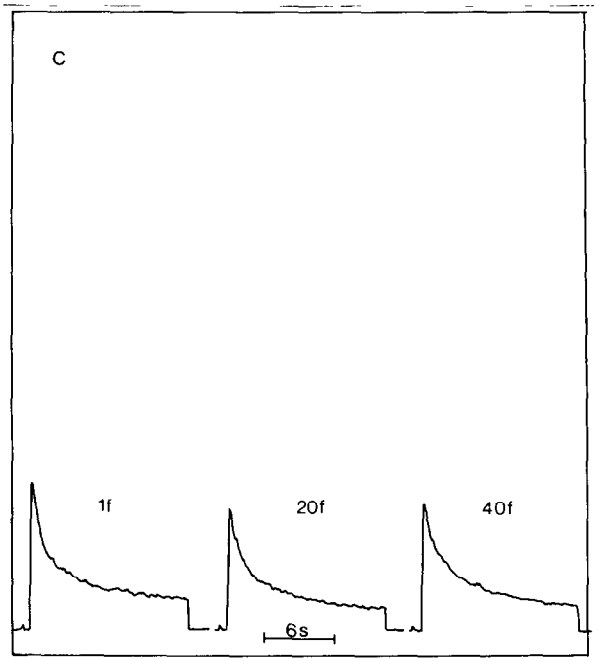


Fig. 2. The effect of many flashes upon luminescence in spinach chloroplasts. Luminescence was recorded after, 1, 20 and 40 flashes. A, untreated chloroplasts; B, in the presence of gramicidin (1 μM) and C, in the presence of both gramicidin (1 μM) and methyl viologen (100 μM). Additions of gramicidin and methyl viologen were done in darkness. The same sample was used to record traces after 1, 20 and 40 flashes with 5 min dark adaptation between each experiment. A separate sample was used for each treatment A–C.

with DCMU and little luminescence is present 5 s after the flash. This luminescence could be due to $Z^+Q_B^-$ recombination but the decay could at least partly be defined by loss of Z^+ due to other secondary donation reactions.

Fig. 3C shows thermoluminescence recorded after a single flash in untreated, DCMU treated and Tris-treated chloroplasts. Also shown is the result of a preliminary experiment where thermoluminescence was recorded in a Tris-treated sample in the presence of DCMU. It can be seen that the intensity of the luminescence is diminished and also the peak position is shifted to lower temperature. These effects probably reflect the decreased stability of the charge pair. This band is preliminarily assigned to $Z^+Q_A^-$ recombination. It is of note that the amplitude of the thermoluminescence increased with successive flashes after the first few flashes. This could reflect competition between the forward reaction, $ZP^+Q_A^-Q_B^- \rightarrow Z^+PQ_A^-Q_B$, and the back reaction, $ZP^+Q_A^-Q_B \rightarrow ZPQ_AQ_B$, at this temperature (see [19]). Alternatively, this could reflect the filling up of a more stable acceptor which could function under these conditions, e.g. oxidized low-potential cytochrome b_{559}. Further experiments are required on the Tris-treated system to characterise properly the ther-

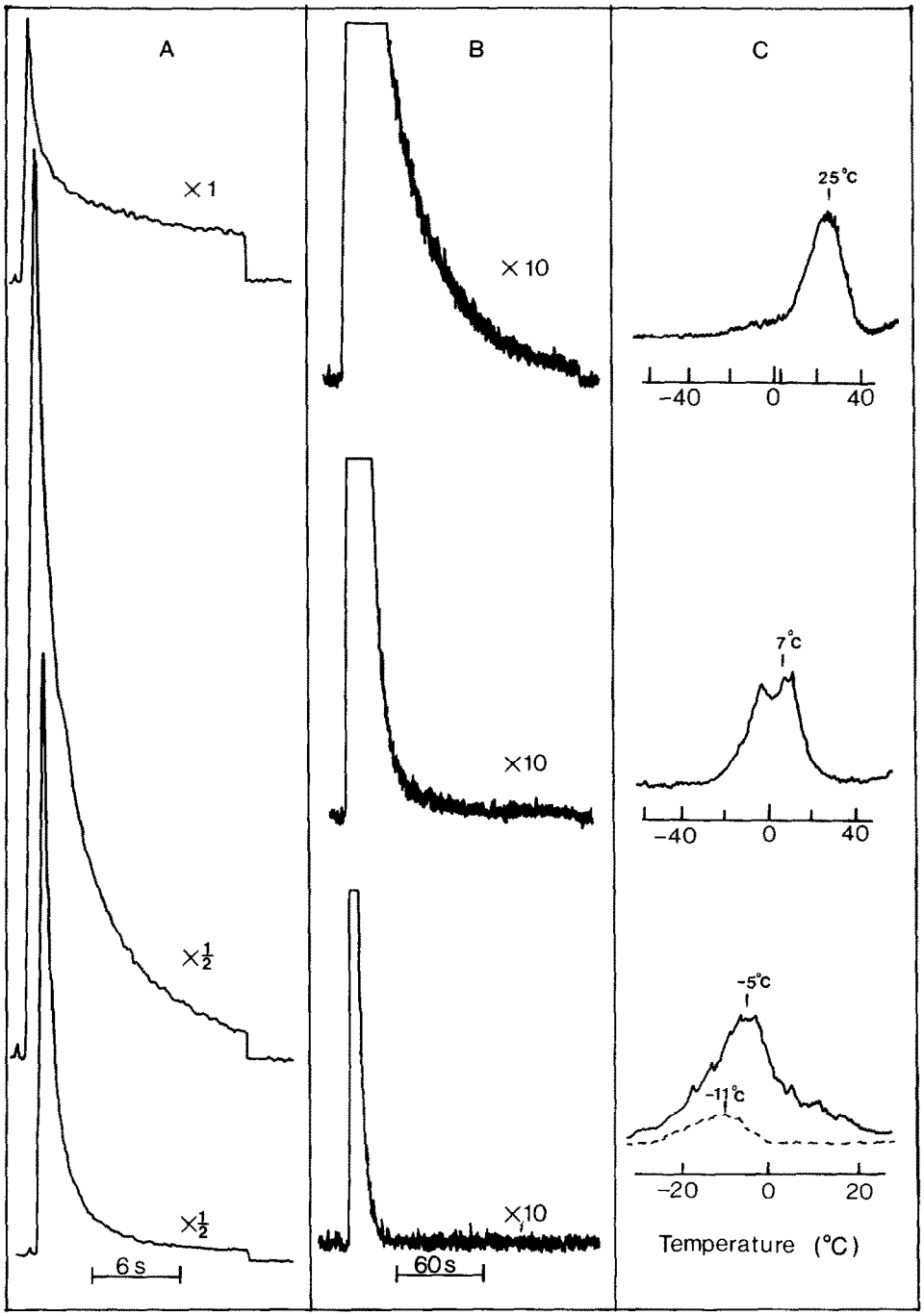


Fig. 3. The effect of DCMU and Tris-treatment upon the delayed luminescence and thermoluminescence in spinach chloroplasts after a single flash. A, luminescence recorded in the seconds time scale; B, luminescence recorded in the 10 s to minutes time scale; C, thermoluminescence. Upper traces, no additions; middle traces, + DCMU; lower traces, after treatment with Tris. In C, the broken line shows the effect of DCMU on Tris-treated chloroplasts. Luminescence and thermoluminescence were measured as described in section 2. Chlorophyll concentrations; A and B, 40 μg/ml; C, 0.8 mg/ml. DCMU, when present, was at a concentration of 2 μM in A and B, and 8 μM in C. In C, lower trace, thermoluminescence of Tris-washed chloroplasts (in 50 μM Mes, pH 6.0) was recorded using a faster heating rate (1°C/s) than that used for the untreated and DCMU-treated chloroplasts (~ 0.3°C/s). Under comparable conditions this trace would be at a slightly lower temperature and have a lower intensity. Flash illumination was given at room temperature in A and B and at −15°C for C.

moluminescence effects. A clear correlation of the emission temperature of the glow peaks with decay kinetics of the luminescence is observed, i.e. thermoluminescence with emission peaks at lower temperatures correspond to delayed luminescence with shorter lifetimes.

4. CONCLUDING REMARKS

Oscillations of luminescence associated with the S states of the O_2-evolving enzyme in the absence of inhibitors have been reported previously but have remained difficult to interpret [1,2,20–23]. At very short times (< 6 ms) oscillations with maxima associated with S_4 have been observed in *Chlorella* [22] while at longer time ranges (240 ms) oscillations associated with S_2 and S_3 have been reported [20]. In neither case is the charge pair which recombines to give the luminescence known, although several possibilities have been discussed [1,2]. The oscillations in slow luminescence reported here for the first time are apparently easier to assign. S_2 and S_3 are known to deactivate in the seconds to tens of seconds time scale and an obvious source of a deactivating electron is Q_B^-, since it is relatively stable and still associated with the reaction center. Moreover the oscillation pattern matches well the pattern predicted from the characteristic 2-electron gate mechanism of Q_B [9]. Although the existence of $S_2Q_B^-$ and $S_3Q_B^-$ recombination had been assumed earlier [17, 24], and this assumption was supported by previous luminescence studies [21], the first clear demonstration of these reactions came from thermoluminescence experiments [9] and, for $S_2Q_B^-$, was confirmed by recent fluorescence data [12]. The luminescence data reported here complement these studies and also confirm that delayed luminescence and thermoluminescence are different manifestations of the same phenomena.

The interpretation of this and previous work [9] indicates that $S_2Q_B^-$ and $S_3Q_B^-$ recombination reactions occur with similar kinetics as measured by delayed luminescence, and at the same emission temperature as measured by thermoluminescence. It has also been implied that these recombination reactions constitute a significant deactivation pathway of the respective S states [9,12,25,26]. For chloroplasts, where S_2 and S_3 deactivation rates are similar [21], this assumption seems justified. However it is of note that in *Chlorella* cells S_3 deactivates much faster than does S_2 [21]. Thermoluminescence measurements using *Chlorella* cells should show markedly different results from those obtained in chloroplast and might help determine if $S_3Q_B^-$ luminescence really occurs as recombination of $S_3Q_B^-$ ($S_3Q_B^- \rightarrow S_2Q_B$ (luminescence)) or is rather due to $S_2Q_B^-$ recombination preceded by a faster, non-recombination deactivation reaction ($S_3Q_B^- \xrightarrow{e^-} S_2Q_B^- \rightarrow S_1Q_B$ (luminescence)) as discussed in [25].

ACKNOWLEDGEMENTS

We would like to thank Dr B.A. Diner (Paris) for critically reading the manuscript and for useful discussion. We also appreciate discussion with Drs Govindjee (Urbana), G. Renger (Berlin) and P. Mathis (Saclay) and also the help of Dr Mamiko Kimimura and Agnès Rutherford for help in the preparation of the manuscript. This work was supported by an STA (Science and Technology Agency) Grant on Solar Energy Conversion by means of Photosynthesis given to RIKEN (The Institute of Physical and Chemical Research) and in part by the Commission of the European Communities (Solar Energy Programme, contract ESD-015-F). A.W.R. was the recipient of a Japanese Government Research Award for Foreign Specialists.

REFERENCES

[1] Lavorel, J. (1975) in: Bioenergetics of Photosynthesis (Govindjee ed.) pp. 233–317, Academic Press, New York.
[2] Malkin, S. (1977) in: Primary Processes of Photosynthesis (Barber, J. ed.) pp. 349–431, Elsevier, Amsterdam, New York.
[3] Joliot, P., Barbieri, G. and Chabaud, R. (1969) Photochem. Photobiol. 10, 309–329.
[4] Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475.
[5] Bouges-Bocquet, B. (1973) Biochim. Biophys. Acta 314, 250–256.
[6] Velthuys, B. and Amesz, J. (1974) Biochim. Biophys. Acta 333, 85–94.
[7] Lavergne, J. and Etienne, A.-L. (1980) Biochim. Biophys. Acta 593, 136–148.

[8] Desai, T.S., Tatake, V.G. and Sane, P.V. (1982) Biochim. Biophys. Acta 681, 383-387.
[9] Rutherford, A.W., Crofts, A.R. and Inoue, Y. (1982) Biochim. Biophys. Acta 682, 457-465.
[10] Demeter, S. (1982) FEBS Lett. 144, 97-100.
[11] Inoue, Y. and Shibata, K. (1978) FEBS Lett. 85, 193-197.
[12] Robinson, H.H. and Crofts, A.R. (1983) FEBS Lett. 153, 221-226.
[13] Arntzen, C.J. and Ditto, C.L. (1976) Biochim. Biophys. Acta 449, 259-279.
[14] Ichikawa, T., Inoue, Y. and Shibata, K. (1975) Biochim. Biophys. Acta 408, 228-239.
[15] Wollman, F.A. (1978) Biochim. Biophys. Acta 459, 351-363.
[16] Inoue, Y. (1983) in: The Oxygen Evolving System of Photosynthesis (Inoue, Y. et al eds.) Academic Press, Tokyo, in press.
[17] Diner, B.A. (1977) Biochim. Biophys. Acta 460, 247-258.
[18] Velthuys, B. (1982) in: Function of Quinones in Energy Coupling Systems (Trumpower, B.L. ed.) pp. 401-408, Academic Press, New York.
[19] Rienman, S. and Mathis, P. (1981) Biochim. Biophys. Acta 635, 249-258.
[20] Barbieri, G., Delosme, R. and Joliot, P. (1970) Photochem. Photobiol. 12, 197-206.
[21] Joliot, P., Joliot, A., Bouges, B. and Barbieri, G. (1971) Photochem. Photobiol. 14, 287-305.
[22] Zankel, K.L. (1971) Biochim. Biophys. Acta 245, 373-385.
[23] Velthuys, B.R. and Amesz, J. (1975) Biochim. Biophys. Acta. 376, 162-168.
[24] Bouges-Bouquet, B. (1975) in: Proc. 3rd Int. Congr. Photosynth. (Avron, M. ed). pp. 579-588, Elsevier, Amsterdam, New York.
[25] Rutherford, A.W., Gonvindjee and Inoue, Y. (1983) in: Proc. 6th Int. Congr. Photosynth., Brussels (Sybesma, C. ed.) Nihoff Junk, The Hague, in press.
[26] Rutherford A.W., Govindjee and Inoue, Y. (1983) Proc. Natl. Acad. Sci. USA, submitted.