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THERMOLUMINESCENCE AS A PROBE OF PHOTOSYSTEM II PHOTOCHEMISTRY

THE ORIGIN OF THE FLASH-INDUCED GLOW PEAKS

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A single flash given at −15°C to chloroplasts results in charge separation in Photosystem II to form a stable state which, upon warming, recombines giving rise to luminescence. This recombination occurs at 25°C in untreated chloroplasts but is shifted to 0°C in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea or weak concentrations of a reducing agent. The luminescence at 0°C is attributed to recombination of the $S_2Q_A^-$ state while that at 25°C is attributed to recombination of $S_2Q_AQ_B^-$ (and $S_3Q_AQ_B^-$ upon further flash illumination). The identification of the thermoluminescence at 25°C is based upon the following experimental evidence: (1) illumination of chloroplasts in the presence of methyl viologen with 710 nm light before and after flash illumination has no effect on the extent or temperature of the thermoluminescence. This is taken as evidence that the plastoquinone pool is not involved in the recombination reaction. (2) Calculations of the extent of thermoluminescence expected after a number of flashes, assuming that $S_2Q_AQ_B^-$ and $S_3Q_AQ_B^-$ are the thermoluminescent reactants, give a good fit to the experimental results. (3) The effect of continuous illumination at 77 K (i.e., donation from cytochrome b-559 to Q_A and thence to Q_B or Q_B^-) results in predictable changes in the extent of flash-induced thermoluminescence.

Introduction

When the PS II reaction centre is excited by light, a special chlorophyll II species, P-680 [1], becomes excited to its first excited singlet state, P-680*. P-680* donates an electron to a nearby pheophytin molecule, Ph [2–8], possibly via another intermediate carrier [9], to form the radical pair $P\text{-}680^+Ph^-$. Under normal circumstances, the charges on $P\text{-}680^+Ph^-$ are rapidly translocated away to secondary donors and acceptors. The primary quinone acceptor, Q_A [10], undergoes reduction shortly followed by oxidation of $P\text{-}680^+$ by a donor, D. The $D^+P\text{-}680PhQ_A^-$ state is formed approx. 30 ns after a flash [11]. The positive charge on this state is further translocated to the water-oxidizing enzyme, charging the S states [12,13], while Q_B accumulates two electrons before donating electrons, two at a time, to the plastoquinone pool, PQ [14,15].

If Q_A is reduced, chemically or photochemically, illumination still results in the formation of the $P\text{-}680^+Ph^-$ radical pair (Refs. 2–8, see also Refs. 17–19). At low temperature this radical pair recombines via a triplet state of P-680 [7]. At higher temperatures $P\text{-}680^+Ph^-$ can back-react,

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Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS, photosystem; Q_A, primary quinone acceptor; Q_B, secondary quinone acceptor; Tricine, *N*-tris(hydroxymethyl)methylglycine; PQ, plastoquinone; Chl, chlorophyll.

forming P-680* which returns to the ground state, presumably by transfering excitation energy to antenna chlorophyll, which loses the energy by fluorescence [20]. It is this recombination fluorescence which is thought to give rise to the phenomena of variable fluorescence [20] and fast delayed light emission [18] when Q_A is reduced.

It seems likely that the same recombination fluorescence is responsible for slower components of delayed light emission (see Ref. 21 for a recent review) and for thermoluminescence. In both of these phenomena, illumination results in the formation of states more stable than $P\text{-}680^+ Ph^-$ (i.e., $D^+ P\text{-}680Ph^-$ [18,22], $S^+ DP\text{-}680PhQ_A^-$ (see Refs. 21, 23 and 24 for reviews), etc.). As a result, more thermal activation energy is required to drive the charge pair back to the $P\text{-}680^+ Ph^-$ state and thence to give rise to recombination fluorescence (hence the delay in the delayed light emission and the thermo in the thermoluminescence).

The different states formed by illumination of PS II under variable conditions can be monitored by the kinetics of delayed light emission, i.e., more stable charge pairs recombining more slowly. Similarly, in thermoluminescence the temperature at which luminescence occurs is an indication of the energy required to get the charge pairs to recombine; i.e., more stable charge pairs recombine at higher tempertares. Unlike delayed light emission, however, the use of thermoluminescence as a probe of PS II photochemistry has been limited, since the whereabouts of the charges responsible for the thermoluminescence bands are largely unknown.

In this work we set out to identify the states responsible for the recombination reaction that results in the flash inducible thermoluminescence bands. Pevious work has indicated that the positive charge is located on the S_2 and S_3 states (Ref. 25, see also Ref. 26), although other workers [27] believe that only the S_3 state is involved. The origin of the negative charge is less clear. Inoue and co-workers [28,29] have considered that the energy required to return the negative charge to the reaction centre is small relative to that needed to drive the positive charge from the donor side back to the reaction centre and therefore that the whereabouts of the negative charge will not contribute to the thermoluminescence. On the other hand, Demeter et al. [30] have suggested that the negative charge comes from the plastoquinone pool (see also Ref. 26). However, much of the phenomenology of thermoluminescence is complex and contradictory (reviewed in Ref. 29). The complexity is not only due to that inherent in PS II (i.e., multiple donors and acceptors, each with a different temperature dependence; reviewed in Ref. 31) but also due to the type of experiments performed (i.e., illumination with continuous light either at temperatures where the electron-transport reactions are not well defined by other techniques and/or while-varying the temperature) [29]. In this work, experiments have been carried out using flash illumination and continuous illumination at 77 K. From the results the states responsible for the flash-induced thermoluminescence bands have been identified.

Materials and Methods

Spinach chloroplasts were made fresh daily from market spinach by stanard methods [32]. Chloroplasts were resuspended at high concentration (8 mg Chl/ml) in 15 mM (or 50 mM in dithionite experiments) Tricine (pH 7.8), 5 mM $MgCl_2$, 10 mM NaCl, 100 mM sorbitol buffer and stored on ice in the dark for over 2 h before use.

Thermoluminescence measurements were carried out on diluted chlorplasts suspensions (0.7 or 2.0 mM Chl) as described previously except that the sample was contained in a cuvette arrangement rather than on filter paper as used previously [33]. This allowed anaerobic experiments to be performed and provided more repeatable results. Since the rate of heating is proportional to the thermolyminescence peak height, in experiments where low concentrations of chlorophyll were used (i.e., under conditions where it was necessary to have a saturating flash) a fast heating rate was used (1°C/s). However, this rate of heating results in a distortion of the peak position, shifting it to higher temperatures. In experiments where a more accurate peak position was required, a slower heating rate (0.3°C/s) and a higher concentration of chlorophyll were used. Under these conditions the flash was not necessarily saturating.

Illumination with far-red light was carried out as follows: chloroplasts in the presence of 100 μM methyl viologen were illuminated at 20°C with 710

nm light (±2.5 nm, intensity 277 $\mu W \cdot cm^{-2}$) for 5 s, dark adapted for 2 min, given one or two flashes and reilluminated for a further 5 s with 710 nm light before being frozen to liquid nitrogen temperature in the dark. The time between flash illumination and freezing was 20 s. 5 s illumination with 710 nm light was the maximum illumination time that was not actinic to PS II under the conditions of the experiment (monitored as the presence of thermoluminescence at 38°C).

Small amounts of sodium dithionite (200 mM) in 100 mM glycine/KOH, pH 10 (final pH 8.0), were added to chloroplast suspensions in an anaerobic vessel under an atmosphere of O_2-free nitrogen. Equilibration for several minutes was allowed after an addition of sodium dithionite before chloroplasts were transferred anaerobically to the thermoluminescence cuvette. The sample was kept under O_2-free N_2 in the cuvette until frozen.

Results

The involvement of Q_A^-

When dark-adapted chloroplasts were illuminated with a single flash at temperatures between 25 and −20°C and cooled quickly to liquid nitrogen temperature, a single thermoluminescence band was observed upon warming. This band has a maximum at 25°C (38°C when warmed at the faster rate) and it has been designated the B band in previous reports [25]. This band is thought to result from the recombination of a positive charge located on the water-oxidation enzyme (in this case the S_2 state) with a negative charge located on an acceptor side-component.

As mentioned earlier, one theory suggests that the whereabouts of the electron is unimportant, since its back-reaction is thought to require small thermal activation relative to that required to drive the positive charge back to P-680 [25,28,29]. Alternatively, other workers have suggested that the plastoquinone pool may be the source of the electron [30].

In order to test these possibilities, experiments have been carried out where electron transport on the acceptor side was blocked by successive chemical reduction of the acceptors (Fig. 1) or by addition of DCMU (Fig. 2) prior to flash illumination.

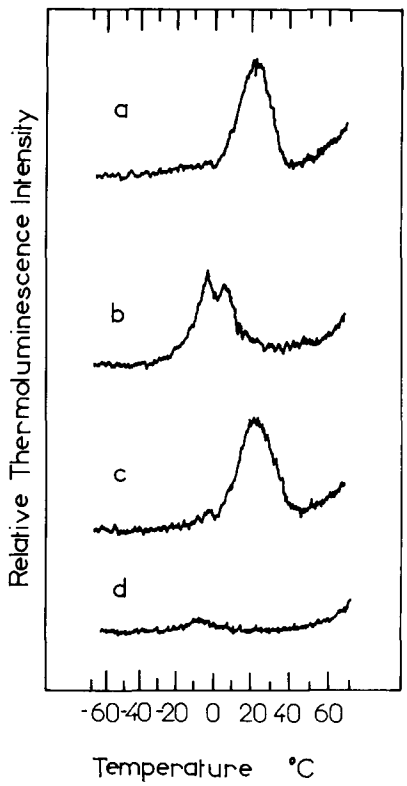

Fig. 1. The effect of lowering the ambient redox potential upon the thermoluminescence band position. Dark-adapted chloroplasts were illuminated with one 5 μs flash (white light, 4.5 J) at −15°C and frozen quickly to 77K. Thermoluminescence was recorded upon warming of the sample at a rate of 0.3°C/s. (a) No additions; (b) in the presence of 2 mM sodium dithionite, anaerobic; (c) in the presence of 2 mM sodium dithionite but having been aerated; (d) in the presence of 40 mM sodium dithionite, anaerobic.

Fig. 1 shows the effect of lowering the ambient potential with small additions of sodium dithionite. The flash-induced thermoluminescence band at 25°C was lost upon the addition of a small amount of dithionite and at the same time a band around 0°C was generated (Fig. 1b). The double peak at 0°C was a result of the solid-liquid transition of water. In the presence of 50% glycerol, a single peak is observed in this region (not shown) but the increased viscosity made anaerobic manipulations of the sample more difficult so aqueous samples were routinely used. When air was bubbled through the partially reduced sample, the 0°C band was lost and the 25°C band was regenerated

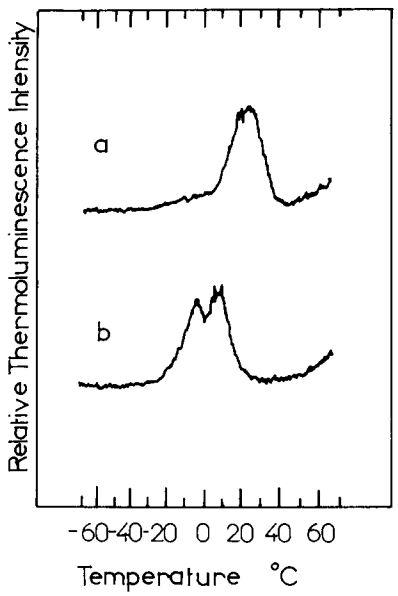

Fig. 2. The effect of 2 μM DCMU upon the thermoluminescence band position. Dark-adapted chloroplasts were given a single flash at −15°C and frozen quickly to 77 K. Thermoluminescence was recorded at a warming rate of 0.3°C/s. (a) No addition, (b) in the presence of 2 μM DCMU.

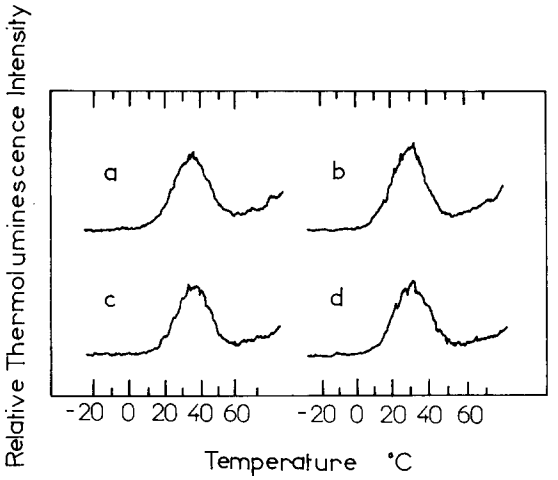

Fig. 3. The effect of far-red light upon the extent of the thermoluminescence band. Dark-adapted chloroplasts in the presence of 100 μM methyl viologen were illuminated by one or two flashes at 20°C and were frozen to liquid N_2 temperature after 20 s. Samples were treated as described in Materials and Methods. (a) One flash; (b) one flash with 5 s illumination with 710 nm light before and after flash illumination; (c) two flashes; (d) two flashes with 5 s of illumination with 710 nm light before and after flash illumination.

(Fig. 1c). Further additions of dithionite resulted in the total loss of any flash-inducible thermoluminescence bands (Fig. 1d). The addition of 2 μM DCMU prior to flash illumination mimicked the lowering of the redox potential in that the 25°C band was replaced by one at around 0°C (Fig. 2).

The 0°C band is interpreted as being due to recombination of the $S_2Q_A^-$ state, since DCMU blocks electron transport between Q_A and Q_B. The lowering of the redox potential reduces Q_B, also blocking electron transport at this point. The loss of the 0°C band with higher concentrations of dithionite probably reflects the chemical reduction of Q_A but the possibility that dithionite reduces S_2 back to S_1 has not been ruled out. It is concluded that the whereabouts of the electron on the acceptor side has a marked effect upon the temperature at which the recombination reaction occurs. The thermoluminescence band at 25°C must arise from recombination of a pair of charges where the negative charge is further from the reaction centre than Q_A.

The involvement of the plastoquinone pool

From the literature it would be predicted that dark-adapted chloroplasts have 70% of the centres were Q_B is present and 30% where Q_B^- is present [16,34,35] *. Thus, every flash generates both Q_B^- and Q_B^{2-} (and hence PQH_2). To test the involvement of PQH_2 in the reaction generating the thermoluminescence, experiments were carried out in which chloroplasts, in the presence of methyl viologen, were illuminated with far-red light before and after flash excitation. This treatment excites PS I causing it to donate electrons, via methyl viologen, to oxygen and at the same time to drain electrons out of the PQ pool. If electrons in the PQ pool can back-react to give rise to the thermoluminescence band, then the photochemical draining of electrons from the PQ pool should lead to a loss of thermoluminescence intensity. Fig. 3 shows that no such effect could be observed after one or two flashes.

* For clarity the redox states of Q_B are represented as $Q_B/Q_B^-/Q_B^{2-}$ without taking the protonation states of the species into account.

It is concluded that PQH_2 and therefore Q_B^{2-} are not the source of electrons in the thermoluminescence-generating back-reaction.

The involvement of Q_B^-

By a process of elimination, it seems likely that the thermoluminescence arises from recombination of the electron on Q_B^- with the positive charge on the S states of the water-oxidation enzyme. If this is the case, using the literature values for the distribution of Q_B and Q_B^- (70 and 30%, respectively) [16, 34, 35] and of S_0 and S_1 (30 and 70%, respectively) (reviewed in Ref. 36) in dark-adapted chloroplasts it should be possible to predict the intensity of thermoluminescence after flash illumination. Table I shows the predicted states present in the dark and after flash illumination and the percentage of centres in those states. The distribution of the four states, S_0Q_B, $S_0Q_B^-$, $S_1Q_B^-$ and S_1Q_B in the dark was obtained using the following assumptions: firstly, in the light an equal number of centres exist in the state Q_B and Q_B^-, and an equal number of centres exist in each of the stable states S_0, S_1, S_2, S_3; and secondly, Q_B^- is reoxidized by a back-reaction but only in centres with a back-reactable S state (S_2 or S_3). It has been assumed that charge recombination giving rise to thermoluminescence occurs only from the $S_2Q_B^-$ and $S_3Q_B^-$ states. The predicted flash pattern shows a maximum upon the first flash and a decrease on the second, third and fourth flashes. This pattern is different from that reported for this thermoluminescence band previously [25,27] where it was shown that a maximum intensity was present on the second and sixth flashes. The flash pattern on the first two flashes was reinvestigated and it was found that the relative extents of thermoluminescence on the first two flashes varied markedly depending upon pretreatment (Fig. 4, solid curves). In chloroplasts dark adapted for 2 h or more, thermoluminescence on the first flash was indeed as big or larger than that on the second flash. From eight different experiments the results of five experiments showed thermoluminescence to be larger on the first flash than on the second (i.e., Fig. 4a), and the results of three experiments showed the first flash thermoluminescence to be the same size or slightly smaller than that on the second flash. Preillumination for 1 min at 25°C followed by 5 min dark adaptation resulted in the intensity of thermoluminescence after the first flash being significantly smaller than that observed after the second flash (Fig. 4b). This pattern was observed even after 1 h dark adaptation at 4°C (not shown). This pattern is similar to that previously reported [25,27,28] and indeed in two of those reports chloroplasts were preilluminated for 1 min, before dark adaptation and subsequent flash illumination was carried out, in order to randomize the S states [25,28]. This then explains the dis-

TABLE I

PREDICTED STATES PRESENT IN DARK-ADAPTED AND FLASH-ILLUMINATED CHLOROPLASTS

The table has been compiled combining the S state model of O_2 evolution, [12,13,36] with the two-electron gating action of Q_B [14,15]. It has been assumed that Q_B^- is stable in the dark only in centres which are in the S_0 or S_1 state. $S_2Q_B^-$ and $S_3Q_B^-$ are assumed to be the thermoluminescent charge pairs and are denoted by an asterisk. TL, thermoluminescence.

% of state in dark	Dark	Number of flashes			
		1	2	3	4
62.5	S_1Q_B	$*S_2Q_B^-$	S_3Q_B	$S_0Q_B^-$	S_1Q_B
12.5	$S_1Q_B^-$	S_2Q_B	$*S_3Q_B^-$	S_0Q_B	$S_1Q_B^-$
12.5	S_0Q_B	$S_1Q_B^-$	S_2Q_B	$*S_3Q_B^-$	S_0Q_B
12.5	$S_0Q_B^-$	S_1Q_B	$*S_2Q_B^-$	S_3Q_B	$S_0Q_B^-$
% of centres that can give TL (ideal)	0	62.5	25.0	12.5	0
% of centres that can give TL (12% misses)	0	55.0	32.6	17.0	7.4

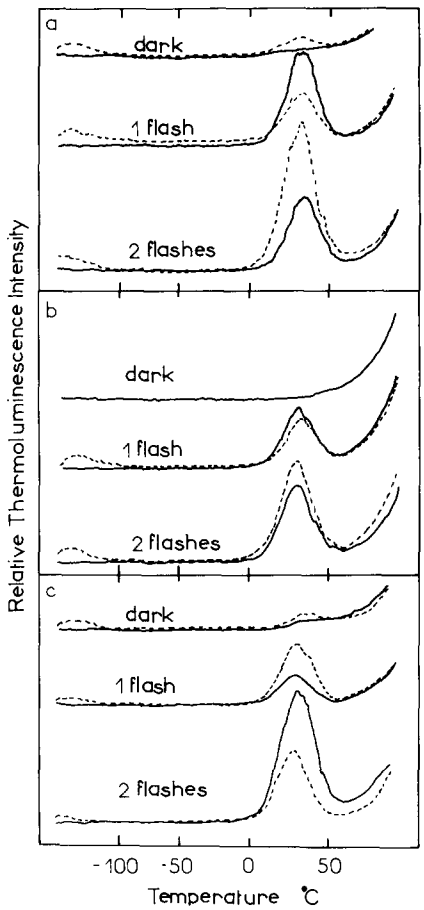

Fig. 4. The extent of the thermoluminescence induced by one or two flashes at 20°C and the effect of various pretreatments. (a) Chloroplasts dark-adapted for over 2 h; (b) illuminated at 20°C with red light, followed by dark adaptation for 5 min; (c) preilluminated at 77 K for 2 min with red light, warmed to 20°C. Samples were frozen to liquid N_2 temperature after dark adaptation or after one or two flashes as marked in the figure. The dashed curves are thermoluminescence measured in samples identical to those shown as solid curves except the chloroplasts received illumination at 77 K for 2 min just before the thermoluminescence was recorded. Illumination at 77 K was with red light (λ 640 nm, 12 mW·cm^{-2}) provided by a 300 W projector.

crepancy between these results and those reported earlier [25,28].

The effect of preillumination upon the flash pattern can be explained if such a treatment results in the formation of an increased proportion of stable Q_B^-. Such an increase would occur if the PQ pool were completely reduced and/or if an increase in the amount of the S_0 or S_1 states relative to the S_2 and S_3 states had occurred during strong continuous illumination. It is of note that a change in the Q_B/Q_B^- ratio from 30/70 to 50/50 could be enough to give a thermoluminescence maximum on the second flash.

The presence of the larger thermoluminescence band after the first flash in dark-adapted chloroplasts fits well with the assignment of the recombination pair as $S_2Q_B^-$ and $S_3Q_B^-$. As a further test of this model, experiments were devised which allow the redox state of Q_B to be manipulated without blocking electron transport and without significantly altering the S states.

The effect of illuminating chloroplasts at 77 K is well characterized [38–40]. Electron transport is blocked between Q_A and Q_B at temperatures below −50°C [41], thus only one electron is extracted from the donor side. The ultimate souce of that electron is largely cytochrome *b*-559 [37–40]. Warming to temperatures above −30°C of chloroplasts that have been illuminated at 77 K results in electron transport from Q_A^- to Q_B [42]. When thermoluminescence measurements were made of dark-adapted chloroplasts that were illuminated for 2 min at 77 K, no thermoluminescence band attributable to cytochrome *b*-559$^+$ Q_B^- recombination could be observed (Fig. 4a, top broken curve). This is taken as an indication that the state is very stable or that cytochrome *b*-559 is rereduced by an alternative pathway. A small fraction (approx. 7%) of the thermoluminescence band at 25°C is formed by illumination at 77 K (Fig. 4a); this reflects a small amount of S_2 formation that can occur under the conditions where cytochrome *b*-559 can better compete as a donor. Thus, after illumination at 77 K and warming to 20°C a single electron is delivered to the acceptor complex while the S states are virtually unaffected. Table II shows the states predicted before and after flash illumination if 77 K illumination is carried out before or after flash illumination. It was predicted that this treatment should result in a small thermoluminescence band after the first flash and a very large thermoluminescence band after the second. Fig. 4 (broken curves and Fig. 4c) shows the effect of 77 K illumination observed experimentally. The predicted effect was very marked in dark-adapted chloroplasts (Fig. 4a) but much less marked in

TABLE II

PREDICTED STATES PRESENT AFTER ILLUMINATION AT 77 K OF DARK-ADAPTED CHLOROPLASTS AND THE EFFECT OF FLASH ILLUMINATION

The table was compiled as in Table I. Oxidized cytochrome *b*-559 may also be present but it has been omitted from the scheme, since it is not involved in a thermoluminescence back-reaction. TL, thermoluminescence.

% of state before flash illumination	States present above 0°C				
	Dark	Number of flashes			
		1	2	3	4
62.5	$S_1Q_B^-$	S_2Q_B	$^*S_3Q_B^-$	S_0Q_B	$S_1Q_B^-$
12.5	S_1Q_B	$^*S_2Q_B^-$	S_3Q_B	$S_0Q_B^-$	S_1Q_B
12.5	$S_0Q_B^-$	S_1Q_B	$^*S_2Q_B^-$	S_3Q_B	$S_0Q_B^-$
12.5	S_0Q_B	$S_1Q_B^-$	S_2Q_B	$^*S_3Q_B^-$	S_0Q_B
% of centres that can give TL (ideal)	0	12.5	75	12.5	0
% of centres that can give TL (12% misses)	0	11.0	60.7	29.9	9.2

preilluminated chloroplasts (Fig. 4b). The close correlation with the predicted results is strong evidence that the thermoluminescence band is generated only from the $S_2Q_B^-$ and $S_2Q_B^-$ states.

Fig. 5a shows the effect of multiple flashes upon the extent of the thermoluminescence band, and the effect of 77 K illumination given after flash excitation. This is compared to the predicted values which were calculated using a 12% miss parameter (Fig. 5b) and a good correlation is obtained.

When this experiment was repeated with an additional preillumination at 77 K carried out before flash excitation, the results shown in Fig. 5c were obtained. It can be seen that the oscillation patterns are almost an inversion of those seen in Fig. 5a.

Some mismatch of experimental results with those predicted is to be expected for the following reasons: firstly, the assumptions required to determine the ratios of the four dark states (Table I) resulted in S_1/S_0 and Q_B/Q_B^- ratios of 75 : 25 rather than 70 : 30 as reported in the literature; secondly, donors other than S states can function at ambient temperatures (i.e., Signal II_{slow} (see, for example, Ref. 43)); and thirdly, in some centres, illumination at 77 K results in S state oxidation when the temperature is raised (Fig. 4a, broken

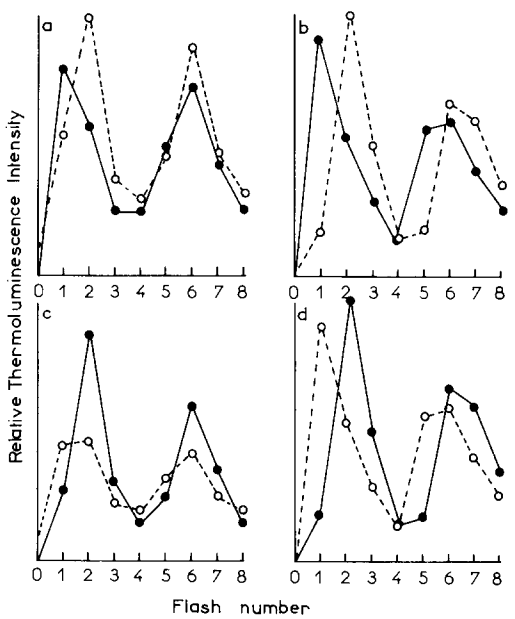

Fig. 5. Oscillations of the flash-induced thermoluminescence band and the effect of illumination at 77 K. Experiments were carried out as described in Fig. 4 (a) Dark-adapted chloroplasts; (b) calculated oscillations for the first eight flashes (calculated as in Tables I and II including a 12% miss parameter); (c) chloroplasts preilluminated at 77 K for 2 min and warmed to 20°C before flash illumination. (d) calculated oscillations for the first eight flashes in chloroplasts preilluminated at 77 K (calculated as for b). (●) No further illumination after the flashes; (○) a further illumination at 77 K was received after the flashes.

curve). The latter factor at least partially explains why the data after 77 K illumination fit the theoretical data less well than those obtained prior to this treatment.

The good fit of the experimental to the theoretical results if further strong evidence that the model and assumptions outlined above are correct.

Concluding Remarks

The identification of the flash-induced thermoluminescence bands at 25°C as originating from recombination of the $S_2Q_B^-$ and $S_3Q_B^-$ states and of the 0°C band as S_2Q_A (and a $S_3Q_A^-$ band would be predicted in the same place if DCMU is added after the first of two flashes) allows the use of thermoluminescence as a powerful technique to probe the photochemistry of PS II.

As well as being a probe of the S_2 and S_3 states of the oxygen-evolving enzyme, the extent of the 25°C band and the 0°C band can be used as a measure of the redox state of Q_B and also, perhaps, Q_A.

The different thermoluminescence peaks attributed to $S_2Q_A^-$ and $S_2Q_B^-$ reflect the different activation energies for the recombination reaction in each of these states to take place. This energy difference may, in part, reflect the different midpoint potentials between the Q_A/Q_A^- and Q_B/Q_B^- redox couples. Similarly, the slight difference in the peak position of the $S_2Q_A^-$ band depending on whether electron transport is blocked with DCMU (Fig. 2b) or by reduction of Q_B (Fig. 1b) may reflect a slight raising of the midpoint potential of Q_A by DCMU. An equally plausible explanation, however, is that the presence of a negative charge (or charges) on Q_B^{2-} may destabilize the electron on Q_A^-.

The discovery that $S_2Q_B^-$ and $S_2Q_B^-$ states are responsible for flash-induced thermoluminescence in untreated chloroplasts is not unexpected. The S_2 and S_3 states are the two S states that are able to back-react, while Q_B^- is the stable location of the electron while it is still within the reaction centre. Once the electron leaves the reaction centre (along with a second electron), by reduction of a pool plastoquinone molecule, the recombination reaction becomes much less favourable, either for energetic reasons (i.e., protonation and/or diffusion effects, or a large midpoint potential difference between Q_B and PQ) or because cyclic electron flow from the pool to the donor side becomes more favourable than the back-reaction.

It seems likely that the flash-induced thermoluminescence bands identified here can be correlated to particular kinetic phases of delayed light emission. Oscillations of luminescence corresponding to $S_2Q_B^-$ and $S_3Q_B^-$ recombination have not been observed in the milliseconds-seconds time scale at ambient temperatures after a series of flashes [44]. However, the addition of DCMU effectively results in back-transfer of electrons from Q_B^- to Q_A [15] forming the less stable $S_2Q_A^-$ and $S_3Q_A^-$ states which then recombine at ambient temperature giving oscillations of luminescence observed on a seconds time scale which are similar to the oscillations reported here for thermoluminescence [44].

Since at ambient temperature the recombination of $S_2Q_A^-$ (0°C thermoluminescence band) results is delayed light on a seconds time scale, the recombination of $S_2Q_B^-$ and $S_3Q_B^-$ (25°C thermoluminescence band) would be expected to occur on a much longer time scale. It is of interest to carry out light measurements over longer time scales or at higher temperatures (perhaps 35°C in thermophilic species) to determine if delayed light emission and thermoluminescence are indeed different manifestations of the same phenomenon.

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