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THERMOLUMINESCENCE AS A PROBE OF PHOTOSYSTEM II

THE REDOX AND PROTONATION STATES OF THE SECONDARY ACCEPTOR QUINONE AND THE O_2-EVOLVING ENZYME

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The thermoluminescence band observed in chloroplasts after flash excitation at ambient temperatures has recently been identified as being due to recombination of the electron on the semiquinone form of the secondary plastoquinone acceptor, Q_B, with positive charges on the oxygen-evolving enzyme, S_2 and S_3 (Rutherford, A.W., Crofts, A.R. and Inoue, Y. (1982) Biochim. Biophys. Acta 682, 457–465). Further investigation of this thermoluminescence confirms this assignment and provides information on the function of PS II. The following data are reported: (1) Washing of chloroplasts with ferricyanide lowers the concentration of Q_B^- in the dark and predictable changes in the extent of the thermoluminescence band are observed. (2) The thermoluminescence intensity arising from $S_2Q_B^-$ is approximately one half of that arising from $S_3Q_B^-$. (3) Preflash treatment followed by dark adaptation results in changes in the intensity of the thermoluminescence band recorded after a series of flashes. These changes can be explained according to the above assignments for the origin of the thermoluminescence and if Q_B^- provides an important source of deactivating electrons for the S states. Computer simulations of the preflash data are reported using the above assumptions. Previously unexplained data already in the literature (Läufer, A. and Inoue, Y. (1980) Photobiochem. Photobiophys. 1, 339–346) can be satisfactorily explained and are simulated using the above assumptions. (4) Lowering the pH to pH 5.5 results in a shift of the $S_2Q_B^-$ thermoluminescence band to higher temperatures while that arising from $S_3Q_B^-$ does not shift. This effect is interpreted as indicating that Q_B^- is protonated and the S_2 to S_3 reaction involves deprotonation while the S_1 to S_2 reaction does not.

Introduction

The flash-induced thermoluminescence observed in chloroplasts has recently been identified. An emission peak at 25–30°C was attributed to $S_2Q_B^-$ and $S_3Q_B^-$ recombination [1] while, under conditions where electron transport is blocked after Q_A, the band at 0–10°C was attributed to $S_2Q_A^-$ [1] and $S_3Q_A^-$ [1,2] recombination, where S_2 and S_3 are charge-storage states of the O_2-evolving enzyme [3,4] and Q_A and Q_B are the primary [5] and secondary [6,7] plastoquinone acceptors of Photosystem II (PS II). These recombination reactions are also manifest as slow phases of delayed luminescence; $S_2Q_B^-$ and $S_3Q_B^-$ recombination luminescence decaying in the tens of seconds to

Abbreviations: Chl, chlorophyll; PS, photosystem; Mes, 4-morpholineethanesulphonic acid; Tricine; *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

minutes time scale [8] while $S_2Q_A^-$ and $S_3Q_A^-$ recombination luminescence decays within a few seconds [8,9]. The common origin of delayed and thermoluminescence is in agreement with the phenomenological correlation observed earlier [10].

The recent breakthroughs in the understanding of thermoluminescence have already been applied to the study of PS II in chloroplasts [1,2,8,11,12] and in leaves [13,14]. The results are helpful in understanding much of the phenomenological data already in the literature (reviewed in Ref. 15) and demonstrate that thermoluminescence can be easily and usefully applied to a number of aspects of PS II research. In this work, the characterization of the flash-induced thermoluminescence has been continued. The results demonstrate the validity of our previous assignments [1] and provide some information on the mechanism of electron-transfer reactions in PS II.

Materials and Methods

Spinach chloroplasts were isolated daily from market spinach using standard techniques [16]. Chloroplasts were resuspended at high concentrations (3–5 mg Chl/ml) in 15 mM Tricine (pH 7.8)/5 mM $MgCl_2$/10 mM NaCl/100 mM sorbitol buffer and stored on ice in darkness for 2 h before use. Thermoluminescence experiments were carried out as described previously [1,17].

Chlorophyll concentration was 0.8 mg Chl/ml in all experiments. The heating rate was approx. 0.35°C/s. Flash excitation was provided using a xenon flash lamp (5 μs, 4.5 J, white light) while continuous illumination was provided by a 300 W projector ($\geq$ 640 nm, red light, 12 mW · cm^{-2}). Ferricyanide washing was done according to the method of Robinson and Crofts [18] with slight modifications. Chloroplasts were diluted to 0.15 mg Chl/ml in buffer (see above) in the presence of 150 μM potassium ferricyanide. The mixture was incubated at 4°C for 1 h, pelleted (10 min, 2000 × g) and resuspended to 8 mg/ml in buffer. After the addition of ferricyanide, all steps were done in darkness. Chloroplasts treated with low pH were resuspended in buffer as above but in which the Tricine was replaced with 50 mM Mes (pH 5.5). Computer simulations were carried out using a Hewlett-Packard microcomputer model 85F.

Results and Discussion

The chemical oxidation of Q_B^-

In dark-adapted chloroplasts, Q_B^- is normally present in about 30% of the centres [19]. Since the thermoluminescence band at about 30°C is thought to arise from $S_2Q_B^-$ and $S_3Q_B^-$ recombination after the first and second flashes, respectively [1], the concentration of these states (and thus the intensity of the thermoluminescence) depends on the initial ratio of Q_B to Q_B^- in the dark. Previous

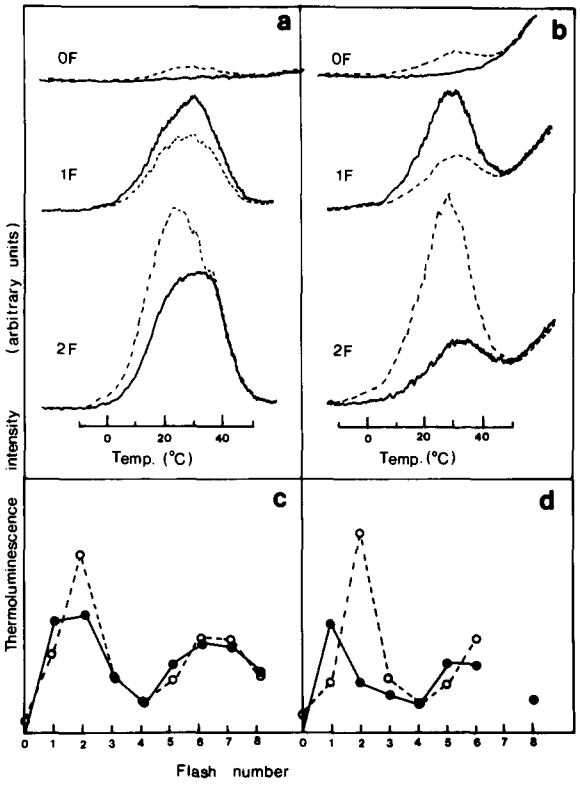


Fig. 1. The effect of washing chloroplasts in 150 μM potassium ferricyanide. The washing procedure was carried out as described in detail in Materials and Methods. (a) Thermoluminescence detected in untreated control samples after none, one and two flashes (F) (solid lines). (c) Oscillation pattern of thermoluminescence in untreated chloroplasts. (b) Thermoluminescence detected in ferricyanide-washed chloroplasts after none, one and two flashes (solid lines). (d) Oscillation patterns of thermoluminescence in ferricyanide-washed chloroplasts. Broken lines are thermoluminescence recorded after identical pretreatment to the solid lines except for an extra period (2 min) of continuous illumination given at 77 K just before thermoluminescence was recorded.

attempts to oxidize Q_B^- by treatment with oxidizing agents proved fruitless due to the loss of thermoluminescence resulting from chemical oxidation of photoinduced Q_B^-. However, when a ferricyanide wash was given, followed by sedimentation of the chloroplasts and resuspension in non-oxidizing buffer (as per Ref. 18), thermoluminescence could once more be observed. Fig. 1b, d shows thermoluminescence recorded after a series of flashes in chloroplasts treated in this way (solid lines). By comparison with untreated control chloroplasts (solid lines Fig. 1a, c) the ferricyanide-washed chloroplasts show an overall decrease in thermoluminescence amplitude (probably due to loss of Q_B^- to remaining ferricyanide) and also an increased intensity of first-flash thermoluminescence relative to that on the second flash. The latter effect would be expected if the concentration of Q_B^- in the dark had been lowered by the ferricyanide wash. The effect of a period of illumination at 77 K given after flash excitation is also shown in Fig. 1 (broken lines). This procedure, which effectively inverts the Q_B/Q_B^- ratio (see Ref. 1 and Scheme I), has a much more marked effect on the ferricyanide-washed chloroplasts. This again reflects a lower Q_B^- concentration in the dark. These results strongly support the identification of $S_2Q_B^-$ recombination as the source of the flash-induced thermoluminescence band at around 30°C.

It is of note in Fig. 1 that ferricyanide induces an increase in thermoluminescence at high temperatures even in the dark. This results in the rising baselines present in Fig. 1b. This phenomenon has been observed previously and may be associated with PS I [20].

The relative thermoluminescence intensity from $S_2Q_B^-$ and $S_3Q_B^-$

In previous work it was considered that the yield of thermoluminescence from $S_2Q_B^-$ was equal to that from $S_3Q_B^-$ recombination [1]. This assumption allowed calculations to be made which provided good qualitative fits to the experimental phenomena [1]. However, closer examination of the data shows a tendency for $S_3Q_B^-$ recombination to result in higher thermoluminescence amplitudes than would be predicted (see Fig. 4 of Ref. 1 and Fig. 2). In order to estimate the relative amplitudes of thermoluminescence originating from $S_2Q_B^-$ and $S_3Q_B^-$, rationale which relies on two experimental tricks was used. Firstly, the S states, which exist either as S_0 or S_1 in a ratio of 30 : 70 in dark-adapted chloroplasts, can be synchronized in the S_1 state by giving a single flash followed by a period of dark adaptation [3] *. Secondly, since PS II centres in the dark can have either Q_B or Q_B^- present, both kinds of centre need to be measured. The thermoluminescence observed after a single flash only reflects those centres that have Q_B present in the dark. However, a period of illumination given at 77 K after the room temperature flash causes an inversion of the Q_B/Q_B^- ratio (Scheme I). The thermoluminescence measured after one flash plus illumination at 77 K reflects those centres that were in the state Q_B^- in the dark. Thus, the amplitude of the luminescence recorded after one flash added to that recorded after one flash plus 77 K illumination should reflect the total amount of luminescence from $S_2Q_B^-$ recombination in 100% of the centres. Similarly, the thermoluminescence recorded after two flashes added to that after two flashes plus 77 K illumination should equal the amount of luminescence from 100% of $S_3Q_B^-$. The results of such experiments are shown in Figs. 2 and 3. After one preflash, it can be seen that $S_3Q_B^-$ luminescence is about 1.7-times greater than $S_2Q_B^-$. In several other experiments, similar values were obtained. After two preflashes, which should also synchronize the S state in S_1, the $S_3Q_B^-$ luminescence was also estimated to be greater than $S_2Q_B^-$ but a factor closer to 1.3. Since more double hits and misses are involved in the two-preflash experiments, the results from the one-preflash experiment are taken as a closer estimation of the true value. These values are only approximate due to a

* Since this work was done, evidence has been presented for an almost 100% population of the S_1 state in dark-adapted chloroplasts [28]. However, due to the existence of a further donor component (inducing a fast S_2 and S_3 decay) the S_i state advancement is retarded by one redox equivalent in approx. 20% of the centres. Under our experimental conditions, this reaction almost certainly occurs to completion so that the assumption of a S_0/S_1 starting state of 30:70 phenomenologically leads to the correct results. Therefore, for the sake of simplicity, the more complex pattern described in Ref. 28 will not be considered explicitly here.

(a) Centres in the state S_1Q_B in the dark

$$S_1Q_AQ_B \xrightarrow[25°C]{1\ \text{flash}} S_2Q_AQ_B^- \underset{(TL)}{} \xrightarrow[\text{cyt } b\text{-}559^+]{h\nu,\ 77\ K;\ \text{cyt } b\text{-}559} S_2Q_A^-Q_B^- \xrightarrow{\text{warm}} S_2Q_AQ_B^{--} \xrightarrow[PQH_2]{PQ} S_2Q_AQ_B\ (\text{no TL})$$

(b) Centres in the state $S_1Q_B^-$ in the dark

$$S_1Q_AQ_B^- \xrightarrow[25°C]{1\ \text{flash}} S_2Q_AQ_B^{--} \xrightarrow[PQH_2]{PQ} S_2Q_AQ_B\ (\text{no TL}) \xrightarrow[\text{cyt } b\text{-}559^+]{h\nu,\ 77\ K;\ \text{cyt } b\text{-}559} S_2Q_A^-Q_B \xrightarrow{\text{warm}} S_2Q_AQ_B^-\ (\text{TL})$$

Scheme I. Centres in the states S_0Q_B and $S_0Q_B^-$ are not considered in the scheme because they do not contribute to thermoluminescence on the first flash. A mixture of (a) S_1Q_B and (b) $S_1Q_B^-$ are normally present. Thermoluminescence (TL) measured after one flash only detects TL from (a). TL recorded after one flash + 77 K $h\nu$, only detects TL from (b). Protonation events are disregarded.

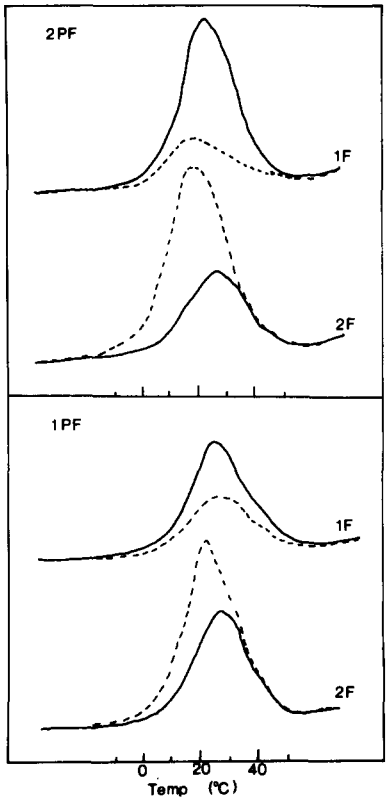


Fig. 2. The effect of one and two preflashes (PF) on flash-induced thermoluminescence with (broken line) and without (solid line) a further period of illumination at 77 K. Data after one or two flashes (F) are shown. A dark adaptation of 5 min at 25°C was allowed between preflashes and flashes.

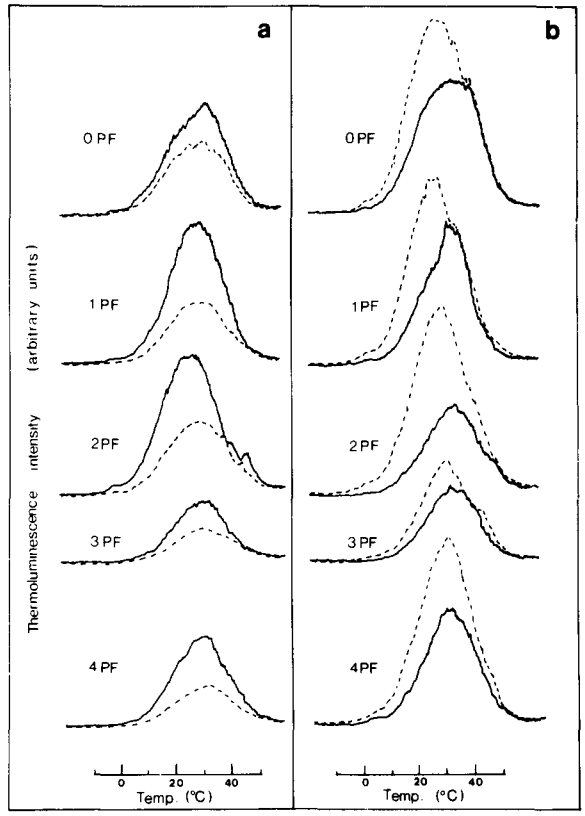


Fig. 3. The effect of one to four preflashes (PF) on flash-induced thermoluminescence. (a) Recorded after one flash and (b) after two flashes. The data were recorded on the same batch of chloroplasts. The effect of a further period of illumination at 77 K is also shown with broken lines.

number of factors (double hits, misses and S-state turnover at 77 K previously estimated at 7% for $S_1 \rightarrow S_2$ [1]) which interfere with the measurement.

It is of note that 77 K illumination technique shown by Scheme I enables us to separately estimate the initial concentrations of Q_B and Q_B^- in the dark. In Fig. 2, the solid line recorded after a single preflash and induced by one flash corresponds to the concentration of Q_B in the dark while the broken line corresponds to that of Q_B^- in the dark, so that the Q_B/Q_B^- ratio is estimated to be 2 : 1, which agrees well with the reported value of 70 : 30 (Ref. 19).

Preflash effects – S state synchronization and recombination deactivation

As explained in the previous section, one or two preflashes followed by a period of dark adaptation results in synchronization of the S states in S_1. Preflash effects measured by thermoluminescence should be affected by the synchronization in predictable ways. The results of preflash experiments are shown in Fig. 3. The thermoluminescence is markedly affected by the preflash treatment. After one and two preflashes, the synchronization of the S states in S_1 results in an increase in the concentration of S_2 produced by the first flash. This gives rise to an increase in thermoluminescence intensity on the first flash (Fig. 3a).

If the Q_B to Q_B^- ratio was unaffected by these preflashes, an increase in thermoluminescence intensity on the second flash (Fig. 3b) should be observed due to the synchronization effect and the larger S_3 luminescence yield. In fact, what is observed in Fig. 3b, is a decrease in intensity on the second flash after one and two preflashes. This can be explained if the preflashes not only effect the redox state of the S states but also of Q_B in the dark. The decrease in the intensity of the band after the second flash indicates a decrease in stable Q_B^- present in the dark. The more marked effect of the Q_B/Q_B^- ratio inversion induced by 77 K illumination (Fig. 3, broken lines) in samples which received one or two preflashes also indicates a decreased amount of stable Q_B^- prior to flash excitation. A decrease in stable Q_B^- induced by one or two preflashes would be predicted if the electron on Q_B^- was an important source of electrons for S-state deactivation.

The effect of three preflashes resulted in a marked decrease in thermoluminescence on both the first and second flashes (Fig. 3a, b). This is due to an increase in the S_0 concentration present after dark adaptation. The samples which received four preflashes should have returned to the original S_0/S_1 ratio of 30 : 70 (disregarding double hits and misses). Indeed the thermoluminescence in such samples was similar to that seen in samples which did not receive any preflash.

The effect on the oscillation pattern of flash-induced thermoluminescence is shown in full for two and four preflashes in Fig. 4. It can be seen that two preflashes result in an increase of thermoluminescence intensity after the first and fifth flashes relative to that on the second and sixth flashes. This again indicates that the number of centres having stable Q_B^- is lower after this treatment. The original oscillation pattern is restored

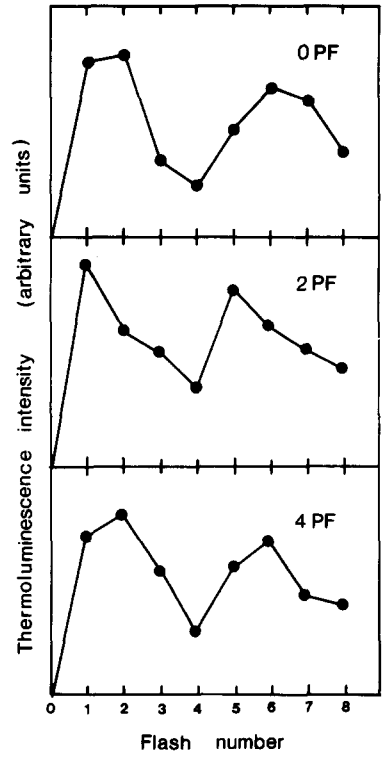


Fig. 4. The oscillation patterns of flash-induced thermoluminescence after a series of flashes and the effect of none, two and four preflashes (PF) given 5 min before the flash series.

by giving four preflashes. The effect of preflash experiments on the thermoluminescence oscillation pattern has already been reported [21] but, until now had remained unexplained. The data of Läufer and Inoue [21] differ from that reported here only in that continuous strong preillumination was given to the chloroplasts before the preflash treatment. This extra pretreatment results in an increase in the number of centres having Q_B^- stable in the dark [1] and, consequently, a flash pattern with marked maxima after two and six flashes in samples treated with none and four preflashes. The fact that treatment with one and two preflashes changes such a pattern to one with maxima on the first and fifth flashes can be interpreted as strong evidence that Q_B^- is an important source of deactivating electrons for the S states.

Computer simulations of the thermoluminescence expected after a series of flashes and the effect of preflash treatment are shown in Fig. 5. Fig. 5a is simulated assuming a Q_B/Q_B^- ratio of 70:30 and should be compared to the data reported in Fig. 4. Fig. 5b is simulated assuming a Q_B/Q_B^- ratio of 50:50 and should be compared to Fig. 1 in Ref. 21.

A 'nonrecombination deactivation' factor is included in the program to account for higher than predicted Q_B^- concentration when $S_2Q_B^-$ and $S_3Q_B^-$ recombination was taken as 100%. This factor needs only be small and values of 12% for $S_2Q_B^-$ and 5% for $S_3Q_B^-$ have been used. A smaller value for the nonrecombination deactivation factor for $S_3Q_B^-$ seems inherently likely since Q_B^- is twice as likely to find a positive charge for its electron in this state.

It is likely that better fits could be obtained by manipulation of the 'nonrecombination deactivation' factors and the dark-adapted starting state appropriately, but this awaits improvements in our understanding and also in the quality of the data. The latter suffers from the time taken to carry out a series of experiments, typically several hours, during which time the lability of the chloroplasts and the gradual changes in the redox state of Q_B in the dark result in significant data scatter.

The preflash data provide strong support for the idea that Q_B^- provides an important source of electrons for S-state deactivation. At the same time, the one or two preflash treatment, which

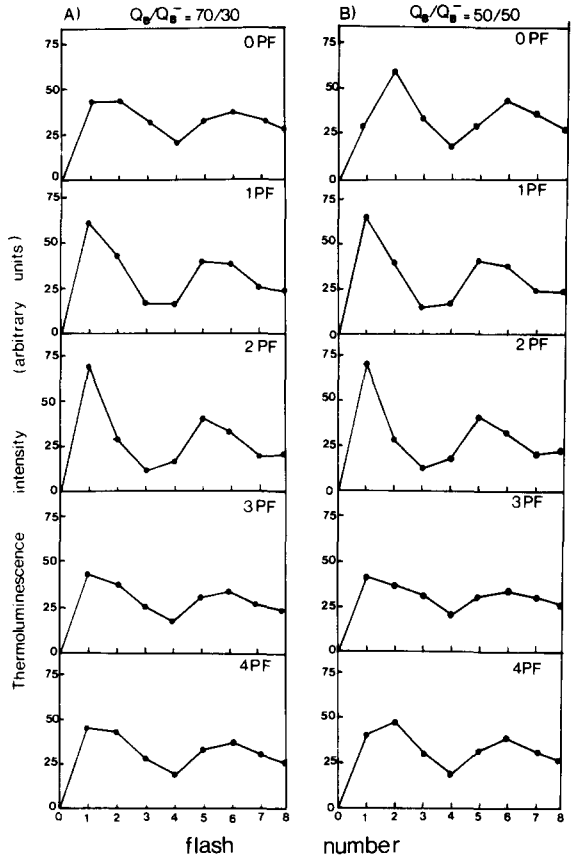


Fig. 5. Computer simulation of thermoluminescence induced by a series of flashes and the effect of preflashes (PF). (A) Assuming the following starting states: 12% misses; 7% double hits; ratio of luminescence yield $S_3Q_B^-/S_2Q_B^- = 1.7$; initial SQ_B states, 21% S_0Q_B, 9% $S_0Q_B^-$, 49% S_1Q_B, 21% $S_1Q_B^-$ (Q_B/Q_B^- = 70:30, S_0/S_1 = 30:70). Nonrecombination factor, 12% for $S_2Q_B^-$ recombination and 5% for $S_3Q_B^-$ recombination. (B) Assuming the following starting states: the same as (A) except the initial SQ_B states of 15% S_0Q_B, 15% $S_0Q_B^-$, 35% S_1Q_B, 35% $S_1Q_B^-$ (Q_B/Q_B^- = 50:50, S_0/S_1 = 30:70).

lowers the concentration of stable Q_B^- in the dark, could prove a useful technique in studying PS II photochemistry since both the S states and the Q_B redox state are effectively synchronized without needing to add an oxidizing agent.

Some pH effects – the involvement of protons in charge accumulation

In previous work, Inoue [22] carried out experiments on chloroplasts resuspended at low pH dur-

ing the course of investigation into the properties of a thermoluminescence band at approx $-10°C$ (the A band). In that work, temperature differences in the emission maxima were observed in the bands at around $+25°C$ and were attributed to effects of pH on S_2 and S_3 [22]. Further investigation of this effect seemed of interest in the light of the new understanding of these thermoluminescence bands [1].

Fig. 6 shows the effects of lowering the pH 5.5 on the flash-induced thermoluminescence bands. A single preflash was given in order to synchronize the S states in S_1 prior to flash excitation. In control experiments at pH 7.5, the position of the thermoluminescence bands generated by the first flash (i.e., $S_2Q_B^-$ recombination) was almost exactly the same as that for the second flash (the $S_3Q_B^-$ recombination). The emission maximum was approx. 27°C in both cases. At pH 5.5, however, the thermoluminescence band after the first flash was shifted to a higher temperature, $+38°C$, while that after the second flash remained close to its original position at normal pH, $+27°C$.

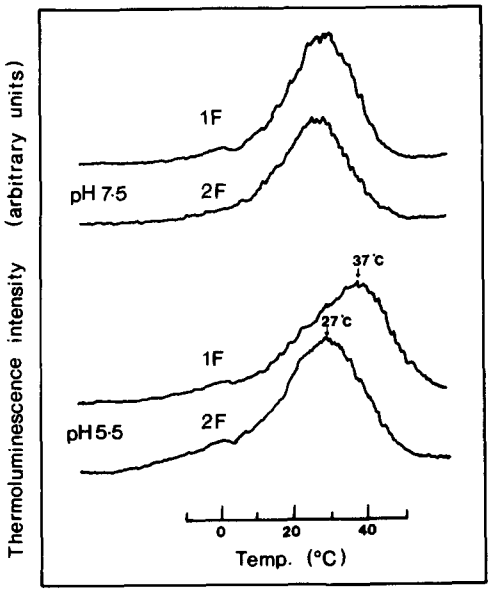


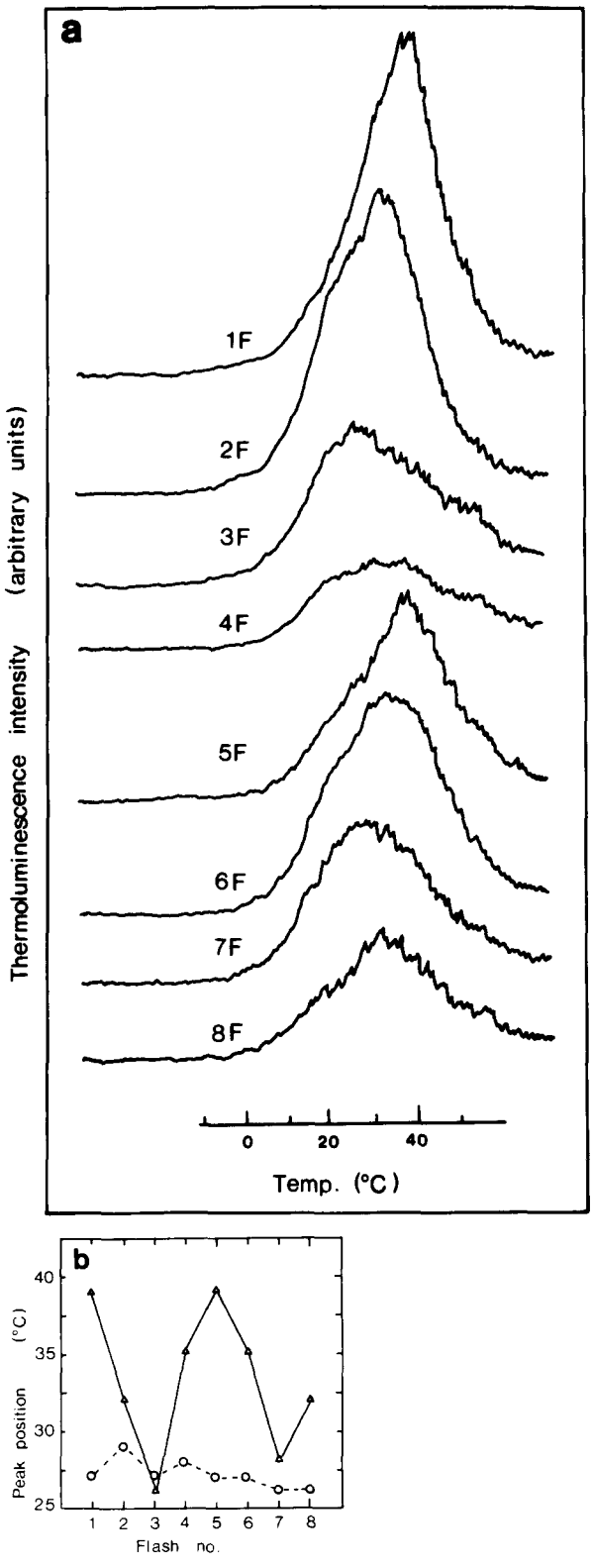
Fig. 6. The effect of pH on the temperature of the emission maxima of the thermoluminescence band generated by one or two flashes (F). Control (pH 7.5) and low pH (pH 5.5) samples are shown. Each sample received one preflash followed by a period of 10 min dark adaptation at 25°C before flash excitation.

Fig. 7a shows thermoluminescence recorded at pH 5.5 after a sequence of flashes (no preflash was given in this experiment). The thermoluminescence band shifted to higher temperatures was present after the first and fifth flashes while that at lower temperatures was present after the other flashes. Fig. 7b shows a plot of the emission maximum of the thermoluminescence band as a function of flash number at pH 5.5 (open triangles). It is clear that high temperature emission maxima are associated with $S_2Q_B^-$ recombination. Also shown in Fig. 7b is the temperature of the emission maximum for the thermoluminescence induced by a series of flashes at pH 7.5 as a control (open circles).

An explanation for the pH effects can be suggested. A shift to higher temperature of a thermoluminescence band is effectively an increase in stability of the charge pair, $S_2Q_B^-$. The fact that this occurs when the pH is lowered indicates an acceptor side proton is involved (i.e., the E_m of the redox couple $Q_B/Q_B^-(H^+)$ will be shifted to a more oxidizing potential as the pH is lowered and $Q_B^-(H^+)$ is more stable at lower pH). The increase in stability of the $S_2Q_B^-$ charge pair at low pH is probably due to this effect. While this paper was in preparation, similar conclusion were published based on other types of experiments [28,29].

Since Q_B^- is involved in $S_3Q_B^-$ recombination also, why is the $S_3Q_B^-$ band not shifted to higher temperatures? The most likely answer is that a proton is associated with the $S_2 \rightarrow S_3$ reaction. If a proton was lost in the formation of S_3, then S_3 would be destabilized by lower pH. This would cancel out the stabilizing effect on the acceptor side and no band shift would be observed.

This interpretation, if correct, provides the following information. Firstly, oxidation of the semiquinone form of Q_B back to the quinone involves a deprotonation event. Secondly, the reduction of S_2 to S_1 does not involve uptake of a proton. Thirdly, the reduction of S_3 to S_2 does involve protonation. These conclusions are derived from measurements of back reactions but it seems likely that the same protonation/deprotonation processes are involved in forward reactions. Indeed, direct measurements of protonation indicate that the S_1 to S_2 change does not involve loss of a proton, while the S_2 to S_3 change does [23–26].



Extending this interpretation further, it would be predicted that the $S_2Q_B^-$ band will stop shifting to higher temperatures as the pH is lowered if a pK is reached on the oxidized form and a shift to lower temperatures should occur with increasing pH until a pK is reached on the semiquinone. Similarly, when these pK values are reached, the $S_3Q_B^-$ band should begin to shift (to lower temperature at low pH, to higher temperatures at high pH). Such experiments remain to be done but could provide much useful information, provided that the pK values are in a range where the oxygen-evolving enzyme remains intact.

Concluding remarks

In this work, the previous assignments of the flash-induced thermoluminescence bands in untreated chloroplasts as being due to charge recombination reactions when PS II centres are in the states $S_2Q_B^-$ and $S_3Q_B^-$ have been confirmed. Whether $S_3Q_B^-$ recombination itself ($S_3Q_B^- \xrightarrow{TL} S_2Q_B$) actually results in the luminescence or whether a nonrecombination deactivation takes place prior to $S_2Q_B^-$ recombination ($S_3Q_B^- \xrightarrow{e} S_2Q_B^- \xrightarrow{TL} S_1Q_B$) is not known. However, the different effects of pH on the thermoluminescence arising from $S_2Q_B^-$ and $S_3Q_B^-$ does indicate that true $S_3Q_B^-$ recombination luminescence is being observed.

The source of deactivating electrons for S_2 (and S_3) in centres where Q_B^- is not present is a matter for speculation. Under some circumstances, reduced plastoquinone from the pool, PQH_2, could act as a source of electrons through the two-electron gate mechanism at Q_B. In the case of S_2 deactivation, this would result in an anomalous amount of $S_1Q_B^-$ in the dark.

$$S_2Q_B + PQH_2 \rightleftharpoons S_2Q_BH_2 + PQ \rightleftharpoons S_1Q_B^-(H^+) + H^+ + PQ$$

Fig. 7. The effect of low pH on the emission maxima of the thermoluminescence bands generated after a series of flashes. No preflash was given. Samples were at pH 5.5. (a) The thermoluminescence bands themselves. (b) Emission maxima plotted as a function of flash number. Solid lines with open triangles, pH 5.5; broken lines with open circles, pH 7.5.

This mechanism (speculatively protonated) could explain the increase in stable Q_B^- in the dark after a period of strong illumination [1], since, in the absence of an added acceptor in this kind of chloroplast preparation, the PQ pool becomes reduced (e.g. Ref.8). It may also be partly responsible for the unexpectedly high Q_B^- concentrations after one and two preflashes which is taken into account by the 'nonrecombination deactivation factor' in the computer simulations above. However, the importance of this reaction under conditions where the PQ pool is largely oxidized is probably small, since estimates of the equilibrium constant for the reaction, $Q_A^- Q_B \rightleftharpoons Q_A Q_B^-$, are high, i.e., 50 [27].

While this manuscript was in preparation, it was shown that S_2 and S_3 can be deactivated by donation from another component and that this, rather than dark-stable S_0, accounts for the proportion of S_1 present on the second of a series of flashes (spaced by 1 s) [28]. This donor when ozidized is probably the component giving rise to signal II_{slow}. It is evident that deactivation of the S states involves a number of mechanisms that depend on the redox states of other donor and acceptor side components.

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