Biochimica et Biophysica Acta, 1184 (1994) 85–92

BBABIO 43968

The origin of 40–50°C thermoluminescence bands in Photosystem II

Giles N. Johnson *, Alain Boussac and A. William Rutherford

Section de Bioenergetique (CNRS URA 1290), Bât 532, C.E.-Saclay, 91191 Gif-sur-Yvette Cedex (France)

(Received 6 July 1993)

Key words: Photosynthesis; Photosystem II; Thermoluminescence; EPR; Calcium depletion

We have used thermoluminescence (TL) and EPR measurements of Photosystem II (PS II) from spinach in order to identify charge pairs responsible for TL bands in the region of 40–50°C including the 'C-band' (peak V) and the TL band from PS II depleted of calcium. In intact PS II membrane preparations, in the presence of DCMU, a TL band at 50°C is induced following illumination at 77 K. This band decays, at 30°C, with a half-time of 10 min. This decay corresponds to the disappearance of the EPR signal arising from Q_A^- and an accelerated decay of the organic free radical Tyr D^+. It is concluded that recombination of this charge pair is probably responsible for the thermoluminescence emission. In PS II preparations that have been depleted of calcium using a salt/EGTA wash followed by rebinding of the extrinsic polypeptides, a TL band is produced at around 45–50°C following 198 K illumination. In such samples a stable S_2 state of the water-splitting complex is present, giving rise to a modified form of the EPR multiline signal. During incubation at 30°C in the dark this signal decays with a half-time around 20–25 min. This decay is not accelerated by the presence of Q_A^- induced by low-temperature illumination of the sample. In contrast, low-temperature illumination does result in an acceleration in the decay of Tyr D^+, indicating that Tyr D^+/Q_A^- recombination is again the dominant origin of thermoluminescence. In PS II depleted of calcium by incubation at pH 4.0, the possibility that TL emission temperature is determined by a change in the mid-point redox potential of Q_A (Krieger, A. and Weis, E. (1992) Photosynthetica 27, 89–98) was investigated by comparing TL from equivalent samples of control and Ca^{2+}-depleted PS II. It was shown that the emission temperature of the high temperature TL band induced by illumination at 77 K did not differ significantly between control and treated samples, suggesting that, under the conditions used, the potential of Q_A does not change significantly.

Introduction

Excitation of higher plant Photosystem II results in the transfer of an electron from the primary donor, P680, to pheophytin and then to the quinones, Q_A and Q_B (for a review see Ref. 1). P680 can be re-reduced by a variety of donors, including reduced cytochrome b_{559}, reduced Tyr D and an accessory chlorophyll. At room temperature, in intact centres, the dominant donor is Tyr Z, which is in turn re-reduced by electrons donated from the water-splitting complex. By rapidly freezing pre-illuminated samples or by illuminating at low temperatures, different charge pairs can be trapped. Subsequent warming of these samples can result in charge recombination, leading to light emission at characteristic temperatures; thermoluminescence [2,3].

A number of different thermoluminescence bands have been shown to arise from PS II; however, the charge pairs responsible for these have been conclusively identified in only two cases. A DCMU-inhibited band at around 20–30°C (B band; peak IV) and a DCMU-enhanced band at 5–15°C (Q band; D band; peak II) have been shown to result from recombination of Q_B^- and Q_A^-, respectively with either the S_2 or S_3 states of the water-splitting complex [4]. Other bands that have been attributed to PS II photochemistry include bands around −10°C (A and A_T) and +50°C (C band; peak V) and a band at a variable temperature, depending on illumination temperature (Z_V). The precise origin of these remains uncertain (see Discussion in Refs. 2,3).

The thermoluminescence band at around 40–50°C can be observed under a variety of conditions (for reviews see Refs. 2,3). Early literature describing this band is confused and sometimes contradictory (e.g., Refs. 5–10), however a consensus has been reached

* Corresponding author. Fax: +33 1 69088717.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene-bis(oxyethylenenitrilo)tetraacetic acid; EPR, electron paramagnetic resonance; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Mes, 4-morpholineethanesulphonic acid; PS II, Photosystem II; TL, thermoluminescence; Tyr, tyrosine.

SSDI 0005-2728(93)E0171-L

that a band around 50°C arises due to recombination reactions occurring within the PS II reaction centre [11]. Some of the early confusion in the assignment of this band to PS II may relate to the occurrence of an artifactual band, in the same temperature range, produced by the interaction of chlorophyll and detergent molecules [12].

The 50°C band has been shown to be enhanced upon treatment of a sample with DCMU, implying that the negative charge involved in recombination is stored on Q_A [6,7,10,11]. The nature of the corresponding positive charge remains unknown. Perhaps the most comprehensive study investigating this band is that of Demeter et al. [11] who showed that, upon giving sequences of single turnover flashes, the amplitude of the band oscillated with a period of four, being maximal on the third and fourth flashes. This oscillation was anti-parallel to that of the 10°C band arising from recombination of $S_{2/3}/Q_A^-$, demonstrating that the 50°C band arises in centres in the states S_0 or S_1. A direct involvement of either of these states in recombination was thought unlikely since a band at 50°C can also be observed in material that had been treated to remove manganese from the water-splitting complex and in systems where the complex is not formed [7,8,11]. Instead, since the S_2 and S_3 states are both able to recombine efficiently with Q_A^-, it is probably the absence of either of these states that gives rise to the oscillatory behaviour.

Other likely candidates as the location of the positive charge involved in formation of the 50°C band include the radical Tyr D^+ or the oxidised form of cytochrome b_{559}. Demeter et al. [11] excluded the latter on the basis of its slow donation time to the reaction-centre chlorophyll; however, these kinetics are probably not relevant to the situation under which TL emission is measured. Tyr D^+ was also excluded, partly because its lifetime was known to be considerably longer than that of the C band. However, estimates of Tyr D^+ lifetime had only previously been made in samples where Q_A^- was absent. Additional evidence excluding Tyr D^+ came from the observation that incubation of chloroplasts with antimycin did not inhibit TL emission. Antimycin has previously been observed to cause the disappearance of the EPR signal II, arising from Tyr D^+ [13]. Demeter et al. [11] did not, however, measure directly the presence or absence of either Tyr D^+ or of oxidised cytochrome *b*-559, so neither species can be excluded. Indeed, on the basis of unpublished EPR data, Tyr D^+/Q_A^- has been suggested to be the charge pair giving rise to the 50°C band (see Ref. 2).

Recently, attention has focused on a variety of preparations in which the donor side of PS II has been impaired due to the removal of calcium (reviewed in Refs. 14–16). Ca^{2+} depletion can be brought about by exposure of PS II to low pH [17] or by incubating at high salt concentrations in the light (see Refs. 14–16). In such systems, in the presence of DCMU, a thermoluminescence band is typically observed at an elevated temperature, replacing the normal $S_{2/3}/Q_A^-$ band emitted at 5–15°C [18–22]. The temperature at which this new band is emitted has been seen to vary depending on the details of the preparation used [18–23].

The TL bands observed at elevated temperatures in Ca^{2+}-depleted preparations have been assumed to reflect a stabilisation of the charge pair S_2/Q_A^- such that the energy required for recombination is higher [18–23]. In some preparations, a stable, modified, form of the multiline signal arising from S_2 has been observed [19–21,24] and it has been suggested that it is this stabilisation that leads to the formation of a high temperature TL band [19–21]. The relationship between the presence of a high-temperature TL band and the presence of a modified stable multiline remains, however, unclear.

Another possible reason for the elevation of TL emission temperature in Ca^{2+}-depleted PS II has been proposed by Krieger and co-workers [25,26], who have made measurements of the redox mid-point potential of the Q_A/Q_A^- charge pair in control and Ca^{2+}-depleted membranes. Such titrations, using chlorophyll fluorescence yield as a probe of Q_A redox state, indicate that, upon removal of Ca^{2+}, there is an increase in the mid-point potential of Q_A from -120 to $+40$ mV. Such a shift would be predicted to increase the TL emission temperature.

In this paper we investigate the origin of high temperature (40–50°C) TL emission from PS II in different systems by following the decay of TL bands and various EPR signals during incubation at 30°C. We present data from control membranes following 77 K illumination (the classic 'C band') and from salt-washed membranes with the extrinsic polypeptides rebound, in which a modified stable form of the S_2 multiline signal is present [24]. In addition, we investigate whether changes in the redox potential of Q_A/Q_A^- are involved in altering the TL emission by studying PS II depleted of Ca^{2+} using mild low pH treatment.

Materials and Methods

Photosystem II-enriched membranes (BBY) were prepared from market spinach during the period September–November 1992 following the method of Ref. 27 as modified by Ref. 28, with the further modifications that for the Triton treatment a chlorophyll concentration of 3 mg ml^{-1} and a Triton concentration of 33% w/v were used, and incubation with Triton was for 30 min. Ca^{2+}-depleted/EGTA-treated PS II membranes were prepared as described by Boussac et al. [23]. Where membranes were depleted of Ca^{2+} using

low pH, the treatment involved incubating membranes in a solution containing 330 mM sorbitol, 10 mM NaCl, 25 mM Hepes, 25 mM Mes, 25 mM glycylglycine (pH 4.0) for 10 min in room light and then for 15 min in the dark following addition of 50 μM EDTA. Membranes were then centrifuged and washed, and resuspended in a solution containing 330 mM sorbitol, 10 mM NaCl, and 20 mM Mes (pH 6.5).

Thermoluminescence was measured using equipment described in Ref. 29. For TL experiments a chlorophyll concentration of approximately 250 (μg Chl) ml^{-1} was used. Dark-adapted samples were cooled to 77 K or 198 K and illuminated for 2 min with a heat-filtered white light from a 150 W quartz-halogen lamp. This illumination was found sufficient to give a saturated TL signal in all cases. Prior to measuring TL emission, samples were warmed first to -40°C for 60 s and then to -5°C for 2 min. This step-wise warming procedure was employed to ensure that the incubation time at 30°C included the minimum possible warming period. Warming from -5°C to 30°C took less than 30 s. Samples were warmed to 30°C for varying amounts of time (0–60 min) prior to being rapidly cooled to -40°C by plunging the sample holder into liquid N_2. Light emission was then recorded during warming from -40°C to 80°C at a heating rate of 0.5°C/s. In all TL measurements DCMU was added at a concentration of 20 μM.

EPR spectra were recorded at liquid helium temperatures using a Bruker ESR 200 X-band spectrometer equipped with an Oxford Instruments cryostat. For EPR, samples were used at a chlorophyll concentration of approximately 8 (mg Chl) ml^{-1}. In all cases 100 μM DCMU was added. Samples were illuminated at 77 K or 198 K for 20 min using a heat-filtered quartz-halogen lamp. This long illumination was found necessary to maximise the EPR signal from Q_A^- in these highly concentrated samples and, even under these conditions, 100% reduction of Q_A^- was not possible (see Results). Samples were warmed first to -5°C and then incubated at 30°C for periods up to 60 min. Conditions for the measurement of different EPR signals are given in the figure legends. The signal from Q_A^- was estimated from a light-dark difference spectrum of either the $g = 1.9$ (control samples) or $g = 1.82$ (Ca^{2+}-depleted samples). For the $g = 1.9$ form this was estimated as the vertical difference between the shoulder at around 3550 G and the trough at around 3700 G, and for the $g = 1.82$ as the peak height at around 3700 G.

Results

The C-band in intact Photosystem II

Thermoluminescence emission at around 50°C was charged in intact PS II membranes by illumination of

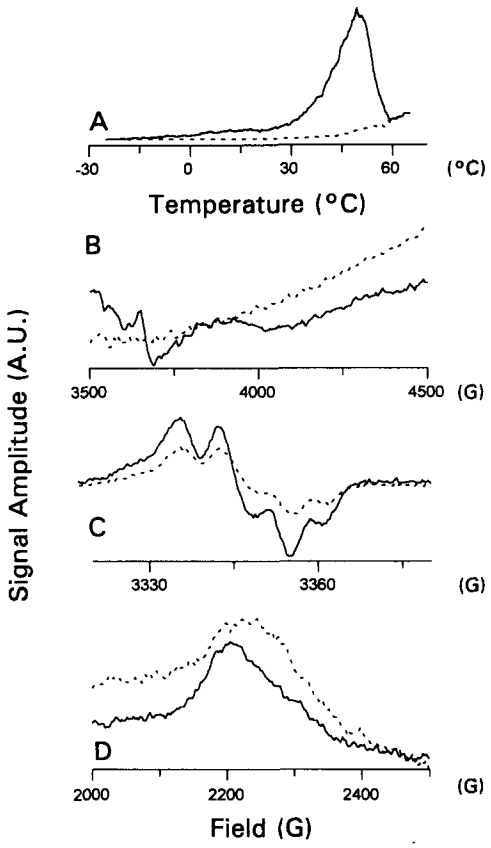

Fig. 1. TL signal (A) and EPR signals arising from Q_A^- (B), Tyr D^+ (C) and cytochrome b_{559} (D) in intact PS II membranes. Data shown are recorded following 77 K illumination for 2 (A) or 20 (B, C, D) min and subsequent warming to -5°C for 2 min (solid lines) or 30°C for 60 min (dashed lines). Conditions for EPR measurments were as follows: (B) Temperature 4.6 K, microwave power 31 mW, modulation amplitude 22 G, modulation frequency 100 kHz; (C) Temperature 15 K, microwave power 0.64 μW, modulation amplitude 2.2 G, modulation frequency 100 kHz; (D) Temperature 15 K, microwave power 6.4 mW, modulation amplitude 22 G, modulation frequency 100 kHz. DCMU was added to all samples.

samples at 77 K in the presence of DCMU. Under such conditions, electron donation to Q_A occurs from cytochrome b_{559} or, in a fraction of centres, from a chlorophyll molecule (data not shown). The Chl^+ radical formed in this way decays rapidly upon warming, presumably largely as a result of electron donation from an unidentified species. Samples were warmed to -5°C for 2 min and then incubated at 30°C. Fig. 1 shows TL and EPR signals recorded from BBY particles following incubation for 2 min at -5°C and 60 min at 30°C. Fig. 2 shows the decay in the amplitude of these signals during incubation at 30°C. Warming of samples from -5°C to 30°C for times less than 5 min resulted in the loss of a variable portion of the 50°C band (up to 30% of total signal, varying between preparations, see Fig. 2A). If samples were warmed to 30°C for 5 min prior to 77 K illumination, the resulting thermoluminescence signal was diminished by an amount corresponding to this fast decay (data not

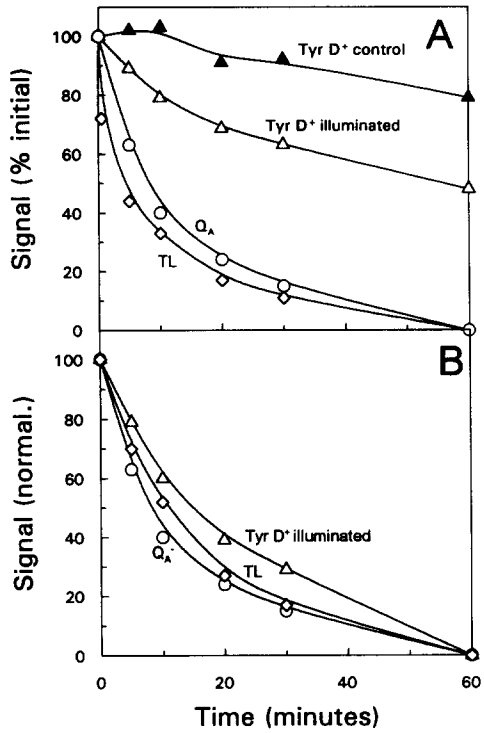

Fig. 2.(A) Changes in the amplitude of TL emission (diamonds) and EPR signals arising from Q_A^- (circles) and Tyr D^+ (open and filled triangles) during incubation at 30°C of intact PS II membranes. Samples were illuminated at 77 K (open symbols) or dark-adapted (closed symbols) before being warmed to −5°C for 2 min (time = 0) and then to 30°C for the time indicated.(B) Normalised decay of TL emission and EPR signals from Q_A^- and Tyr D^+. The initial phase of decay of TL seen in A (< 5 min) was corrected for by plotting data from 5 min onwards in A on a semilogrithmic scale, fitting a straight line and estimating the signal size at time = 0 from the extrapolated line. This value was then set to 100. EPR data were normalised by setting data at time = 0 to 100 and at time = 60 min to zero. DCMU was added to all samples.

shown). This decay did not correspond to a change in any measured EPR signal. It is probable, therefore, that this fast-decaying component in the TL signal does not arise from a charge recombination reaction within the PS II reaction centre, but may correspond to the 'detergent-induced' band observed by Rozsa et al. [12]. Consistent with this assignment, the effect was not observed upon warming of intact thylakoids (data not shown).

Incubation for periods longer than 5 min led to a mono-exponential decay of the 50°C band with a half-time of 10 min (Fig. 2). This decay corresponded well with the decay time of the EPR signal arising from Q_A^-. The EPR signal from Tyr D^+ (Signal II) is normally present in the majority of centres, even after moderate periods of dark-adaptation. During 1 h incubation of dark-adapted samples at 30°C, when no Q_A^- is present, around 20% of the signal from Tyr D^+ is lost. In 77 K illuminated samples the proportion of Tyr D^+ signal lost was increased to 50%. The presence of an electron on Q_A^- leads to an enhancement of Tyr D^+ decay. The only other stable EPR signal detected in PS II was that from oxidised cytochrome *b*-559. The amplitude of this signal increased following 77 K illumination but remained constant throughout the subsequent incubation.

If charge separation occurs in all centres, it can be seen from Fig. 2A that the decay in Tyr D^+ cannot account for the entire loss of Q_A^-. Over 1 h of incubation the signal arising from Q_A^- decays to close to zero whilst there is only a 50% loss of Tyr D^+. There are, however, a number of factors that contribute to this discrepancy. Firstly, the efficiency of Q_A reduction at 77 K is less than 100%. Illumination of the samples at 198 K following 77 K illumination and 60 min incubation at 30°C yielded a signal 140% of that induced at 77 K (data not shown). Hence Q_A^- was formed in no more than 75% of centres. This is also likely to be an underestimation, since there may be damage occurring to the reaction centre during the period of incubation. Secondly, it is unlikely that all Q_A^- loss during incubation occurs via charge recombination. It seems possible that, in some centres, electrons will also be lost via a non-recombinative pathway, thereby giving a smaller than expected loss of Tyr D^+ signal. In Fig. 2B, data are normalised to equalise the decay during 1 h, so allowing direct comparison of the kinetics of decay. For TL data, the initial signal is calculated by extrapolating back the mono-exponential decay recorded from 5 min onwards. This figure shows that there is a good correspondence between the decay of Q_A^- and the TL signal. The decay kinetics of Tyr D^+ match the decay of Q_A^- reasonably well. The slight discrepancy in this decay may be due to failure to correct for non-recombinative loss of Tyr D^+ and possibly Q_A^- in some centres.

Incubation of thylakoids with antimycin has previously been observed to lead to the disappearance of the EPR signal associated with Tyr D^+ [13]. Demeter et al. [11] found that antimycin did not affect the amplitude of the 50°C TL band and so concluded that Tyr D^+ could not be involved in the TL emission observed. Under our experimental conditions, however, we were unable to detect any effect of antimycin on the concentration of Tyr D^+, even after addition of concentrations up to 800 μM and incubation for 60 min (data not shown). Whilst the reasons for the discrepancy between earlier data [13] and our results are not clear, the effects of this or similar reagents, in the absence of a direct measure of Tyr D^+, cannot be used as an argument to exclude the involvement of this species in TL emission.

We conclude that, under the experimental conditions we have used, charge recombination is occurring between Q_A^- and Tyr D^+ and that this recombination

is probably the predominant origin of the TL band observed at 50°C.

The high temperature band in calcium-depleted PS II

Fig. 3 shows the thermoluminescence signal arising following 198 K illumination of BBY particles depleted of Ca^{2+} using a NaCl/EGTA wash followed by rebinding of polypeptides as described by Boussac et al. [23]. As in Fig. 1, data are shown following 2 min at −5°C and 60 min at 30°C. As has been observed in preparations where calcium has been depleted by citrate/pH 3.0 treatment [18,19], low temperature illumination of such samples gives rise to a single TL band peaking at 45–50°C. Also shown in Fig. 3 are EPR

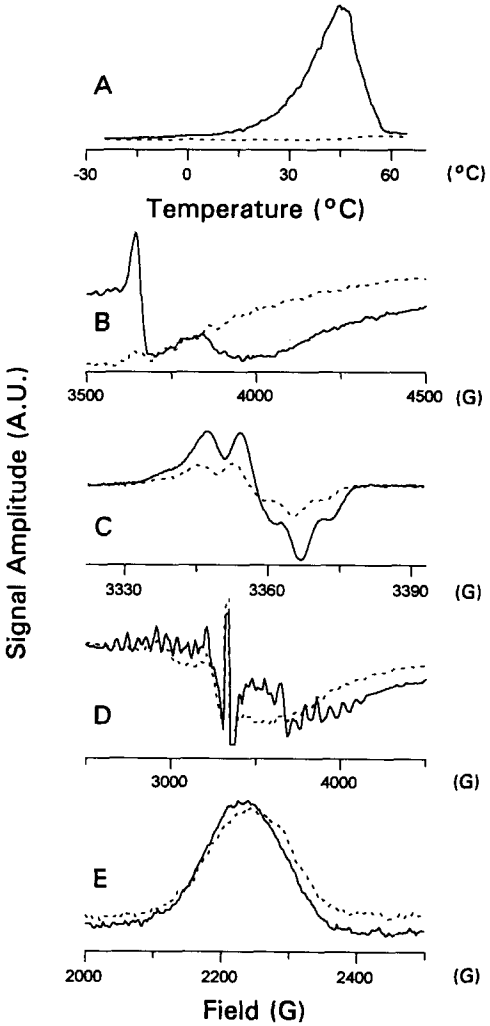

Fig. 3. TL signal (A) and EPR signals arising from Q_A^- (B), Tyr D^+ (C), S_2 (D) and cytochrome *b*-559 (E) in NaCl/EGTA-washed PS II membranes reconstituted with extrinsic polypeptides. Data shown are recorded following 198 K illumination for 2 (A) or 20 (B, C, D, E) min and subsequent warming to −5°C for 2 min (solid lines) or 30°C for 60 min (dashed lines). Conditions for EPR measurments were as follows: (B, C, E) as Fig. 1B, C, D, respectively; (D) Temperature 10 K, microwave power 31 mW, modulation amplitude 22 G, modulation frequency 100 kHz. DCMU was added to all samples.

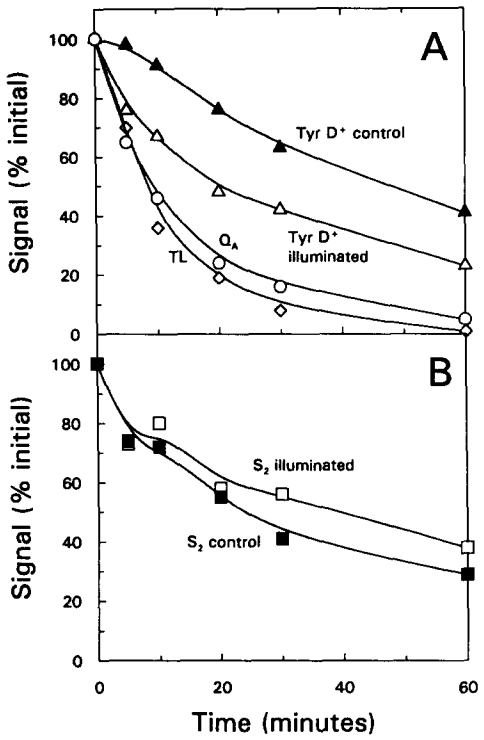

Fig. 4. Changes in the amplitude of TL emission (diamonds) and EPR signals arising from Q_A^- (circles), Tyr D^+ (open and filled triangles) and S_2 (open and closed squares) during incubation at 30°C of NaCl/EGTA-washed PS II membranes reconstituted with extrinsic polypeptides. Samples were illuminated at 198 K (open symbols) or dark-adapted (closed symbols) before being warmed to −5°C for 2 min (time = 0) and then to 30°C for the time indicated. TL data are normalised to the extrapolated signal size at time = 0, as in Fig. 2B. All EPR data are normalised to the signal amplitude at time = 0. DCMU was added to all samples.

signals recorded in equivalent samples. In such samples the manganese complex is in a stable modified S_2 state and cytochrome *b*-559 is pre-oxidised [23]. Upon 198 K illumination donation of electrons to Q_A^- is, therefore, predominantly from chlorophyll, giving rise to a free radical EPR signal (data not shown). EPR measurements following 198 K illumination of Ca^{2+}-depleted samples did not give rise to a detectable split S_3 signal (see Ref. 24). The large free radical signal arising from Chl^+ that was formed was stable at 198 K. Incubation at −40°C for 20–30 min resulted in the complete disappearance of this signal and a loss of about 20% of the signal from Q_A^- (data not shown).

Fig. 4 shows the decay of thermoluminescence and EPR signals during incubation of Ca^{2+}depleted BBYs at 30°C. As with control membranes, there was seen to be an initial rapid loss of TL emission following warming from −5°C to 30°C that was non-exponential and did not correlate with a change in any EPR signals detected. Data for the TL signal in Fig. 4 has been corrected for this, as in Fig. 2B. In dark-adapted samples incubated at 30°C, there was found to be a significant loss of EPR signals from both Tyr D^+ and S_2. The

extent of the loss of these signals after 1 h varied between different preparations of BBYs and typically resulted in the decay of 50–80% of the initial signal. When the decay of signals in pre-illuminated samples was compared to that of identical samples that had not been illuminated, it was consistently found that the decay of Tyr D^+ was accelerated in the presence of Q_A^-. In all cases, the decay of the multiline signal from S_2 was unaffected, or possibly slightly slowed, by the presence of Q_A^- (Fig. 4B). From these data, it seems that recombination of the charge pair Tyr D^+/Q_A^- is likely to be the dominant source of TL emission. There is no evidence that recombination occurs between Q_A^- and the stable S_2 state.

Attempts were made to investigate the origin of TL emission in samples that had been depleted of Ca^{2+} using salt/EDTA-washing in the light but in which the 23 and 17 kDa polypeptides were not rebound [18]. We found, however, that such samples were insufficiently stable to withstand incubation at 30°C, making it impossible to determine whether recombination could occur between Q_A^- and the stable modified S_2 state. In such samples we found, however, that TL emission generated by 77 K illumination was at 50°C, similar to the observations made by Homann and Madabusi [22] (compare results in Ref. 23).

The effect of Q_A^- mid-point redox potential on TL emission temperature in low-pH-treated membranes

Recently, Krieger and co-workers [25,26] have presented evidence that, in experimental systems where the donor side of PS II has been inhibited (e.g., by low pH treatment or by hydroxylamine washing) a shift in the mid-point potential of Q_A^- occurs from -120 mV to $+40$ mV. In such samples a 40–50°C TL band is observed [26]. The emission temperature of this band was assumed to reflect the shift in the Q_A potential. Comparison of Figs. 1A and 3A show that the peak temperature of TL emission in each case is very similar, inconsistent with Q_A having a different potential in control and Ca^{2+}-depleted samples. Fig. 5 shows TL emission from control PS II membranes and from membranes depleted of Ca^{2+} using a method similar to that in Ref. 26. For the data shown, 50% inhibition of O_2 evolution was attained. In Fig. 5 it can clearly be seen that the peak of the high temperature TL band formed following illumination at 77 K, arising, presumably, from Tyr D^+/Q_A^-, is very similar in the two samples, being slightly lower in the Ca^{2+}-depleted sample. This observation is inconsistent with there having been a shift in the mid-point potential of Q_A.

A possible reason for the discrepancy of results from TL and Krieger et al.'s data [25,26] would be that the high temperature TL band in control samples arose only from a percentage of reaction centres that were inactive and that therefore had Q_A in the modified,

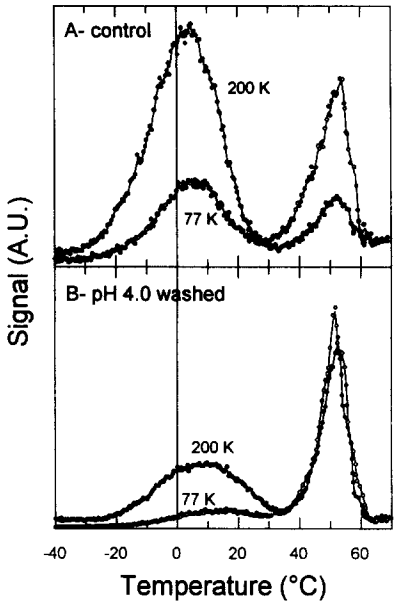

Fig. 5. TL signals arising from control (A) and pH 4.0-washed (B) PS II-enriched membranes following illumination at 77 K (open symbols) or −198 K (closed symbols). DCMU was added to all samples.

high potential state. However, illumination of our control membranes at 198 K, which allows turnover from S_1 to S_2 in intact centres, causes a dramatic increase in the intensity of the TL band at around 5°C with a corresponding diminution of the 50°C band (Fig. 5), implying that active centres are giving rise to the 50°C band. In low-pH-treated membranes no large decrease in 50°C band intensity was observed following 198 K illumination, relative to that after 77 K illumination. In addition, Demeter et al. [11] observed an oscillation of C-band intensity, with number of flashes, that was antiparallel to that of the band arising from $S_{2/3}/Q_A^-$, also implying that the band arises from active centres.

In samples washed at pH 4.0, we were unable to detect the presence of a stable modified multiline (data not shown). Upon illumination of such samples at 198 K, an S_2 multiline signal was formed that was like that observed in control samples.

Discussion

In this paper we have presented evidence that recombination of the charge pair Tyr D^+/Q_A^- leads to the formation of a thermoluminescence band in the region of 50°C. In the presence of the tyrosine radical, recombination does not occur between Q_A^- and cytochrome b-559$^+$ and, in calcium-depleted/EGTA treated systems, there is no evidence for charge recombination between Q_A^- and the modified S_2 state of the manganese complex. It cannot be totally ruled out that other reactions contribute to light emission in this temperature region. Indeed, as discussed above, bands

of this type can be produced from trapping of excited states of free chlorophyll interacting with detergent [12]. Bands in this temperature range may also arise due to other reactions in different experimental systems. However, it appears that, under our conditions, Tyr D^+/Q_A^- recombination is the dominant reaction occurring.

Although a quantitative match between the decay of the thermoluminescence and the EPR signals from Q_A^- and Tyr D^+ was not obtained, it is not, perhaps, to be expected, even if Tyr D^+ is the only positive charge involved in recombination. Our experimental protocol involved long periods of incubation at a temperature that was sub-optimal for the recombination reaction. Hence, it is likely that, over the course of the incubation, there was a significant degree of Q_A^- loss due to non-recombinative reactions. The 50°C band that could be induced by 77 K illumination was always somewhat smaller than the S_2/Q_A^- recombination band at 5°C, induced by illumination at 0°C (data not shown). This suggests that the 50°C band does not reflect a reaction occurring in all centres. The similarity between the decay of Q_A^- and the TL signal in each case (Figs. 2B, 4A) indicates that at any point during the incubation, the concentration of Q_A^- is largely responsible for determining the TL amplitude.

In functional PS II membranes, we conclude, from the enhanced decay of Tyr D^+, that charge recombination between this species and Q_A^- is probably the predominant source of TL emission at 50°C. This conclusion fits with the observations of Demeter et al. [11] in which the 50°C band was shown to correlate with the presence of both S_0 and S_1. Tyr D^+ is present in all of the S states, but is only involved in recombination reactions when energetically more favourable reactions cannot occur. Thus, when S_2 and S_3 are present, Tyr D^+/Q_A^- recombination does not occur; when they are absent, it does.

It has been discussed earlier that S_0 and S_1 might be involved in recombination reactions associated with the 50°C band [11]. It is now known, however, that the S_0 state is able to donate electrons to Tyr D^+ [34]. Hence a putative recombination involving S_1 would also result in the electron from Q_A^- being donated to Tyr D^+. We have no kinetic evidence indicating that such an intermediate reaction occurs. Recombination involving other hypothetical charge pairs, including a formal S_{-1}/S_0 pair, cannot be totally excluded. However, in the absence of hard data indicating the existence of other reactions and how they might bring about Tyr D^+ loss, such considerations remain speculative.

The observation that in samples that have been depleted of calcium, charge recombination does not occur between Q_A^- and the stable S_2 state, is perhaps somewhat unexpected. There are a number of reports in the literature that relate temperature changes in TL emission to the stability of S_2. In particular, it has recently been reported that binding of extrinsic polypeptides [22,23] or of chelators [22] to the donor side of PS II modulate the temperature of TL bands in calcium-depleted PS II membranes. These data have been interpreted as indicating that the binding of these effector molecules induces a change in the redox potential of the manganese complex, in line with earlier EPR data [30]. If it is assumed that our findings can be extended to such systems, then these data need to be reinterpreted. The implication of our results is that such modulation in band temperature must reflect an alteration in the redox properties of Tyr D^+, Q_A^- or possibly of an intermediate in the recombination reaction. The properties of Q_A are known to be modulated by the binding of various molecules, including many carboxylic acids. These are thought to act by displacing bicarbonate from a site close to the non-haem iron located between Q_A and Q_B [32]. Bicarbonate displacement (or carboxylate binding) causes a change in the EPR signal arising from the Q_A–Fe interaction, including a shift from $g = 1.9$ to 1.82 [31]. A transition between these two signal forms is seen comparing control and Ca^{2+}-depleted/EGTA-treated membranes [24] (compare also Figs. 1B and 3B). It is quite possible that different chelators binding to the iron could have different effects on the mid-point redox potential of the Q_A/Q_A^- pair.

It is unlikely that binding of the 23 kDa polypeptide could affect Q_A in precisely the same way as chelators, but it may act on either this or on Tyr D^+ by causing conformational changes within the PS II complex. Evidence for slight conformational changes upon binding of the 23 and 17 kDa proteins has been obtained from EPR studies [30]. An apparent upshift in the potential of Q_A has been observed upon debinding of the 33 kDa extrinsic protein [33]. (It is worth noting that addition of purified 23 kDa polypeptide, at least in the study by Ono and Inoue [24], involved simultaneous addition of significant amounts of EDTA, which may, therefore, at least contribute to the effects observed.)

As noted above, we were unable to establish the charge pair involved in TL emission in systems lacking the extrinsic polypeptides, since they were insufficiently stable to withstand incubation at 30°C. Hence, in such experimental systems, it is possible that recombination occurs between Q_A^- and S_2. If, as has been suggested [22,23], the binding of the 23 kDa polypeptide does modulate the mid-point potential of S_1/S_2 then it is possible that, in the absence of the extrinsic polypeptides, the potential of this pair is shifted to below that of Tyr D/Tyr D^+. It is expected that, under any given conditions, recombination will involve the least stable positive charge.

An alteration in the mid-point redox potential of the Q_A/Q_A^- charge pair modulated by calcium release

from the donor side of PS II has recently been proposed by Krieger and co-workers on the basis of titrations of Q_A redox state by the measurement of fluorescence yields. Krieger et al. [26] have also made measurements of TL emission in their system and observed similar signals to those seen following Ca^{2+} depletion using citrate/pH 3.0 treatment [18,19], or here using NaCl/EGTA followed by reconstitution of polypeptides or using pH 4.0 incubation. Comparing TL data in Figs. 1A and 3A, we observe TL bands with peak emission at 45–50°C, being marginally lower in Ca^{2+}-depleted membranes. This is also true comparing control membranes and membranes incubated at pH 4.0 (Fig. 5). These observations are difficult to reconcile with a 160 mV shift in the mid-point potential of Q_A having occurred.

In conclusion, it seems that, in the absence of normal S_2 or S_3 (or less stable positively charged species), the dominant species involved in charge recombination with Q_A^- leading to TL emission is Tyr D^+, and this gives rise to a TL band at around 50°C. This implies that, in systems inhibited by removal of Ca^{2+}, the mid-point potential of the S_1/S_2 couple is altered such that recombination of Tyr D^+/Q_A^- occurs more readily than S_2/Q_A^-. Additionally, kinetic limitations in one or more electron transfer steps may contribute to favouring Tyr D^+ over the modified S_2 in charge recombination.

Acknowledgements

We would like to thank Paula Booth for providing us with Ca^{2+}-depleted/EGTA-treated PS II membranes, Jean-Marc Ducruet for the time and effort he has put into building the TL apparatus and various members of the group, especially Anja Krieger, for useful discussions. G.N.J. is funded by an SERC/NATO fellowship.

References

1 Hansson, O. and Wydrzynski, T. (1990) Photosynth. Res. 23, 131–162.
2 Sane, P.V. and Rutherford, A.W. (1986) in Light Emission of Plants and Bacteria (Govindjee, Amesz, J. and Fork, D.C., eds.), pp. 329–360, Academic Press, New York.
3 Vass, I. and Inoue, Y. (1992) in The Photosystems: Structure, Function and Molecular Biology (Barber, J., ed.), pp. 259–294, Elsevier, Amsterdam.
4 Rutherford, A.W., Crofts, A.R. and Inoue, Y. (1982) Biochim. Biophys. Acta 973, 350–353.
5 Rubin, A.B. and Venediktov, P.S. (1969) Biofizika 1, 105–109.
6 Desai, T.S., Sane, P.V. and Tatake, V.G. (1975) Photochem. Photobiol. 21, 345–350.
7 Ichikawa, T., Inoue, Y. and Shibata, K. (1975) Biochim. Biophys. Acta 408, 228–239.
8 Inoue, Y., Furuta, S., Oku, T. and Shibata, K. (1976) Biochim. Biophys. Acta 449, 357–367.
9 Inoue Y., Ichikawa T. and Shibata, K. (1976) Photochem. Photobiol. 23, 125–130.
10 Sane, P.V., Desai, T.S., Tatake, V.G. and Govindjee (1977) Photochem. Photobiol. 26, 33–39.
11 Demeter, S., Vass, I., Horvath, G. and Laufer, A. (1984) Biochim. Biophys. Acta 764, 33–39.
12 Rosza, Zs., Droppa, M. and Horvath, G. (1989) Biochim. Biophys. Acta 973, 350–353.
13 Esser, A.F. (1974) Photochem. Photobiol. 20, 173–181.
14 Yocum, C.F. (1991) Biochim. Biophys. Acta 1059, 1–15.
15 Rutherford, A.W., Zimmermann, J.-L. and Boussac, A. (1992) in The Photosystems: Structure, Function and Molecular Biology (Barber, J., ed.), pp. 179–229, Elsevier, Amstrerdam.
16 Debus, R.J. (1992) Biochim Biophys. Acta 1102, 269–352.
17 Ono, T.-A. and Inoue, Y. (1988) FEBS Lett. 227, 147–152.
18 Ono, T.-A. and Inoue, Y. (1989) Biochim. Biophys. Acta 973, 443–449.
19 Ono, T.-A. and Inoue, Y. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), pp. 701–708, Kluwer, Dordrecht.
20 Ono, T.-A. and Inoue, Y. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), pp. 741–744, Kluwer, Dordrecht.
21 Ono, T.-A. and Inoue, Y. (1990) Biochim. Biophys. Acta 1020, 269–277.
22 Homann, P.H. and Mdabusi, L.V. (1992) Photosynth. Res. 35, 29–39.
23 Ono, T.-A. and Inoue, Y. (1992) Biochemistry 31, 7648–7655.
24 Boussac, A., Zimmermann, J.-L. and Rutherford, A.W. (1989) Biochemistry 28, 8984–8989.
25 Krieger, A. and Weis, E. (1992) Photosynthetica 27, 89–98.
26 Krieger, A., Weis, E. and Demeter, S. (1993) Biochim. Biophys. Acta 1144, 411–418.
27 Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 61, 231–234.
28 Ford, R.C. and Evans, M.C.W. (1983) FEBS Lett. 160, 159–164.
29 Ducruet, J.-M. and Miranda, T. (1992) Photosynth. Res. 33, 15–27.
30 Boussac, A., Zimmermann, J.-L. and Rutherford, A.W. (1990) FEBS Lett. 277, 69–74.
31 Vermass, W. and Rutherford, A.W. (1984) FEBS Lett. 160, 159–164.
32 Blubaugh, D.J. and Govindjee (1988) Photosynth. Res. 19, 85–128.
33 Vass, I., Ono, T.-A., Homann, P.H., Gleiter, H. and Inoue, Y. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. 1, pp. 649–652, Kluwer, Dordrecht.
34 Styring, S. and Rutherford, A.W. (1987) Biochemistry 26, 2401–2405