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Participation of the $g = 1.9$ and $g = 1.82$ EPR forms of the semiquinone–iron complex, $Q_A^- \cdot Fe^{2+}$ of photosystem II in the generation of the Q and C thermoluminescence bands, respectively

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Following illumination at 200 K, the charge recombination reactions and the origin of the thermoluminescence (TL) bands appearing at about 0°C (Q band) and +50°C (C band) in the glow curve were investigated by comparative TL and EPR measurements in DCMU-treated photosystem II particles. Decay half-time measurements carried out at −25°C and +25°C, respectively, suggest that the S_2 state (multi-line signal) undergoes charge recombination with the $g = 1.9$ form of the semiquinone–iron complex, $Q_A^- \cdot Fe^{2+}$, resulting in the appearance of the Q band, and that the $g = 1.82$ form of $Q_A^- \cdot Fe^{2+}$ back-reacts with the oxidized tyrosine, Y_D^+ (Signal II_s), accounting for the generation of the C band.

Photosystem II; Semiquinone–iron complex; Thermoluminescence; Charge recombination; EPR

1. INTRODUCTION

Thermoluminescence (TL) originates from recombination of positive and negative charges separated in the photosynthetic light energy conversion process and subsequently stored on different components of the electron transport chain of photosystem II (PS II). Corresponding to the various interactions between the positively charged donors and negatively charged acceptors, several TL bands can be observed in the TL glow curve [1–3].

In the presence of DCMU, an inhibitor of electron transport between the primary (Q_A) and secondary quinone electron acceptor (Q_B), illumination of PS II particles results in the transfer of an electron from the water-splitting system to Q_A. Back-reaction of the reduced Q_A^- with the donor side of PS II leads to the appearance of two main TL bands in the glow curve between 0 and 10°C and at about 50°C [4–6]. These bands are designated as Q and C bands, respectively. The Q band has been suggested to originate from charge recombination of the $S_2Q_A^-$ redox couple [4,5]. The C band has been attributed to charge recombination of Q_A^- with an unspecified donor, D^+ [6] or with Y_D^+ [1]. The pH dependence of the Q and C bands exhibits complementary behaviour [7,8]. By lowering the pH the Q band decreases with a concomitant intensification of the C band.

It has been demonstrated that the $Q_A^- \cdot Fe^{2+}$ semiquinone–iron complex gives rise to two EPR signals at $g = 1.9$ and $g = 1.82$ [9]. At higher pH the $g = 1.9$ form predominates whereas at lower pH the $g = 1.82$ form is observed [9]. The similar pH dependence of the Q and C TL bands and the $g = 1.9$ and $g = 1.82$ EPR forms raises the possibility that the Q and C bands are associated with the $g = 1.9$ and $g = 1.82$ resonance forms of the semiquinone–iron complex of PS II, respectively. The present work was undertaken to investigate this possible correlation.

2. MATERIALS AND METHODS

Oxygen evolving PS II particles were isolated from spinach or pea leaves [10] and stored at 77 K in a medium containing 0.4 M sucrose, 15 mM NaCl, 5 mM $MgCl_2$ and 20 mM MES, pH 6.0. Before TL and EPR measurements, samples were incubated at room temperature in the dark for 1 h in the presence of 50 mM sodium oxalate or 100 mM formate or without any addition. DCMU was added 3 min before freezing. TL was measured in an apparatus described in [6]. 0.4 ml aliquots of samples containing 100 μg chlorophyll were excited by white light of 10 W/m^2 at 200 K for 1 min and heated at a rate of 20°C/min. EPR spectra were obtained using a Bruker ER 200 D-SRC spectrometer interfaced to a PC and equipped with an Oxford ESR 900 cryostat. In the EPR measurement samples (3–6 mg Chl/ml) were illuminated at 200 K for 8 min with a projector lamp. The decay courses of the EPR signals were measured by subsequent warming and cooling the same sample to the incubation temperature and back to the measuring temperature. It is of note that after complete relaxation of the $g = 1.9$ and $g = 1.82$ signals they could be regenerated to their original amplitudes by a second illumination at 200 K. In the formate-

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Abbreviations: PS II, photosystem II; Q_A and Q_B, primary and secondary quinone acceptors of PS II; TL, thermoluminescence; EPR, electron paramagnetic resonance; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl-urea; Y_D, tyrosine-160 on the D_2 protein, accessory electron donor in PS II; Chl, chlorophyl.

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treated samples during the measurements of decay courses at +25°C, the fast-decaying components were eliminated by incubating the samples for 1 min at 10°C. The remaining levels of signals are used as the 0 min initial values of the decay courses. The amplitudes measured after excitation of samples at 200 K are considered as the 100% values of signals.

3. RESULTS

Fig. 1 shows the effects of various treatments on the Q and C TL bands (Fig. 1A) and on the $g = 1.9$ and $g = 1.82$ EPR signals (Fig. 1B). In the glow curve of isolated PS II particles excited at 200 K, four TL bands can be distinguished at about −70°C, +15°C, +50°C and +65°C (Fig. 1A, curve a). The low temperature band at about −70°C (Z_v) and the minor band at +65°C are not examined in the present work.

The main band appearing at about 15°C in the glow curve of our PS II particles originates from $S_2Q_B^-$ charge recombination and is designated as B band [1–3]. The trough at 0°C is an artifact due to a distortion of the band caused by the solid–liquid phase transition of water [11]. The small band at about 50°C is denoted as C band [6]. In the semiquinone–iron region of the EPR spectrum three signals can be distinguished at $g = 1.95$, $g = 1.9$ and a small signal at $g = 1.82$ (Fig. 1B, curve a). While the $g = 1.95$ signal was found to decay quickly even at 200 K, the $g = 1.9$ signal did not show any decay at this temperature. Therefore, the $g = 1.95$ signal observed in the present work does not seem to correlate with the $g = 1.95$ feature which has been assigned to an excited state of the $g = 1.9$ conformation [12]. Due to its decay at the temperature of illumination (200 K) we have not investigated the $g = 1.95$ signal further. The $g = 1.9$ and $g = 1.82$ signals represent two alternative conformations of the semiquinone–iron complex [9].

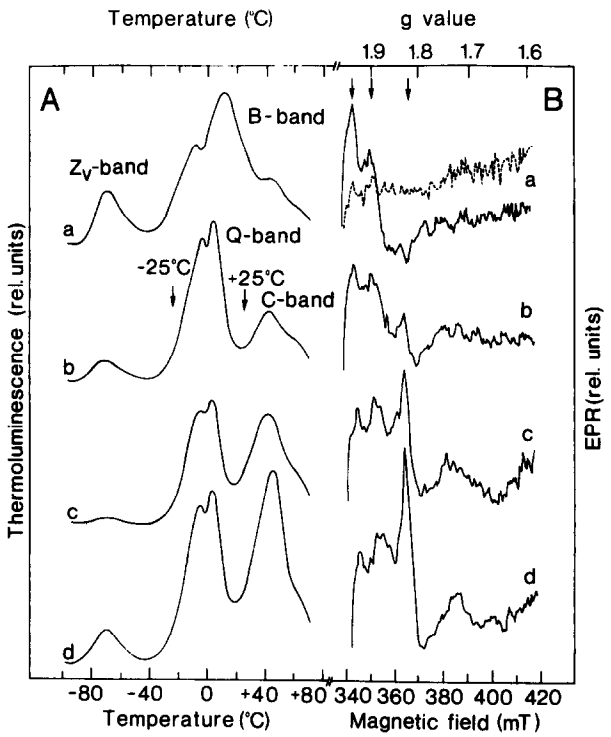

Fig. 1. Effect of various treatments on the TL (A) and EPR (B) spectra of PS II particles. (a) No addition, pH 6.0. (b) Addition of 20 μM (A) or 150 μM DCMU (B) at pH 6.0. (c) The same as (b), at pH 5.5. (d) Addition of 50 mM sodium oxalate and 20 μM (A) or 150 μM DCMU (B) at pH 6.0. Dashed line shows the spectrum recorded in the dark prior to illumination. EPR conditions: temperature, 4.4 K; microwave frequency, 9.42 GHz; microwave power, 31 mW; modulation amplitude, 16 G. Spectra shown are averages of 9 scans between 340 and 390 mT.

Following DCMU addition the B band, as in whole chloroplasts [5], was replaced by the Q band peaking at about 0°C and the amplitude of the C band increased (Fig. 1A, curve b). DCMU addition also increased the amplitude of the $g = 1.82$ signal of $Q_A^- \cdot Fe^{2+}$ (Fig. 1B, curve b) in agreement with [13]. Even a small decrease in the pH of the medium from pH 6.0 to 5.5 approximately doubled the C band (Fig. 1A, curve c) and the $g = 1.82$ signal (Fig. 1B, curve c). Addition of a carboxylate anion, oxalate, which is known to increase the $g = 1.82$ signal in the absence of DCMU [14], in the presence of DCMU gave rise to a large enhancement of both the C band (Fig. 1A, curve d) and the $g = 1.82$ signal (Fig. 1B, curve d).

The relationship between the Q and C bands and the $g = 1.9$ and $g = 1.82$ resonance forms was further investigated by comparing the dark decay half-times of the Q and C bands and of the $g = 1.9$ and $g = 1.82$ EPR signals at −25°C and +25°C (Figs. 2 and 3). The incubation temperatures, for the investigation of the dark relaxation of the excited PS II samples, were chosen at the rising sides of the Q and C bands in order to produce relatively long decay times [6,11] (see Fig. 1A, curve b).

Fig. 2 shows the decay of the Q and C bands as well as that of the $g = 1.9$, $g = 1.82$ and S_2 multi-line [15] signals at −25°C. The Q band ($t_{1/2} \approx 5$ min; Fig. 2A), the $g = 1.9$ signal ($t_{1/2} \approx 4$ min; Fig. 2B, solid line) and the multi-line signal ($t_{1/2} \approx 5$ min; Fig. 2B, dashed line) decay with similar half-times. Surprisingly, the $g = 1.82$ signal exhibits a biphasic decay at −25°C (Fig. 2B). Approximately 40% of it decays within 1 min. The fast-decaying portion of the signal indicates that almost half of the $g = 1.82$ form relaxes below −25°C and can not be associated with the slowly decaying Q or C TL bands (Fig. 2). Therefore, this phase has not been investigated in the present work. It probably participates in the generation of a low temperature TL band. Possible candidates are the Z_v band at about −65°C [1–3] or other hidden bands of very low intensity peaking in the glow curve at −55 and −35°C [16]. The slowly decaying phase of the $g = 1.82$ signal, like the C TL band, is stable at −25°C and hardly shows any decay during the 12 min period (Fig. 2).

The comparable decay kinetics measured after incubation at samples at +25°C were accelerated, resulting in a complete decay within 1 min of the S_2 multi-line and

the $g = 1.9$ signals, as well as the Q TL band (not shown). The $g = 3.08$ signal of cytochrome b_{559} did not exhibit any decay during the measuring period (not shown). The $g = 1.82$ signal decays with a half-time of about 7 min (Fig. 3B), a value which matches the 6 min decay half-time of the C band fairly well (Fig. 3A). The decay of the $g = 1.82$ signal is not accompanied by a similar decay of Signal II_s (Fig. 3B). However, increasing the amplitude of the $g = 1.82$ form by formate treatment [17] resulted in a fast decay of a large part of Signal II_s almost in parallel with that of the $g = 1.82$ signal (Fig. 3C). In the presence of formate the half-time of the $g = 1.82$ form ($t_{1/2} \approx 13$ min) and that of Signal II_s ($t_{1/2} \approx 16$ min) are somewhat longer than that of the $g = 1.82$ form in the untreated sample ($t_{1/2} \approx 7$ min). The decay kinetics of the C band in formate-treated samples are not presented in Fig. 3 because, as has been reported [18], formate quenches the TL emission and dampens the amplitude of the Q and C bands. Formate treatment (100 mM) decreased the amplitude of the S_2 multi-line signal to 60–70% of that observed in the untreated PS II particles. At −25°C its decay kinetics (not shown) were somewhat slower than in the untreated sample in Fig. 2B. At +25° the S_2 multi-line signal decayed within 1 min. It is of note that incubation of the formate-treated or untreated PS II samples at +10°C for 1 min also resulted in an almost complete disappearance of the S_2 multi-line signal (not shown).

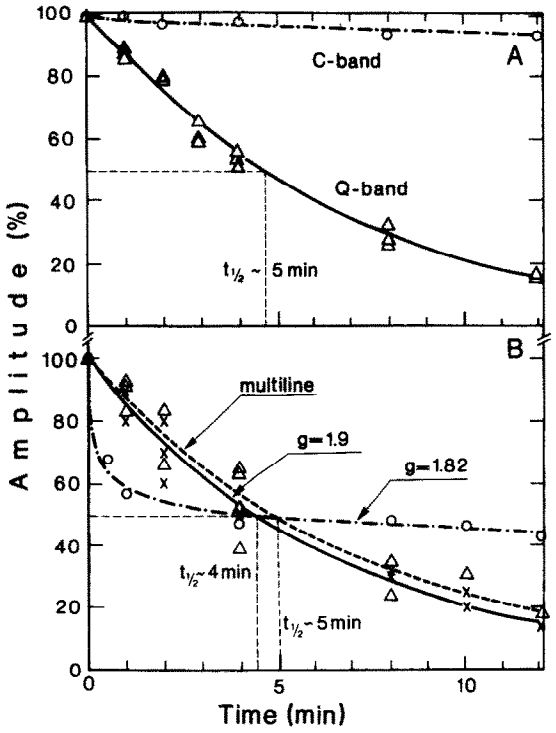

Fig. 2. Dark decay of the amplitude of the Q (△) and C (○) TL bands (A) and the S_2 multiline (△), $g = 1.9$ (×) and $g = 1.82$ (○) EPR signals (B) in PS II particles at −25°C. All samples were treated with DCMU as in Fig. 1b. After excitation at 200 K the samples (pH 6.0) were incubated in darkness at −25°C for the times indicated before measurements. EPR settings for the $Q_A^- \cdot Fe^{2+}$ and multi-line signals are as in Fig. 1 except that the measuring temperature of the S_2 multi-line signal was 10 K.

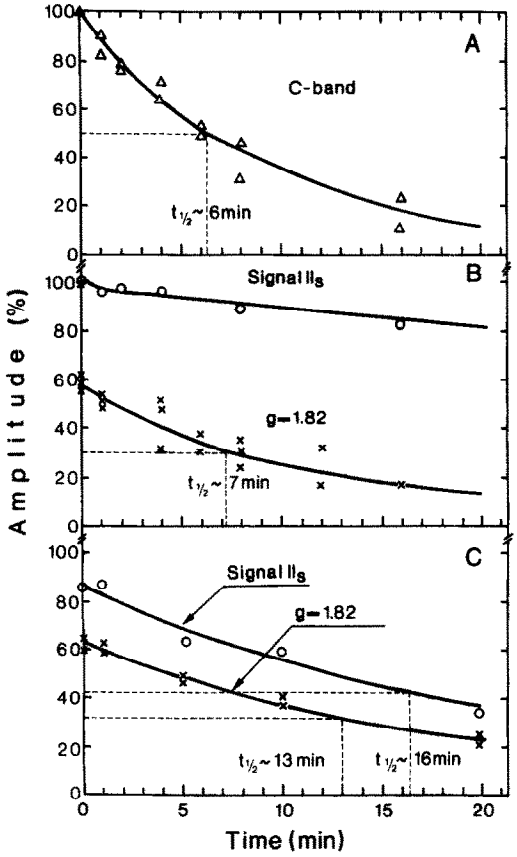

Fig. 3. Dark decay of the amplitude of the C (△) TL band (A) and that of the $g = 1.82$ signal (×) and Signal II_s (○) (B and C) at +25°C. A and B are the decays in untreated PS II particles, C in 100 mM formate-treated samples. All samples were treated with DCMU as in Fig. 1b. The samples (pH 6.0) were excited at 200 K, thawed and incubated in darkness at 10°C for 1 min and afterwards at +25°C for the times indicated. EPR settings for the $g = 1.82$ signal are as in Fig. 1; for Signal II_s; temperature, 13 K; microwave frequency, 9.42 GHz; microwave power, 200 nW; modulation amplitude, 4 G.

4. DISCUSSION

The aim of the present comparative TL and EPR study was to investigate the charge recombination in PS II particles in the presence of DCMU and to determine the origin of the Q and C bands.

The Q TL band was abolished at −25°C with a decay half-time of about 5 min. The positively charged S_2 state of the water-splitting system, which is represented by the multi-line signal, and the $g = 1.9$ form of the semiquinone–iron complex exhibited very similar decay half-times of 5 and 4 min, respectively. No other electron transport component was found to decay within the same time range. It has been reported that the $g = 4.1$ EPR signal of the S_2 state [19,20] and the Chl^+

free radical [21] decay rapidly at lower temperatures. Contrary to this, the $g = 3.08$ signal of oxidized cytochrome b_{559} did not display any decay during the measuring period of 16 min. Consequently, we suggest that it is the S_2 state, represented by the multi-line signal, which undergoes charge recombination with the $g = 1.9$ form of the semiquinone–iron complex, resulting in the generation of the Q TL band.

The reported decay half-times of the S_2 state [22] are longer than the value obtained in our measurements. However, these half-times were measured in the absence of DCMU, when the S_2 state recombines with Q_B^- instead of Q_A^-. It should be noted that in addition to the charge recombination with Q_A^- and Q_B^-, another possible decay route of the S_2 state involves a direct reduction by Y_D [23,24]. However, this reaction is slower ($t_{1/2}$ = 10–20 s at room temperature), especially at low pH, than the $S_2Q_A^-$ back-reaction ($t_{1/2}$ = 1–3 s at room temperature [1,5]).

The enhancement of the amplitude of both the C band and the $g = 1.82$ signal by DCMU, oxalate or low pH treatment supports our hypothesis that these signals are associated with each other. A relationship between the two signals is further corroborated by the measurements of decay half-times. At +25°C the decay half-time of the slowly decaying phase of the $g = 1.82$ signal ($t_{1/2} \approx 7$ min) and that of the C band are almost the same ($t_{1/2} \approx 6$ min). Therefore, we suggest that the C band can be assigned to the $g = 1.82$ form of the $Q_A^- \cdot Fe^{2+}$ semiquinone–iron complex.

The question arises as to the positively charged counterpart undergoing charge recombination with the slowly decaying phase of the $g = 1.82$ form. The S_2 state can be excluded because it interacts with the $g = 1.9$ form (see above) and its decay kinetics are faster (see also [25]) than that of the $g = 1.82$ form of $Q_A^- \cdot Fe^{2+}$. Moreover, the C TL band can be generated in manganese-free particles, as well, as in which the S_2 state is not functional [1,6,7]. We can assume that after 1 h dark incubation of our PS II samples, about 75% of the centers are in the S_1 state and 25% in the S_0 state. Illumination of the dark-adated samples at 200 K in the presence of DCMU induces only the $S_1 \rightarrow S_2$ and $S_0 \rightarrow S_1$ transitions of the water-splitting system. Consequently, in Fig. 2 the S_2 multi-line signal represents a dominating fraction of the centers which undergoes charge recombination with the $g = 1.9$ form and decays with the characteristic half-time of the Q band. In the S_1 state the water-splitting system has no net positive charge. In this smaller fraction of the PS II centers Q_A^- can enter into a charge recombination reaction only with an unidentified oxidized donor (D^+) generating the C TL band [6]. Thus, following illumination of the dark-adapted PS II samples at 200 K, the C band originates mainly from centers in the state S_1–D^+–Q_A^- ($g = 1.82$) that came from centers that were in the S_0 state before illumination. An additional part of the C band can probably originate from centers possessing an inactive water-splitting system.

The most likely candidates as positively charged donors participating in the generation of the C band are cytochrome b_{559} and Signal II_s [1]. However, at +25°C no decay of the $g = 3.08$ EPR signal of oxidized cytochrome could be observed during our measuring time of 16 min, eliminating the participation of cytochrome b_{559} in the generation of the C band. Surprisingly, in untreated PS II particles the decay of the $g = 1.82$ signal was not accompanied by a significant decay of Signal II_s either. We assumed that although Signal II_s (Y_D^+) undergoes charge recombination with the $g = 1.82$ form, as suggested in [1], the available small pool size of the $g = 1.82$ form is not enough to induce an apparent decay of Y_D^+ which possesses a much larger pool. It has been demonstrated that formate, which replaces bicarbonate at the non-heme iron, gives rise to a 10-fold increase in the amplitude of the $g = 1.82$ signal [17]. We expected that in the presence of formate a larger pool of the $g = 1.82$ form of the semiquinone–iron complex would result in the decay of a larger part of Signal II_s. Indeed, in formate-treated samples a large part of Signal II_s decayed almost in parallel with the $g = 1.82$ signal (Fig. 3C). The longer decay half-times of the $g = 1.82$ signal and of Signal II_s in formate-treated sample ($t_{1/2} \approx 13$ min and 16 min, respectively) than that of the $g = 1.82$ form measured in the absence of formate ($t_{1/2} \approx 7$ min) might be attributed to a formate-induced change in the non-heme iron environment. Moreover, the half-time of Signal II_s may be a little over-estimated due to the contribution of a small slowly decaying component [23,24].

On the basis of the simultaneous decays and the similar half-times of Signal II_s and the $g = 1.82$ signal in formate-treated particles, we suggest that Y_D^+ is the recombining partner of the $g = 1.82$ form of the semiquinone–iron complex in the generation of the C band. The observation that the dark decay of Signal II_s depends on the pool size of the $g = 1.82$ signal indicates that the redox state of the $Q_A^- \cdot Fe^{2+}$ complex can affect the dark equilibrium at the donor side of PS II (e.g. amplitude of Signal II_s). Interestingly, the recombination half-time of Y_D^+ with Q_A^- ($g = 1.82$) proved to be similar to that of the reduction of Y_D^+ by the S_0 state ($t_{1/2}$ = 10–40 min [23,24]).

Corresponding to the higher peak temperature of the C band relative to that of the Q band, the redox span of the redox pair responsible for the C band is larger by about 150 mV than that of the redox couple assigned to the Q band [8]. This means a difference in the E_m values of either the donors or acceptors participating in the generation of the Q and C bands. If we accept that the redox potential of the $g = 1.9$ and $g = 1.82$ forms are almost the same [26], then the redox potential of the donor responsible for the C band should be less positive by about 150 mV than that of the multi-line form of the

S_2 state. The 170–180 mV redox potential difference estimated between the Y_D^+/Y_D and S_2/S_1 couples [24] agrees well with that of the redox spans associated with the Q and C TL bands. Consequently, Y_D^+/Y_D satisfies the requirement for the E_m of the donor which can account for the C band.

However, as a second alternative, it can not be excluded at present that the E_m of the $g = 1.82$ form is more positive in comparison with that of the $g = 1.9$ form [27]. This would imply that the redox potentials of the donors (S_2 and Y_D) responsible for the Q and C bands respectively, should be approximately the same. To select between the two alternatives (see also [8]) the determination of the exact E_m values of the $g = 1.9$ and $g = 1.82$ forms as well as that of Signal II_s is required.

The assignment of the $g = 1.9$ and $g = 1.82$ EPR forms to the Q and C bands provides a means of investigating the semiquinone–iron complex. The considerably larger amplitude of the Q band in comparison with that of the C band in isolated chloroplasts and in alga cells [1–3] provides evidence that the $g = 1.9$ form is the native form of the semiquinone–iron complex, as previously suggested [9]. Interestingly, a small C band of variable amplitude is always present in the glow curve of chloroplasts and alga cells. Several factors which are influencing the rate of electron transport (low pH, bicarbonate depletion etc.) result in a conversion of the $g = 1.9$ form and the Q band into the $g = 1.82$ form and the C band, respectively [8,9,17,28]. It would be interesting to examine the role of the two forms of the semiquinone–iron complex in regulating the rate of in vivo electron transport.

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Note added in proof

At the completion of this work the authors were informed of a study by Johnson, G.N., Boussac, A. and Rutherford, A.W. (Biochim. Biophys. Acta, in press) on the origin of TL bands in the region of 40–50°C. There is agreement with the identification of Y_D^+ as the donor to the C band but Johnson et al. do not discriminate between the two conformations of the $Q_A^- \cdot Fe^{2+}$ state.