ELSEVIER

Biochimica et Biophysica Acta 1364 (1998) 46–54

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Thermoluminescence measurements on chloride-depleted and calcium-depleted photosystem II

Anja Krieger *, A. William Rutherford, Caroline Jegerschöld

Séction de Bioénergétique (CNRS URA 2096) Bât 532, CEA Saclay, 91191 Gif-sur-Yvette, France

Received 3 October 1997; revised 20 January 1998; accepted 21 January 1998

Abstract

Photosystem II (PSII) in which O_2 evolution was inhibited by depletion of either chloride or calcium ions was studied by thermoluminescence (TL) and luminescence (L) measurements in the presence and absence of 3-(3′,4′-dichlorophenyl)-1,1-dimethyl urea (DCMU). Cl^--depleted PSII gives rise to TL and L signals which are similar to those in untreated controls i.e., DCMU shifts the TL band from 30°C to 8°C and suppresses the L component with $t_{1/2} = 10–15$ s. In Ca^{2+}-depleted PSII a TL-band at around 50°C and a slow luminescence decay ($t_{1/2} = 60$ s) is observed. Under these conditions, DCMU does not lead to a downshift of the peak temperature of the TL-band nor does it accelerate the decay kinetics of the luminescence. This indicates that in Ca^{2+}-depleted PSII the Q_A/Q_B electron transfer is inhibited prior to the addition of DCMU while in Cl^--depleted PSII Q_A/Q_B electron transfer seems unaffected. These results are consistent with previous fluorescence measurements which showed that the midpoint potential of the redox couple Q_A/Q_A^- is unchanged in Cl^--depleted PSII compared to the control while in Ca^{2+}-depleted PSII it is shifted towards a more positive value [A. Krieger, A.W. Rutherford, Biochim. Biophys. Acta, 1319 (1997) 91–98]. In the literature there are several conflicting reports concerning the TL in Ca^{2+} and Cl^--depleted material so we attempted to understand the origin of some of these discrepancies. We find that in the absence of cryoprotectants, excitation of TL at low temperatures leads to an upshift of TL-bands in Cl^--depleted PSII, both in the presence and absence of DCMU, while the peak temperature of TL-bands in control and Ca^{2+}-depleted PSII are downshifted. When TL is excited at 20°C or at low temperature in the presence of a cryoprotectant then there was no shift of the peak temperature of TL-bands. These unexpected results suggest that the formation of the charge pair triggers modifications in its environment and that the exact nature of these modifications differs depending on the temperature of excitation. It seems that once these modifications have occurred at a given temperature they remain ‘locked in’ being unaffected by subsequent temperature changes until charge recombination has occurred.

Keywords: Photosynthesis; Photosystem II; Thermoluminescence; Cryoprotectant

Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethyl urea; Em, midpoint redox potential; L, luminescence; PSII, Photosystem II; Q_A, the first quinone acting as an electron acceptor in PSII; Q_B, the second quinone electron acceptor in PSII; TL, thermoluminescence; S-states, oxidation states of the Mn cluster; Tyr, tyrosine

* Corresponding author. Present address: Institut für Biochemie, Universität Freiburg, Schänzlestr. 1, 79104 Freiburg, Germany. Fax: +33-1-69088717.

PII S0005-2728(98)00009-7

1. Introduction

Photosynthetic oxygen evolution is reversibly inhibited by depleting PSII of chloride or calcium ions [1–3]. Cl^-- and Ca^{2+}-depletion lead to comparable lesions on the donor-side of PSII [3].

Despite the comparable state of inhibition of the water splitting complex, Ca^{2+}- and Cl^--depleted PSII are different with regard to the redox potential (Em) of Q_A, the primary quinone acceptor of PSII. In Ca^{2+}-depleted PSII, the redox potential of Q_A is shifted by about 150 mV towards a more positive potential (high potential form) [4,5]. In Cl^--depleted PSII the Em of Q_A stays at its normal low potential [6]. In Ca^{2+}-depleted PSII, the electron transfer from Q_A to Q_B is inhibited [7,8] as predicted from thermodynamic considerations based on the increased midpoint potential of Q_A [7]. On this basis electron transfer between Q_A and Q_B is not expected to be inhibited in Cl^--depleted PSII.

Luminescence (L) and thermoluminescence (TL) measurements can be used to test whether the electron transfer between Q_A and Q_B is inhibited [for reviews see Refs. [9–11]] and these methods should provide support for our contention that Ca^{2+}-depleted PSII is impaired while Cl^--depleted PSII should be unimpaired at this electron transfer step.

The light emitted in TL and L originates from recombination of trapped charge pairs. The charge pairs involved can be identified either by their decay kinetics (luminescence measurements) or their emission temperature (thermoluminescence). The main advantage of TL compared to L measurements is that the components are more easily resolved than luminescence decay phases. The peak position of a TL band strongly depends on the redox potentials of the charge pair involved. For example, recombination of the S_2 or the S_3 state of the water-splitting complex with the semiquinone, Q_B^-, yields a TL band (B-band) around 30°C [12] while in the presence of DCMU the recombination between the S_2 and/or S_3 state and Q_A^- yields a TL band (Q-band) around 10°C [12]. In luminescence measurements the half-times for the decay components correspond to the peak temperatures in TL. For $S_2Q_A^-$ the $t_{1/2} \sim 3$ s [13,14] and for the $S_2Q_B^-$ the $t_{1/2} \sim 20$ s [14].

The literature contains several studies of Ca^{2+}-depleted PSII and Cl^--depleted PSII but the data are sometimes contradictory. Ca^{2+}-depleted PSII centres, which are inactive in water splitting and have Q_A with a high redox potential, show a TL emission between 45°C and 55°C [15–18,29]. While this band has been attributed to a modified $S_2Q_A^-$ charge pair by some workers [e.g., Ref. [15]], it was suggested this band could arise from the charge pair Tyr_D^+ Q_A^- by analogy to the suggested assignment of a TL band at similar temperatures (known as the C-band) found in control PSII [29].

It was also reported that Cl^--depletion of PSII leads to an upshift of the peak temperature of the Q- and B-band [19–24]. This up-shift in the peak temperature in Cl^--depleted PSII was interpreted as a stabilization of the S_2 state due to a decrease of its redox potential by 60–80 mV [20]. In this case, however, the results are complicated by some reports in which no shift in the peak temperature was observed. The pH-value of the medium and the presence of the extrinsic proteins seem to play an important role for the peak temperature of Q-and B-band in Cl^--depleted PSII [19,21,22].

In the present study, we measured thermoluminescence and luminescence from Cl^--depleted, Ca^{2+}-depleted and control PSII membranes prepared using the same methods which were used earlier for our studies of the Em of Q_A [6]. In this way we tested and compared the influence of the Em of Q_A on electron transfer and charge recombination in PSII inhibited by Ca^{2+} or Cl^--depletion.

2. Materials and methods

PSII-enriched membrane fragments from spinach were prepared essentially as described by Ref. [25] with modification as described by Ref. [29]. The activity of these samples was about 500 μmol O_2/mg chl $*$ h. Ca^{2+}-depletion was performed by incubation of PSII samples at room temperature for 5 min in room light (10–12 μmol quanta m^{-2} s^{-1}) in a buffer containing 300 mM sucrose, 50 mM KCl, 5 mM $MgCl_2$, 25 mM succinic acid (pH 4.2). Incubation was stopped by adding a buffer containing 300 mM sucrose, 50 mM KCl, 5 mM $MgCl_2$, 80 mM MES (pH 6.5). The residual activity measured at pH 6.5 was very low (20–50 μmol O_2/mg chl $*$ h). By readdition of $CaCl_2$, 70–80% of the activity of a

control sample was obtained. Cl^--depletion was done by alkaline pH-treatment in room light as described by Ref. [26] and 'Superpur' sucrose was used to reduce the chloride contamination. PSII samples were incubated at pH 10 for 30 s before lowering the pH. This reduced the activity to less than 50 μmol O_2/mg chl $*$ h when measured at pH 6.5. By readdition of NaCl, 80% of the activity of a control sample was obtained in these samples. Cl^--depletion by sulfate treatment was performed as described [20].

Thermoluminescence and luminescence were measured with the equipment described by Ref. [27]. The presence of cryoprotectants during the measurement is given in the figure legends. TL was charged by giving one single-turnover flash with a Stroboslave lamp followed by rapid freezing in liquid N_2. The temperature of illumination was varied between the different measurements and is given in the figure legends. Excitation of TL at room temperature resulted in lower TL intensity than excitation at -20°C or at -40°C, because recombination reactions occurred already during the cooling and before the actual measurement was started. The TL signal was then recorded during warming to 80°C at a heating rate of 0.5°C/s. Luminescence decays were measured using the same set up, the temperature was kept at 20°C during the illumination and the measurement. Luminescence traces were treated with a fitting procedure as described in Ref. [27].

3. Results

Fig. 1 shows thermoluminescence curves from control, Cl^--depleted and Ca^{2+}-depleted PSII-enriched membrane fragments in the absence (Fig. 1A) and in the presence of DCMU (Fig. 1B). TL was excited by a single-turnover flash at -20°C (Fig. 1A) or -40°C (Fig. 1B), in a medium containing 25% glycerol. In the presence of DCMU, a lower excitation temperature was chosen in order to obtain the complete TL emission curve. In control and Cl^--depleted PSII, similar TL bands were observed: a band at 30°C (the B-band), arising from $S_2Q_B^-$ recombination, (Fig. 1A) and a band at approximately 8°C (the Q-band), arising from $S_2Q_A^-$ recombination, was present when treated with DCMU (Fig. 1B).

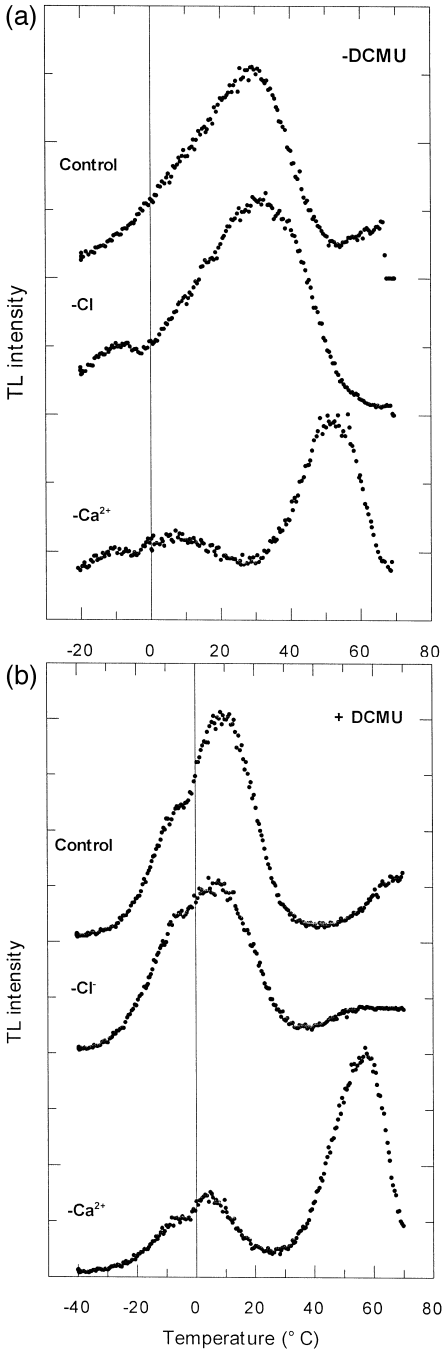

Fig. 1. Thermoluminescence signals from control, Cl^-- and Ca^{2+}-depleted PSII particles in the absence (A) and presence (B) of DCMU. TL was excited by giving a flash at -20°C (A) or -40°C (B). The medium contained 25% glycerol.

In the presence of DCMU Ca^{2+}-depleted PSII shows a different behaviour: the peak temperature was upshifted. In the absence of DCMU, a band with

a peak temperature at 51°C (Fig. 1A) was observed, which was slightly shifted to 57°C in the presence of DCMU (Fig. 1B). Similar peak temperatures between 45° and 55°C were reported earlier for Ca^{2+}-depleted PSII [15,17,29].

In Ca^{2+}-depleted PSII a small Q-band around 5°C is present, in addition to the high temperature band. In the absence of DCMU, the emission is broader and can be fit by two bands corresponding to the Q- and B-bands. These bands might reflect PSII not affected by the Ca^{2+}-depletion: either active PSII centres containing Q_B (B-band) or active centres which lost Q_B during the preparation (Q-band). It is understandable that the latter centres should be preferentially protected against Ca^{2+} depletion since Ca^{2+} is much more rapidly lost from the S_3 state [28], a state which would be absent in centres lacking electron transfer to Q_B. In the control and Cl^--depleted PSII a certain proportion of a Q-band also seems to be present (Fig. 1A).

Luminescence decays were measured in control, Cl^-- and Ca^{2+}-depleted PSII. In the absence of DCMU, the luminescence decays were fit with three components. The half-times and relative contributions of the areas of these components are given in Table 1. Component 2 reflects luminescence arising from $S_2Q_A^-$ recombination with a half-time between 1–5 s, component 3 reflects $S_2Q_B^-$ recombination with a half-time between 9–15 s and component 1 corresponds to faster recombination reactions which were not well resolved using the experimental set-up [13,14]. As expected, DCMU leads to a faster decay in Cl^--depleted and untreated PSII, the decay kinetics can be fit well by two components. In the absence of DCMU, the luminescence decay of Cl^--depleted PSII was only slightly faster than in the control. This is largely attributable to the fraction of damaged centres (i.e., those which do not recover activity upon Cl^- addition and which probably have lost their Mn) which are expected to give rise to an increase in the fast phase (t_1). The luminescence results are in agreement with the results in Fig. 1 where the TL peak emissions were comparable for the control and Cl^--depleted PSII.

Table 1
Luminescence measurements of control, Ca^{2+}-depleted and Cl^--depleted PSII enriched membrane fragments in the absence and presence of DCMU

−DCMU	t_1	A_1	t_2	A_2	t_3	A_3
Control	0.23	9	2.74	33	15	58
$-Cl^-$	0.23	20	1.1	34	9.88	46
$-Ca^{2+}$	0.21	7	2.18	31	57.2	62

+DCMU	t_1	A_1	t_2	A_2	t_3	A_3
Control	0.86	55	3.21	45	−	−
$-Cl^-$	0.29	50	4.31	50	−	−
$-Ca^{2+}$	1.0	28	5.44	21	58.5	41

The luminescence decays were fit according to the program by Ducruet and Miranda [27]. Half-times in seconds and areas (%) of the components are given. Samples in the absence of DCMU and Ca^{2+}-depleted PSII in the presence of DCMU were measured with a time resolution of 100 ms. Control and Cl^--depleted PSII in the presence of DCMU were measured with a time resolution of 50 ms and fit with two components. The measurements were repeated 15 times.

The luminescence from Ca^{2+}-depleted PSII is very different to control and Cl^--depleted PSII. The luminescence decay which is fit with three components is much slower and is dominated by a slow phase ($t_{1/2} = 60$ s) which does not occur in the other samples. This slow phase in luminescence may reflect the high temperature band in thermoluminescence. As in TL (Fig. 1), no significant effect of DCMU can be observed on the luminescence decay kinetics of Ca^{2+}-depleted PSII.

The TL measurements of Cl^--depleted PSII shown here are in disagreement with earlier observations in which an upshift of the peak temperature of both the Q-band and the B-band in Cl^--depleted samples were reported [19–23,25]. The question arises why previously a higher peak temperature in TL was found in Cl^--depleted PSII than in control samples [19,20], while we find that the temperature maxima of the thermoluminescence bands are the same (Fig. 1). Upon comparing experimental conditions, we noted that these studies in the literature were done in the absence of glycerol in the medium while we included 25% glycerol in the medium. Therefore, we repeated the experiment in the absence of glycerol and found that Cl^--depleted PSII gave rise to thermoluminescence which was essentially the same as that reported earlier (Fig. 2) [19,20,23]. The B-band was upshifted in Cl^--depleted PSII by 8°C from 31°C in the presence of glycerol to 39°C in the absence of glycerol (Fig. 2A). In the presence of DCMU, the upshift of

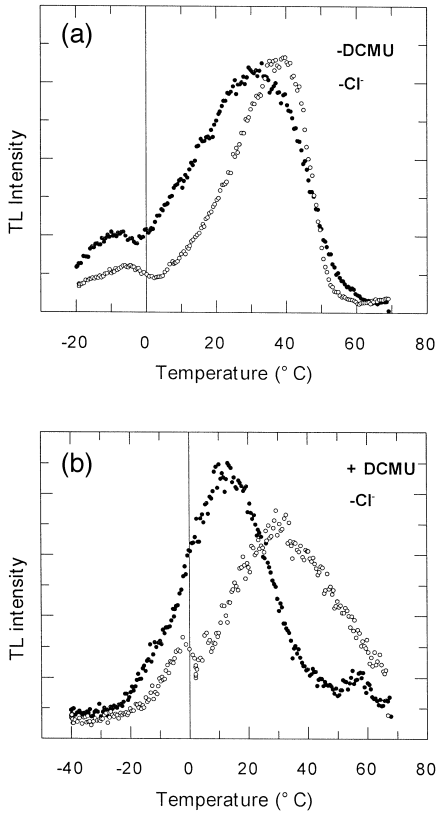

Fig. 2. Thermoluminescence signals from Cl^- depleted PSII particles in the absence (A) and in the presence (B) and of DCMU. Signals were measured in the presence (filled circles) and absence (open circles) of 25% glycerol. Samples were excited at −20°C (A) or at −40°C (B).

the peak temperature was much more pronounced: the Q-band was upshifted to approximately 32°C compared to approximately 10°C in the presence of glycerol (Fig. 2B).

These results indicate that the upshift in the peak temperature of the TL-bands in Cl^--depleted PSII depends on the composition of the medium and is not an intrinsic change of the stability of the S_2 state induced by Cl^--depletion.

Another difference between the present work and several literature reports is the method used for depleting Cl^- in that a sulfate treatment was used earlier [19,20] rather than the high pH treatment used here. When we performed Cl^--depletion by sulfate treatment, we obtained exactly the same peak temperatures as shown in Fig. 1 (presence of glycerol) and Fig. 2 (absence of glycerol) for the Cl^--depletion

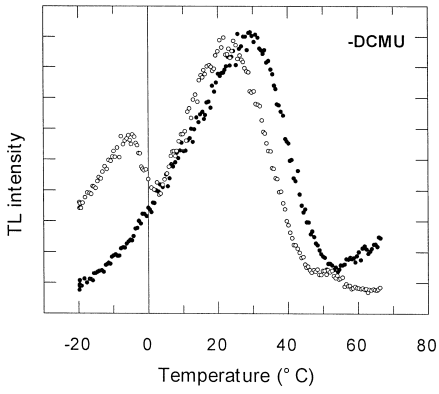

Fig. 3. Thermoluminescence signal of control PSII particles. Signals were measured in the presence (filled circles) and absence (open circles) of 25% glycerol in the absence of DCMU. Samples were excited at −20°C.

obtained by short incubation at pH 10. This indicates that this difference in terms of the method of Cl^--depletion has no influence on the peak temperatures (data not shown).

Fig. 3 shows that a glycerol effect on TL-bands exists also for control samples when excitation energy is given at low temperatures (−20°C). In Fig. 3 and Table 2, we focused on the B-band. In the presence of glycerol, the B-band is observed around 30°C which is shifted by 8°C to a lower temperature in the absence of glycerol. The peak position of the Q-band was also shifted to a similar extent to lower temperatures in the absence of glycerol (data not shown). The effect of glycerol-addition on the peak position of the B-band is opposite in control and Cl^--depleted PSII: an upshift in the case of the control and a downshift of the peak temperature in the case of Cl^--depleted PSII. Glycerol addition also leads to an upshift by approximately 9°C of the

Table 2
Effect of different cryoprotectants on the peak position of the B-band

	Peak temperature of TL (°C)
No addition	22
25% glycerol	30
25% ethyleneglycol	30
10% DMSO	28

TL of untreated PSII enriched membrane fragments. TL was excited by one flash at −20°C.

thermoluminescence peak of the C-band in Ca^{2+}-depleted PSII. In the absence of glycerol, the peak position was at 42°C compared to 51°C in the presence of glycerol (not shown).

We investigated if other cryoprotectants have an effect on the peak temperature of TL-bands similar to that induced by glycerol. Table 2 shows the effect of different cryoprotectants on the peak position of the B-band in control samples excited by a single turnover flash at −20°C. Addition of 25% ethylene glycol or 10% DMSO to the medium gives the same result as addition of glycerol, the maximum emission of the B-band is at 30°C in the presence of cryoprotectant instead of 22°C in the absence of any cryoprotectant. This shows that the described effect are not due to an effect specific to glycerol but might be obtained by cryoprotectants in general.

Given the observation that cryoprotectants seem to influence the thermoluminescence peak temperature, we wished to determine if the effects seen with cryoprotectants were due a direct effect of the cryoprotectant (due for example to viscosity) manifest at all temperatures or rather were they due to a low temperature-induced perturbation which was eliminated by the cryoprotectant. Therefore, we tested the influence of the temperature of excitation on the emission temperature of the B-band. In the following TL measurements, a single-turnover flash was given at 20°C followed by cooling to −20°C in a medium without glycerol and the peak positions of the B-band in Cl^--depleted PSII and the control were monitored. As shown in Fig. 4, when the flash was given at 20°C it resulted in TL emission peaking at the same temperature (∼35°C) in both control and the Cl^--depleted samples. Furthermore, for the Ca^{2+}-depleted PSII, freezing after a flash at room temperature (Fig. 4) or flashing at −20°C in the presence of glycerol (Fig. 1A) gives a TL peak temperature at around 50°C in both cases.

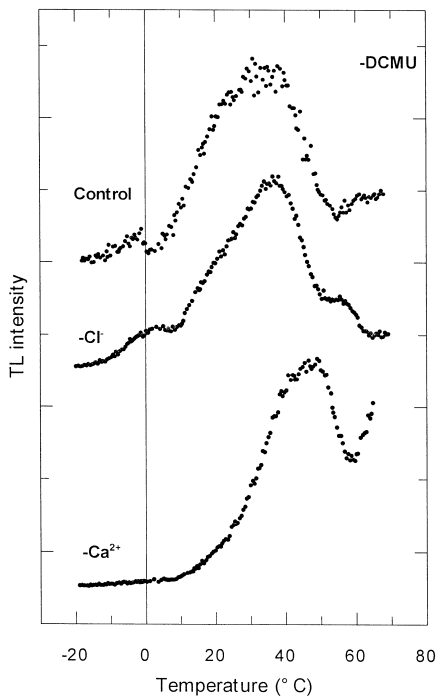

Fig. 4. Thermoluminescence signals from control, Cl^-- and Ca^{2+}-depleted PSII particles. Samples were excited at 20°C and then quickly frozen to −20°C. Signals were measured in the absence of glycerol and DCMU.

Table 3
Effect on the excitation temperature (−20°C or 20°C) on the peak position of the B-band in different material in the absence of cryoprotectants

	TL peak (°C), one flash at −20°C	TL peak (°C), one flash at 20°C
PSII particles	22	33
Thylakoid membranes	22	35
Intact chloroplasts	35	35
Spinach leaves	35	35

We measured the B-band of untreated PSII after excitation at 20°C and −20°C in a range of samples that varied in terms of how native was the biological material. As shown in Table 3, a downshift of the peak temperature of the B-band after excitation at −20°C is seen both in PSII particles and thylakoid membranes. In contrast, intact chloroplasts and leaves showed the B-band emission maximal at 35°C and independent of the excitation temperature.

4. Discussion

The results of the thermoluminescence and luminescence measurements show that in Ca^{2+}-depleted PSII the pair of radicals formed with a single flash is perturbed compared to control material. In Ca^{2+}-depleted PSII DCMU does not lead to a downshift of

the TL peak temperature, this may be taken as indicating that electron transfer from Q_A to Q_B is perturbed prior to DCMU-addition. This is consistent with what is expected as a thermodynamic consequence of a shift in the Q_A midpoint potential by approximately +150 mV making it close to that of Q_B [5,7]. This result then confirms the indications from fluorescence measurements that Q_A to Q_B electron transfer is impaired in Ca^{2+}-depleted PSII [7,8].

It was also suggested earlier that another consequence of the Q_A Em upshift would be to make the charge recombination via the P^+Ph^- radical pair less favourable [7]. This was predicted to lead to less P680 triplet formation (and therefore less singlet oxygen-mediated photodamage) and less luminescence. The slow time-scale of the luminescence decay seen in Ca^{2+}-depleted material and the high temperature of the thermoluminescence band may be consistent with this idea in that a large activation energy may be needed for this recombination reaction.

Cl^--depleted centres, in which Q_A is in its normal low potential form [6], behave in thermoluminescence and luminescence like controls. After excitation of L and TL by one single turnover flash, no difference can be detected between these samples. Electrons are transferred to Q_B and the addition of DCMU results in an inhibition of the electron transfer from Q_A to Q_B. It seems that the radical pairs formed in Cl^--depleted PSII are not perturbed compared to those of the control. This is also in accordance with recent results where redox titrations showed that the Em of Q_A in Cl^--depleted centres was the same as in the control [6]. This result also implies that the S_2 state in Cl^--depleted PSII is unchanged in terms of its redox potential. This result seems to contradict a number of earlier reports [19,20,23,30,31] in which it was suggested that the S_2 state in Cl^--depleted PSII was significantly stabilized. The discrepancy with some reports (those done at a similar pH value to that used here) can be attributed to the use of low temperature excitation to charge the Q-band in the absence of cryoprotectants [19–22]. When cryoprotectants are present (Fig. 1A,B) or when excitation for the B-band is given at room temperature (Fig. 4, Ref. [20]) no shift is observed. In some cases in the literature excitation at room temperature was used and yet stabilization was observed [20,23]. It seems that this occurs at higher pH values (i.e., pH 7.5; [20,23]) than used here. A study of the pH dependence of the stability of the $S_2Q_B^-$ (and $S_2Q_A^-$) has been published [19] and indicates that Cl^--depletion induced a stabilization which is much more marked at higher pH-values. [1] In what follows, we discuss some technical aspects of the TL measurements which are relevant to these apparent discrepancies.

The results show unexpected effects of the temperature of excitation on the stability of the charge pairs responsible for TL. In control samples (and Ca^{2+}-depleted samples) it was found that low temperature excitation resulted in TL at a lower temperature than the TL generated by excitation given at higher temperature. In the Cl^--depleted sample, the reverse was seen: low temperature excitation gave TL at a temperature higher than that seen when TL was generated at 20°C. The effect of low temperature excitation was eliminated by the presence of a cryoprotectant. The presence of the cryoprotectant itself had no influence on the peak position as demonstrated by (a) the experiments which showed identical TL peak positions with and without cryoprotectant when illumination was given at 20°, and (b) the similarities between the luminescence results (room temperature excitation, no cryoprotectants) and the thermoluminescence results of Fig. 1 (low temperature excitation, with glycerol).

The results indicate that in the absence of a cryoprotectant the charge pairs responsible for TL are energetically different and that this depends on the temperature of their formation. Once formed at a given temperature, the different charge pairs retain their energetic properties irrespective of subsequent temperature changes. This suggests that modifications (e.g., conformation and/or protonation changes in the protein and/or cofactors) in the environment of

[1] In addition, there are two reports in the literature where excitation is given at 5°C [21,22]. In this case, Cl^--depleted PSII exhibited no shift despite being illuminated at low temperature in the absence of cryoprotectants. We have studied the excitation temperature dependence and found that by coincidence, 5°C is the critical temperature for the observed effect. Flashing at 5°C or higher leads to no upshift of the B-band while excitation below 5°C results in the upshift. It, thus, seems possible that the data in Refs. [21,22] are equivalent to those obtained at room temperature.

the charge pair are triggered by its formation and that these modifications are dependent on the temperature at which the charge pair is formed. The effect of the cryoprotectants seems to be to maintain the native (room temperature) reactions even at low temperatures.

The phenomena reported here, with its excitation temperature-dependent shifts in the TL temperature, shifting either to higher or lower temperature depending on the biochemical pretreatment, are difficult to interpret in terms of specific acceptor or donor side effects. Indeed, it seems likely that effects on both halfs of the charge pair are involved. At least for the case of the chloride-depleted PSII, where the excitation temperature dependent shifts can be quite large (i.e., a 20°C stabilisation for the TL band formed in the presence of DCMU), it is worth considering the possibility that the cofactor bearing the positive charge may be different when excited at low temperature (without a cryoprotectant) compared to that formed when excited at high temperature. Indeed such an effect might contribute to the bewildering range of phenomena reported in the literature on chloride-depleted PSII [2,3,32–35]. More direct spectroscopic methods are needed to test this possibility.

In earlier work, an effect of the binding of extrinsic polypeptides has been shown to influence the TL peak position in chloride and calcium depleted PSII [16,18,21,26]. It is possible that the binding of the polypeptides may play a role in the phenomenology reported here [see also Ref. [36]], however specific experimentation is required to address this question. Of note is the recent report that the presence of glycerol allows reconstitution of the native polypeptide environment on the donor side of PSII [37], so further investigation may be of interest.

The results also show that although the excitation temperature-dependent effects were evident in both thylakoids and PSII-enriched membranes, such effects were absent in intact chloroplasts and in leaves. We propose that the absence of such effects in the more native material is due to the presence of osmolytes which act as intrinsic cryoprotectants. The present results provide information which is technically useful to researchers using TL to study PSII: it seems clear that comparisons of TL bands generated by excitation at different temperatures should be avoided unless a cryoprotectant is present.

Acknowledgements

We would like to thank Peter Homann for stimulating discussions. C.J. was supported by a FEBS post-doctoral fellowship, A.K. was supported by the European Science Foundation and by a grant from the Deutsche Forschungsgemeinschaft (DFG). This work was supported by a grant from the Human Capital and Mobility Network program (HCM Grant ERBCHRXCT940525).

References

[1] C.F. Yocum, Calcium activation of photosynthetic water-oxidation, Biochim. Biophys. Acta 1059 (1991) 1–15.
[2] R.J. Debus, The manganese and calcium ions of photosynthetic oxygen evolution, Biochim. Biophys. Acta 1102 (1992) 269–352.
[3] A.W. Rutherford, J.-L. Zimmermann, A. Boussac, Oxygen evolution, in: J. Barber (Ed.), The Photosystems: Structure, Function and Molecular Biology, Elsevier, Amsterdam, 1992, pp. 179–229.
[4] A. Krieger, E. Weis, Energy-dependent quenching of chlorophyll-a-fluorescence: the involvement of proton-calcium exchange at photosystem II, Photosynthetica 27 (1992) 89–98.
[5] A. Krieger, A.W. Rutherford, G.N. Johnson, On the determination of the redox midpoint potential of the primary quinone acceptor, Q_A, in photosystem II, Biochim. Biophys. Acta 1229 (1995) 193–201.
[6] A. Krieger, A.W. Rutherford, Comparison of chloride and calcium depleted photosystem II: the midpoint potential of Q_A and susceptibility to photodamage, Biochim. Biophys. Acta 1319 (1997) 91–98.
[7] G.N. Johnson, A.W. Rutherford, A. Krieger, A change in the midpoint potential of the quinone Q_A in photosystem II associated with photoactivation of the primary quinone acceptor Q_A, Biochim. Biophys. Acta 1229 (1995) 201–207.
[8] L.E. Andréasson, I. Vass, S. Styring, Ca^{2+}-depletion modifies the electron transfer on both donor and acceptor sides in photosystem II from spinach, Biochim. Biophys. Acta 1230 (1995) 155–164.
[9] P.A. Jursinic, Delayed fluorescence: Current concepts and status, in: Govindjee, J. Amesz, D.C. Fork (Eds.), Light Emission from Plants and Bacteria, Academic Press, London, 1986, pp. 291–328.
[10] P.V. Sane, A.W. Rutherford, Thermoluminescence from photosynthetic membranes, in: Govindjee, J. Amesz, D.C. Fork (Eds.), Light Emission from Plants and Bacteria, Academic Press, London, 1986, pp. 329–362.
[11] I. Vass, Y. Inoue, Thermoluminescence in the study of Photosystem II, in: J. Barber (Ed.), The Photosystems: Structure, Function and Molecular Biology, Elsevier, Amsterdam, 1992, pp. 259–294.

[12] A.W. Rutherford, A.R. Crofts, Y. Inoue, Thermoluminescence as a probe of photosystem II photochemistry. The origin of the flash-induced glow peaks, Biochim. Biophys. Acta 682 (1982) (1982) 457–465.
[13] J. Lavergne, A.L. Etienne, Prompt and delayed fluorescence of chloroplasts upon mixing with dichlorophenyldimethylurea, Biochim. Biophys. Acta 593 (1980) 136–140.
[14] A.W. Rutherford, Y. Inoue, Oscillation of delayed luminescence from PSII: recombination of $S_2Q_B^-$ and $S_3Q_B^-$, FEBS Lett. 165 (1984) 163–170.
[15] T. Ono, Y. Inoue, Removal of Ca^{2+} by pH 3.0 treatment inhibits S_2 to S_3 transition in photosynthetic oxygen evolving system, Biochim. Biophys. Acta 973 (1989) 443–449.
[16] T. Ono, S. Izawa, Y. Inoue, Structural and functional modulation of the manganese cluster in Ca^{2+}-depleted photosystem II induced by binding of the 24-kilodalton extrinsic protein, Biochemistry 31 (1992) 7648–7655.
[17] A. Krieger, E. Weis, S. Demeter, Low pH induced Ca^{2+} ion release in the water splitting system is accompanied by a shift in the redox potential of the primary quinone acceptor Q_A, Biochim. Biophys. Acta 1144 (1993) 411–418.
[18] P.H. Homann, L.V. Madabusi, Modification of the thermoluminescence properties of Ca^{2+} depleted photosystem II membranes by the 23 kDa extrinsic polypeptide and by oligocarboxylic acids, Photosynth. Res. 35 (1993) 29–39.
[19] P.H. Homann, H. Gleiter, T. Ono, Y. Inoue, Storage of abnormal oxidants $\Sigma_1,\Sigma_2,\Sigma_3$ in photosynthetic water oxidases inhibited by Cl^- removal, Biochim. Biophys. Acta 850 (1986) 10–20.
[20] I. Vass, T. Ono, Y. Inoue, Stability and oscillation properties of thermoluminescence charge pairs in the O_2-evolving system depleted of Cl^- or the 33 kDa extrinsic protein, Biochim. Biophys. Acta 892 (1987) 224–235.
[21] A. Rashid, P.H. Homann, Properties of iodide-activated photosynthetic water-oxidizing complexes, Biochim. Biophys. Acta 1101 (1992) 303–310.
[22] P.H. Homann, Thermoluminescence properties of the S_2-state in chloride-depleted water oxidizing complexes after reconstituting treatments with various monovalent anions, Photosynth. Res. 38 (1993) 395–400.
[23] T. Ono, H. Nakayama, H. Gleiter, Y. Inoue, A. Kawamori, Modification of the properties of the S_2 state in photosynthetic O_2-evolving center by replacement of chloride with other anions, Arch. Biochem. Biophys. 256 (1987) 618–624.
[24] T. Ono, T. Noguchi, M. Kusunoki, Y. Inoue, H. Yamaguchi, H. Oyanagi, Properties of the Mn-cluster of photosynthetic oxygen evolving center in Ca^{2+} and/or Cl^- depleted photosystem II, in: P. Mathis (Ed.) Photosynthesis: from light to biosphere, Vol. II, Kluwer, Netherlands, 1995, 365–368.
[25] D.A. Berthold, G.T. Babcock, C.F. Yocum, A highly resolved, oxygen-evolving photosystem II preparation from spinach thylakoid membranes, FEBS Lett. 134 (1981) 231–236.
[26] P.H. Homann, The chloride and calcium requirement of photosynthetic water oxidation: effects of pH, Biochim. Biophys. Acta 934 (1988) 1–13.
[27] J.-M. Ducruet, T. Miranda, Graphical and numerical analysis of thermoluminescence and fluorescence Fo emission in photosynthetic material, Photosynth. Res. 33 (1992) 15–28.
[28] A. Boussac, A.W. Rutherford, Ca^{2+}-binding to the oxygen evolving enzyme varies with the redox state of the Mn cluster, FEBS Lett. 236 (1988) 432–436.
[29] G.N. Johnson, A. Boussac, A.W. Rutherford, The origin of 40–50°C thermoluminescence bands in photosystem II, Biochim. Biophys. Acta 1184 (1994) 85–92.
[30] A. Muallem, J. Farineau, M. Laine-Böszormenyi, S. Izawa, Charge storage in Cl^--depleted chloroplasts, in: G. Akoyunoglou (Ed.), Photosynthesis II. Electron transport and photophosphorylation, Balaban International Science Services, Philadelphia, 1981, pp. 435–443.
[31] T. Ono, J.L. Zimmermann, Y. Inoue, A.W. Rutherford, EPR evidence for a modified S-state transition in chloride-depleted photosystem II, Biochim. Biophys. Acta (1986) 193–201.
[32] A. Boussac, A.W. Rutherford, Secrets of the Universe unfolded before your very eyes, J. Biol. Chem. 269 (1994) 12462–12467.
[33] M. Haumann, W. Drevenstedt, M. Hundelt, W. Junge, Photosystem II of green plants. Oxidation and deprotonation of the same component (histidine?) on $S_1* \rightarrow S_2*$ in chloride-depleted centres as on $S_2 \rightarrow S_3$ in controls, Biochim. Biophys. Acta 1273 (1996) 237–250.
[34] P. van Vliet, A.W. Rutherford, Properties of the chloride-depleted oxygen-evolving complex of photosystem II studied by electron paramagnetic resonance, Biochemistry 35 (1996) 1829–1839.
[35] H. Wincencjusz, H.J. van Gorkom, C.F. Yocum, The photosynthetic oxygen evolving complex requires chloride for its redox state $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions but not for $S_0 \rightarrow S_1$ or $S_1 \rightarrow S_2$ transitions, Biochemistry 36 (1997) 3663–3670.
[36] P.H. Homann, Stabilization of the water oxidizing polypeptide assembly on photosystem II membranes by osmolytes and other solutes, Photosynth. Res. 33 (1992) 29–36.
[37] M. Haumann, M. Hundelt, P. Jahns, S. Chroni, O. Bögerhausen, D. Ghanotakis, W. Junge, Similar stoichiometries are stabilized in thylakoids and PSII core particles by glycerol, FEBS Lett. 410 (1997) 243–248.