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Inactivation of photosynthetic oxygen evolution by UV-B irradiation: A thermoluminescence study

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Abstract

The influence of UV-B irradiation on photosynthetic oxygen evolution by isolated spinach thylakoids has been investigated using thermoluminescence measurements. The thermoluminescence bands arising from the $S_2Q_B^-$ (B band) and $S_2Q_A^-$ (Q band) charge recombination disappeared with increasing UV-B irradiation time. In contrast, the C band at 50 °C, arising from the recombination of Q_A^- with an accessory donor of Photosystem II, was transiently enhanced by the UV-B irradiation. The efficiency of DCMU to block Q_A to Q_B electron transfer decreased after irradiation as detected by the incomplete suppression of the B band by DCMU. The flash-induced oscillatory pattern of the B band was modified in the UV-B irradiated samples, indicating a decrease in the number of centers with reduced Q_B. Based on the results of this study, UV-B irradiation is suggested to damage both the donor and acceptor sides of Photosystem II. The damage of the water-oxidizing complex does not affect a specific S-state transition. Instead, charge stabilization is enhanced on an accessory donor. The acceptor-side modifications decrease the affinity of DCMU binding. This effect is assumed to reflect a structural change in the Q_B/DCMU binding site. The preferential loss of dark stable Q_B^- may be related to the same structural change or could be caused by the specific destruction of reduced quinones by the UV-B light.

Abbreviations: Chl – chlorophyll; DCMU – 3-(3,4,-dichlorophenyl)-1,1-dimethylurea; PS II – Photosystem II; Q_A – first quinone electron acceptor of PS II; Q_B – second quinone electron acceptor of PS II; Tyr-D – accessory electron donor of PS II; S_0–S_4 – charge storage states of the water-oxidizing complex

Introduction

Photosystem II (PS II) is a multifunctional pigment-protein complex in the thylakoid membrane, that catalyses the light-driven oxidation of water and the reduction of the plastoquinone pool (for recent reviews, see Andersson and Styring 1991, Debus 1992). The heart of PS II is the reaction center complex, consisting of the heterodimer of the D1 and D2 proteins (Nanba and Satoh 1987). The D1/D2 heterodimer binds several and perhaps all of the redox cofactors of light-induced PS II electron transport including the catalytic manganese cluster of water oxidation (see Andersson and Styring 1991).

During illumination, the water-oxidizing complex cycles through five oxidation states denoted S_0,...,S_4, releasing molecular oxygen in the S_3–S_4–S_0 transition (Kok et al. 1970). S_0 represents the most reduced state, while the higher S states represent successively higher oxidation states. The oxidative power in the higher S states is stored, at least partially, on Mn ions constituting the catalytic site of water oxidation. The electrons, which are extracted from the water-oxidizing complex, are transferred to a mobile pool of plastoquinone molecules via the Q_A and Q_B quinone electron acceptors.

The delicate machinery of PS II is sensitive to various environmental stress factors. These include, perhaps surprisingly, light itself. Strong illumination in the photosynthetically active wavelength range (400–700 nm) results in the impairment of electron transport through PS II (Powles 1984) and causes subsequently the damage and degradation of the D1 reaction center protein (Kyle et al. 1984). PS II function and structure is also damaged by the photosynthetically inactive ultraviolet radiation in the 280–320 nm (UV-B) region (for a review see Bornmann 1989).

The harmful effects of UV-B radiation on PS II function are manifested as lower rates of electron transport (Kulandaivelu and Noorudeen 1983), decreased yield of charge separation (Iwanzik et al. 1983) and lower yield of variable chlorophyll fluorescence (Tevini and Pfister 1985). The degradation of the D1 reaction center protein also occurs as a consequence of UV-B irradiation (Trebst and Depka 1990, Melis et al. 1992).

The precise molecular targets of UV-B radiation in PS II are controversial. In addition to the clear demonstration of the impairment of the water-splitting function (Renger et al. 1989), direct damage of acceptor side quinone components has also been reported (Renger et al. 1986a, Melis et al. 1992).

In the present work, flash-induced thermoluminescence measurements were applied to monitor changes in the function of PS II brought about by exposure to UV-B irradiation. The results showed that UV-B treatment of thylakoids adversely affects the efficiency of charge stabilization in the water-oxidizing complex and on the Q_A and Q_B quinone acceptors, indicating nearly concurrent damage of both donor- and acceptor side components of PS II.

Materials and methods

Thylakoid membranes were isolated from spinach with standard methods and were stored at –80 °C until use in 0.4 M sucrose, 5 mM $MgCl_2$, 10 mM NaCl and 40 mM Hepes (pH 7.5) at 2–3 mg Chl/ml. UV-B irradiation was performed by using a VL-215M (Vilber Lourmat) lamp giving an intensity of about 70 Wm^{-2}. The maximal emission from the lamp is at 312 nm with about 20 nm half bandwidth. Thus, contaminating radiation in the UV-A and UV-C spectral ranges is not significant. Thylakoids, at 300 μg Chl/ml in a 2 mm layer, were illuminated in a Petri dish at 10 cm from the sample surface, with slow stirring on ice. Thermoluminescence was measured at 20 °C/min heating rate with a setup similar to that described previously (Vass et al. 1981) but equipped with computer data acquisition. Flash excitation of samples was performed at –20, –10 or 5 °C as indicated in the text. Deconvolution of the measured thermoluminescence (TL) curves to individual components was performed as previously described (Vass et al. 1981). The flash-induced oscillation of the B thermoluminescence band was simulated as described by Demeter and Vass (1984) including the additional assumption that the TL yield of the $S_2Q_B^-$ recombination is only 50% that of the $S_3Q_B^-$ recombination (Rutherford et al. 1984, Demeter et al. 1985). Steady state rates of oxygen evolution were measured in the above buffer, at 25 μg Chl/ml in the presence of 1 mM dimethyl-*p*-benzoquinone, using a Clark-type oxygen electrode.

Results

UV induced damage to the water-oxidizing complex (Renger et al. 1989) and to the quinone electron acceptors of PS II (Trebst and Pistorius 1965, Renger et al. 1986a, Melis et al. 1992) are well documented. However, the literature data are not in agreement concerning the time course of the damage, i.e. which of the two deleterious effects of UV-B radiation occurs first?

A useful technique to monitor the functioning of the water-oxidizing complex and of the quinone electron acceptors is thermoluminescence (TL) (for reviews see DeVault et al. 1983, Sane and Rutherford 1986, Demeter and Govindjee 1989, Vass and Inoue 1992). Recombination of positive charges stored in the S_2 (or S_3) oxidation states of the water-oxidizing complex with electrons stabilized on the reduced Q_A (or Q_B) acceptors gives rise to characteristic TL signals. The shape and peak position of these TL signals is indicative of the energetic stability of the recombining charge separated state, while the flash-induced oscillation of their amplitude reflects the S-state turnovers and the functioning of the Q_AQ_B acceptor complex.

Illumination of spinach thylakoids with a single saturating flash induces a TL band at around 25 °C (Fig. 1A). This band is dominated by a component at 26–28 °C (B band), which arises from the

recombination of the $S_2Q_B^-$ charge pair (Rutherford et al. 1982, DeVault et al. 1983, Demeter and Vass 1984). Small intensity components at 0, 16 and 75 °C are also present as shown by the deconvolution of the measured TL curves in Fig. 1A. The component at 0 °C most likely arises from the recombination of the S_2 state with Q_A^-, which is present in a small amount even after a single-flash illumination due to the equilibration of the electron between $Q_A^-Q_B$ and $Q_AQ_B^-$. The origin of the 16 °C component is not clear, but this is likely the same component that was observed previously under continuous illumination (Vass et al. 1981). Due to its partial suppression by DCMU (Fig. 1B), the electron pool for the 16 °C component should be located on reduced Q_B (or plastoquinone). The 75 °C component has recently been assigned to chlorophyll chemiluminescence promoted by free radicals in damaged thylakoid membranes (Hideg and Vass 1993).

Irradiation with UV-B light induces a rapid loss of the 28 °C component without a significant change in its peak position. In contrast to this, the 16 °C component is transiently increased, which leads to a broadening and down-shift in the peak position of the band after 5 min or longer exposure to the UV-B light (Fig. 1A). In the UV-B treated thylakoids a new TL component appears at around 50 °C. This

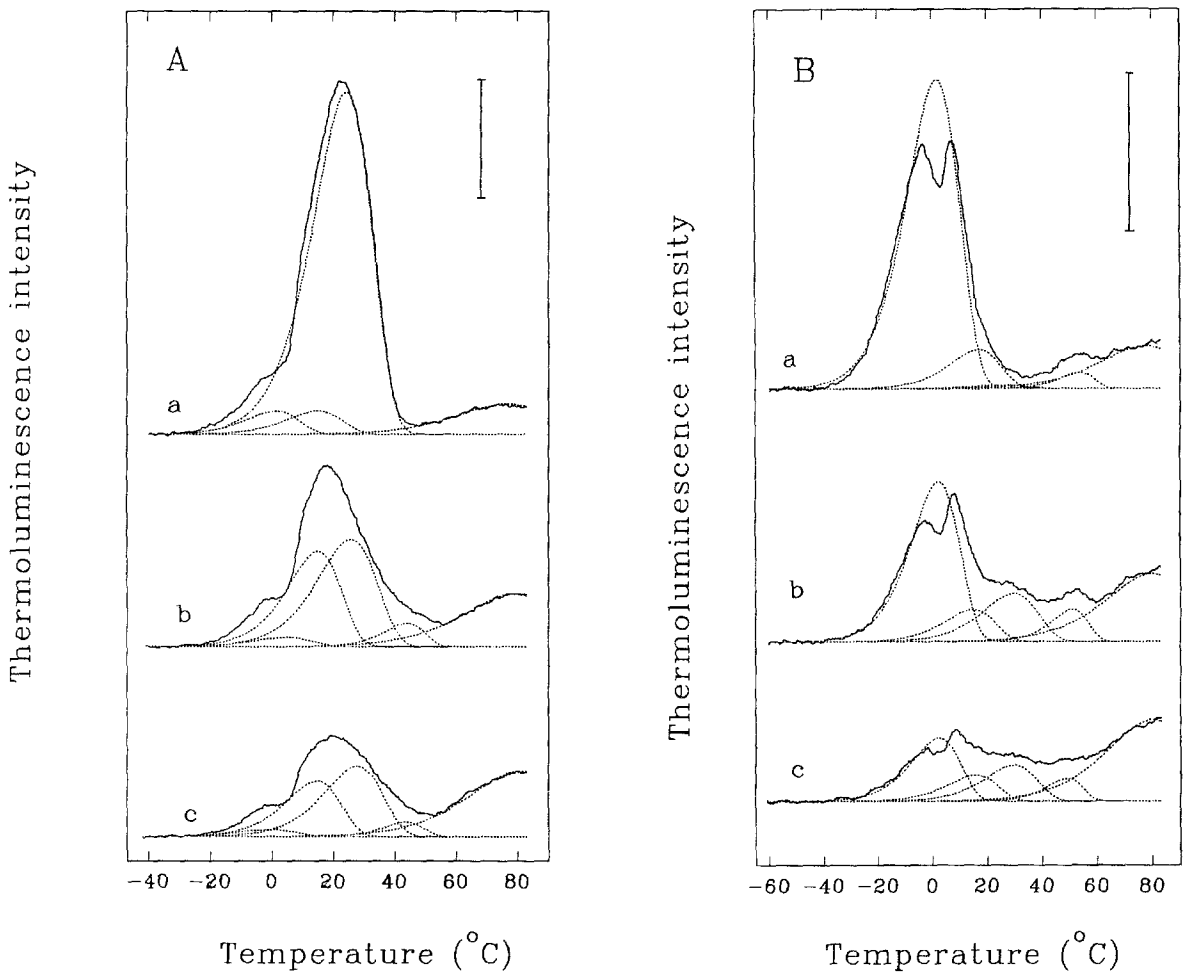

Fig. 1. The effect UV-B radiation on the single-flash induced thermoluminescence of isolated thylakoids. Thermoluminescence was measured after excitation with a single flash at −10 °C in the absence (A) or at −20 °C in the presence of 10 μM DCMU (B). The measurements were performed after 0 (a), 5 (b) and 20 minutes (c) of UV-B irradiation. Besides the measured TL signal (solid line) the deconvoluted components (dashed lines) are also shown. The artifactual shoulder or peak at 0 °C, which is caused by the solid-liquid phase transition (melting) of the samples, was omitted from the curve fitting procedure. Since the intensity scale on the A and B part of the figure is different, vertical bars indicate the same intensity unit.

component can be assigned to the so called C band and will be discussed below. UV-B irradiation induced also an increase in the 75 °C band. Since this TL component reflects membrane damage by free radicals (Hideg and Vass 1993) its enhancement indicates a UV-B induced disruption of thylakoid membrane structure.

In the presence of DCMU the single-flash induced TL curve is dominated by the Q band at around 0 °C (Fig. 1B), which arises from the recombination of the $S_2Q_A^-$ charge pair (Rutherford et al. 1982, Demeter et al. 1982). Small contributions from the 16 °C and 50 °C components are also present, but the 28 °C component is completely suppressed by DCMU in the control thylakoids. UV-B irradiation induced a quick loss of the Q band without changing its shape and peak temperature (Fig. 1B). In contrast to this, the 16, 28 and 50 °C components were transiently enhanced.

The changes in the intensities of the resolved TL components are summarized in Fig. 2. The B band ($S_2Q_B^-$ recombination) decreases sharply in the first two minutes of the irradiation period, followed by a slower decline (Fig 2A, open triangles). The Q band ($S_2Q_A^-$ recombination) is more resistant against the UV-B effect, and its relative intensity (open circles) is considerably higher than that of the B band in the 1–20 min illumination period. This difference in the decline of the Q and B bands indicates a destabilization of Q_B^- binding in the UV-B irradiated thylakoids.

In the presence of DCMU, three components are enhanced transiently by the UV-B irradiation. The C band is increased in the first two minutes of irradiation, and then declines (Fig. 2B, squares). In contrast, the 16 and 28 °C components (circles and squares, respectively) increase slowly to reach maxima after 10–20 min, and are relatively stable during further irradiation.

The observation of the 28 °C band in the UV-B treated samples in the presence of DCMU is surprising. The presence of this component shows that DCMU can only partially block the Q_A to Q_B electron transfer, which may indicate decreased affinity of DCMU binding after UV-B irradiation. This effect was confirmed by measuring the efficiency of different concentrations of DCMU to inhibit oxygen evolution in control and UV-B illuminated thylakoids. The results in Fig. 3 demonstrate that the half-inhibitory concentration of

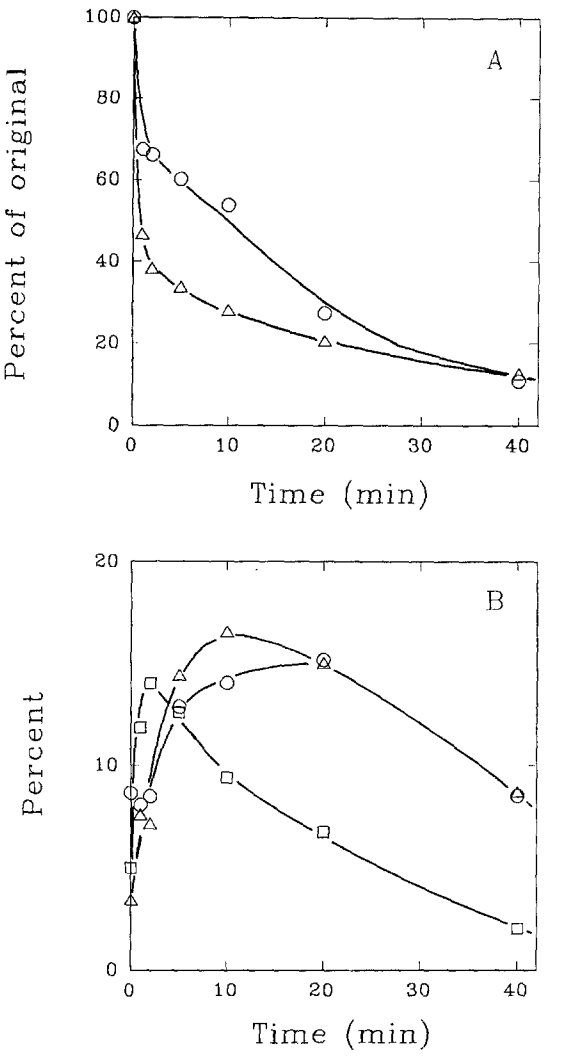

Fig. 2. The time course of UV-B induced decline of various thermoluminescence components. TL curves were measured after different periods of UV-B irradiation and deconvoluted into components as in Fig. 1. The integrated intensity of the resolved components is shown as a function of UV-B irradiation time. A. (△) B band, (○) Q band measured in the presence of 10 μM DCMU. The TL intensities are shown as a percentage of their respective value in the 'non-irradiated' control. B. (□) C band, (○) 16 °C and (△) 28 °C component measured in the presence of 10 μM DCMU. The values are shown as a percentage of the Q-band intensity in the non-irradiated control.

DCMU was increased by 5 min of UV-B irradiation (from 70 nM to 340 nM under our experimental conditions).

The transient induction of the C band (Figs. 1B, 2B) is similar to previous observations in isolated thylakoids and intact leaves upon UV-C (271 nm)

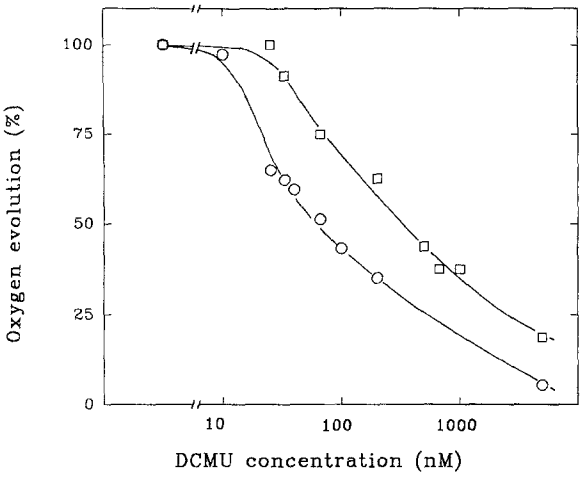

Fig. 3. The effect of UV-B irradiation on the sensitivity of oxygen evolution to inhibition by DCMU in isolated thylakoids. Oxygen evolution was measured in thylakoids after 0 min (○) or 5 min UV-B irradiation (□) in the presence of various concentrations of DCMU as described in the 'Materials and methods'. The oxygen evolution rates are shown as percentage of the respective values measured in the absence of DCMU. In the irradiated sample this reference rate, measured without DCMU, was 60 % of that in the control thylakoids.

irradiation (Desai 1990). The C band was suggested to arise from the recombination of Q_A^- with a donor component of PS II, which is different from the S-states of the water-oxidizing complex (Demeter et al. 1984, Sane and Rutherford 1986, Vass and Inoue 1992). Since this donor, possibly Tyr-D or Cyt *b*-559, is expected to compete with the S states for positive charge stabilization, the enhancement of the C band indicates the inactivation of the water-oxidizing function in the early phase of UV-B irradiation. The origin of the UV-B enhanced C band from PS II is supported by its presence in isolated PS II membranes (not shown).

In order to clarify if UV-B irradiation induces a selective blockage of a definite S-state transition or not, the flash-induced oscillation of the B thermoluminescence band was also measured (Fig. 4). For this experiment, the flash illumination was given at +5 °C to avoid any temperature effect on light-induced cycling of the S-states. Under these conditions, the B band dominates and only a small 16 °C and no 0 °C component was observed (not shown). After two or more flashes the B band arises from the recombination of the $S_2Q_B^-$ and $S_3Q_B^-$ charge pairs (Rutherford et al. 1982, Demeter and Vass 1984). Consequently, the flash-induced oscillation in the

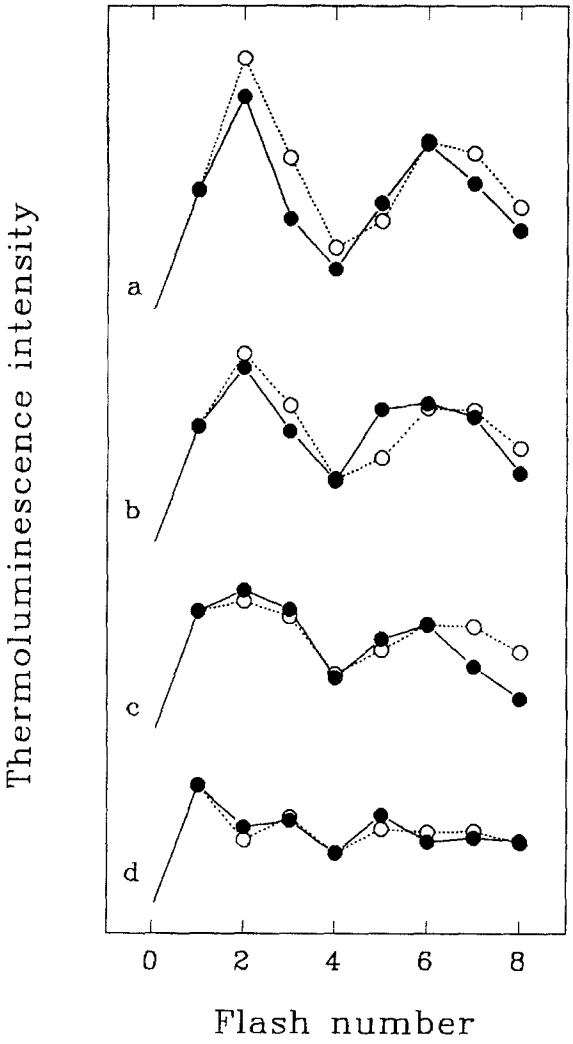

Fig. 4. The flash-induced oscillation of the B thermoluminescence band in UV-B treated thylakoids. Thermoluminescence was excited with one to eight flashes, 1 Hz frequency, at +5 °C. The total amplitude of the B band (●) is plotted as a function of flash number after 0 (a), 3 (b), 5 (c) and 7 min (d) of UV-B irradiation. The different oscillatory patterns are shown after normalization to the B-band intensity obtained after the first flash. In comparison with the measured data the simulated oscillation of the B-band is shown (○), which was calculated as described in the 'Materials and methods.' Assumptions for the simulation: S_0: S_1 = 30: 70, miss = 15%, double hit = 3 % for all samples. Initial distribution of Q_B: Q_B^- = 50: 50 for 0 min, 60: 40 for 3 min, 72: 28 for 5 min and 100: 0 for 7 min UV-B irradiation.

B-band intensity reflects the cyclic formation of the S_2 and S_3 states superimposed with the flash-induced changes in the amount of Q_B^-. In the non-irradiated control samples, the intensity of the B band undergoes a period-four oscillation (see Sane and Ruther-

ford 1986, Vass and Inoue 1992). The appearance of maximal intensities after the second and sixth flashes (Fig. 4) is consistent with the approximately 50:50 distribution of the centers in the Q_B and Q_B^- state before the flash illumination (Rutherford et al. 1982, Demeter and Vass 1984). This situation is characteristic of samples that have been dark adapted for a short period. Irradiation of the samples with UV-B light induced a dramatic change in the oscillatory pattern of the B band. The increase of TL intensity after the second flash was gradually decreased relative to that after the first flash. After 7 min of UV-B irradiation, the oscillation showed a clear binary pattern, with the first maximum appearing after the first flash (Fig. 4). Such modification of the B-band oscillatory pattern was previously observed in thoroughly dark adapted thylakoids and was shown to reflect the shift of the Q_B:Q_B^- distribution towards Q_B (Demeter and Vass 1984). The simulated oscillation in Fig. 4 indicates a similar change in the Q_B:Q_B^- distribution in the UV-B treated samples. Modifications of the TL oscillatory pattern can also indicate the block of a specific S-state transition. This is shown by the interruption of TL oscillation after 1st, 2nd or 3rd flash for the block of the S_1–S_2, S_2–S_3 or S_3–S_4–S_0 transition, respectively (see e.g. Vass et al. 1989). Such modification of the TL oscillatory pattern was not observed here (Fig. 4). Therefore, in agreement with the earlier finding of Renger et al. (1989), UV-B irradiation does not block a specific S-state transition.

Discussion

There is a general consensus that PS II is the most UV-B sensitive part of the photosynthetic apparatus (see Bornmann 1989). However, the exact molecular targets within the PS II complex are not yet identified. Literature data clearly demonstrate that UV-B irradiation impairs the functioning of the water-oxidizing complex (Renger et al. 1989) and inactivates the quinone electron acceptors (Renger et al. 1986a, Melis et al. 1992). However, the relationship between these deleterious effects is not understood, and it is not clear if they occur sequentially or simultaneously.

Here we used thermoluminescence measurements in an attempt to monitor UV-B induced damage at the donor and acceptor sides of PS II. Due to the recombinative nature of TL formation, the observed TL components always reflect the characteristics of a donor-acceptor pair. Thus, the separation of donor- and acceptor side effects requires the comparison of TL bands where one redox component (either at the donor or acceptor side) is kept constant but the counterpart in the electron-hole pair is different.

TL signals studied in the present work showed differential responses to UV-B irradiation. Major differences were observed when comparing the UV-B induced changes of the B and Q bands and also in the response of the Q and C bands, allowing us to separate the effect of UV-B irradiation on the acceptor and donor sides of PS II, respectively. In the presence of DCMU the intensity of the Q band, from the $S_2Q_A^-$ recombination, was gradually decreased during UV-B irradiation indicating inhibition of charge stabilization in the $S_2Q_A^-$ state. In contrast, the C band was transiently increased at the beginning of the irradiation period, which was followed by a subsequent decrease, in agreement with the previous finding of Desai (1990) on the effect of UV-C irradiation. Due to its enhancement by DCMU, we assign the C band, observed in our study, to the electron transport-dependent C band, observed by Desai et al. (1975), Demeter et al. (1984) and Desai (1990), which is different from the 50 °C band in photosynthetically inactive samples (Rózsa et al 1989, Vass et al. 1989). The C band arises from the recombination of Q_A^- with an accessory donor of PS II, possibly Tyr-D+ or oxidized Cyt *b*-559. Therefore, its transient enhancement under the conditions where the Q band is already decreased is interpreted as an indication that UV-B irradiation inactivates the water oxidizing complex more rapidly than the formation of Q_A^-. This opens the way for charge stabilization on the alternative donor, whose recombination with Q_A^- gives rise to the increased C band. The subsequent decrease of the C band during continued UV-B irradiation could be caused either by the destruction of Q_A or of the donor component.

Besides the inhibition of donor-side functions, UV-B irradiation induces changes also on the acceptor side of PS II. One manifestation of this effect is the appearance of the 28 °C TL component ($S_2Q_B^-$) in UV-B treated thylakoids in the presence of DCMU. This suggests the decreased affinity of DCMU to block the Q_A to Q_B electron transfer, which was confirmed by oxygen evolution measurements. Our observation agrees well with the decreased atrazine binding reported in UV-B irradiated PS II preparations (Renger et al. 1989), and indicates a structural modification of the PS II

reaction center by UV-B irradiation. It is of note that an earlier study did not find change in atrazine binding by UV-B irradiation (Tevini and Pfister 1985). A possible reason for this discrepancy might be the large admixture of UV-A and visible light used in the above report (Tevini and Pfister 1985) in contrast to the selective UV-B irradiation applied in the present study and by Renger et al. (1989).

UV-B induced effect on the PS II acceptor side is also demonstrated by the more rapid loss of charge recombination from the $S_2Q_B^-$ than from the $S_2Q_A^-$ state. We explain this effect by a modified Q_B binding site, which leads to the destabilization of bound Q_B^-. This explanation is supported by the characteristic change in the B-band oscillatory pattern, which is consistently described by the gradual decrease of dark stable Q_B^- during UV-B irradiation. In non-irradiated thylakoids a similar change in the distribution of Q_B:Q_B^- requires several hours of dark adaptation (Demeter and Vass 1984) or treatment with oxidizing agents. Since UV-B light is not expected to oxidize the stable Q_B^-, we assume that the change in the Q_B: Q_B^- ratio is due to a specific UV-B effect. This is possibly related to decreased affinity of Q_B^- to the binding site due to a conformational change of the reaction center complex. Such UV-B induced conformational change, which has already been proposed by Renger et al. (1989), might affect directly the Q_B binding region, or might be an indirect consequence of the inactivation of the water-oxidizing complex. Donor-side induced alteration of acceptor side functions has also been suggested or indicated in PS II centers, in which the water-oxidizing complex is inactivated by hydroxylamine (Jursinic and Stemler 1983, Renger et al. 1986b) or modified in the absence of the 33 kDa extrinsic protein (Vass et al. 1986, 1992).

An alternative explanation for the UV-B induced loss of centers with dark stable Q_B^- is the direct destruction of quinone acceptors by the UV-B light. This effect, which was suggested as a major cause of PS II inactivation (Melis et al. 1992), is likely to affect more efficiently Q_B^- (and reduced plastoquinone) than Q_B (and oxidized plastoquinone) due to the pronounced differential absorption of reduced minus oxidized plastoquinones in the UV-B region (Dekker et al. 1984). The selective destruction of Q_B^- would obviously increase the proportion of centers which have Q_B in the dark, and could explain the change in the B-band oscillatory pattern. However, this mechanism cannot explain the slower loss of recombination from the $S_2Q_A^-$ state (Q band) relative to that from the $S_2Q_B^-$ state (B band). Both of these states arise from the same type of centers, which were in the $S_1Q_AQ_B$ state before the flash, with or without blocking the Q_A to Q_B electron transport by DCMU.

In summary, we conclude that UV-B irradiation causes damage to both the donor and acceptor side components of PS II. The damage to the water-oxidizing complex, which seems to occur first, does not affect a specific S-state transition. Instead, charge stabilization is enhanced on an accessory donor. The inhibition of the water-oxidizing complex is followed by acceptor-side modification(s). This is manifested by the decreased affinity of DCMU binding, the faster loss of charge recombination from Q_B^- relative to that from Q_A^- and by the shift of the Q_B: Q_B^- ratio towards Q_B. These effects might all be related to a structural change in the Q_B/DCMU binding site(s) caused by the UV-B irradiation directly or indirectly, via the inactivation of the water-oxidizing complex. The preferential loss of dark-stable Q_B^- could also be caused by the specific destruction of reduced quinones by the UV-B light.

Acknowledgements

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