

## Effect of UV-A radiation on photosynthetic electron transport

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**ABSTRACT** We have studied the inhibition of photosynthetic electron transport by UV-A (320-400nm) radiation in isolated spinach thylakoids by flash-induced oxygen and thermoluminescence (TL) measurements. The flash pattern of oxygen evolution showed an increased amount of the  $S_0$  state in the dark, which indicate a direct effect of UV-A radiation in the water-oxidizing complex. TL measurements revealed the UV-A induced loss of PSII centers. Flash-induced oscillation of the B TL band, originating from the  $S_2Q_B^-$  recombination, indicated a UV-A induced decrease in the amount of  $Q_B^-$  relative to  $Q_B$ . In conclusion, our data show that UV-A radiation is highly damaging for PSII, whose electron transport is affected both at the water oxidizing complex, and the binding site of the  $Q_B$  quinone electron acceptor.

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Light is a well-known stress factor of plants and the mechanisms of its harmful effects are well characterized in the photosynthetically active (400-700 nm) and UV-B (290-320 nm) spectral ranges (Bornman 1989; Vass 1996). Although previous literature data indicate that UV-A (320-400 nm) radiation also exerts harmful effects on photosynthetic organisms and may target PSII (Klein 1965; Joshi et al. 1994), the mechanism of UV-A damage was not studied and understood in detail.

The evidence that UV-B and PAR inhibits photosynthetic electron transport by different mechanisms prompted us to study the inhibitory effects of UV-A in isolated spinach thylakoids.

### Materials and Methods

**Sample preparation.** Thylakoid membranes were isolated from spinach with standard methods and were stored at  $-80^\circ\text{C}$  until use in 0.4 M sucrose, 5 mM  $\text{MgCl}_2$ , 10 mM NaCl and 40 mM Hepes (pH 7.5) at 2-3 mg  $\text{mL}^{-1}$ .

**UV-A treatment.** UV-A irradiation was performed in open, cylindrical glass containers in which the thylakoid suspension of 200 mg Chl  $\text{mL}^{-1}$  formed a layer of 10 mm height, with continuous stirring at room temperature. A Vilbert Lourmat VL-215A lamp was used as a UV-A light source, with maximal emission at 365 nm, and about 40 nm bandwidth.

**Variable chlorophyll fluorescence measurements.** Steady-state variable fluorescence was measured by a PAM machine (WALZ, Effeltrich, Germany), using a  $Q_A$ -Data (Turku, Finland) computer controlled data acquisition system.

**Flash-induced oxygen yield measurements** were performed by using a home-built bare platinum electrode system as described earlier (Vass et al. 1992). Samples were preilluminated with 50 flashes, and dark adapted for 3 min. Oxygen evolution was induced by a series of 20 flashes, given at 1 Hz frequency.

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Thermoluminescence (TL) was measured with a home-built apparatus as in Vass et al. (1992). Flash excitation of the samples was performed at  $-20^\circ\text{C}$  in the absence or presence of 10 mM DCMU. The TL curves were recorded from  $-40$  to  $+80^\circ\text{C}$ , at  $20^\circ\text{C min}^{-1}$  heating rate. Deconvolution of the measured TL curves was done according to Vass et al. (1981).

### Results

#### The effect of UV-A on variable chlorophyll fluorescence

UV-A irradiation caused a gradual loss of the maximal fluorescence yield ( $F_{\text{max}}$ ), and a decrease in the rate of fluorescence rise in the absence of DCMU. The decrease of the  $F_{\text{max}}$  level shows that the amount of PSII centers, which are able to reduce  $Q_A$  is decreased. The slower fluorescence rise could arise from a slower rate of  $Q_A$  reduction due to a decreased absorption cross section of the PSII associated light harvesting antenna, or it could be the consequence of retarded electron flow from the donor side of PSII to the PQ pool that should be reduced before  $Q_A$ . In order to access this point, the fluorescence induction measurements were also performed in the presence of DCMU, which blocks electron transport beyond  $Q_A$ . In that case the  $F_{\text{max}}$  level was lost to a similar extent as seen in the absence of DCMU. However, the rate of fluorescence rise was not affected, which indicates that the cross section of PSII for light energy capture is not affected by UV-A radiation.

#### UV-A effects on the flash pattern of oxygen evolution

UV-A irradiation induced a substantial loss in the amplitude of the flash-oxygen signal, and the oxygen yield after the 4<sup>th</sup> flash was increased relative to that of after the 3<sup>rd</sup> flash. Analysis of the oxygen data showed that the amount of centers in the  $S_0$  state and the miss factor was increased by 60 and 25% during 180 min UV-A treatment. These alterations indicate that UV-A not only inactivates PSII

electron transport, but also modifies the functioning of the water-oxidizing complex in the still active centers.

### UV-A effects on the thermoluminescence characteristics

Illumination of spinach thylakoids with a single saturating flash results in a TL band at around 28°C. This is the so called B band which arises from the recombination of the  $S_2Q_B^-$  charge pair (Rutherford et al. 1982). UV-A treatment leads to a rapid loss in the amplitude of the B band, however, its peak temperature and half-band width is not affected. In the presence of DCMU, which blocks electron transfer from  $Q_A$  to  $Q_B$ , the single-flash induced TL band is shifted to lower temperatures (+7°C). This band is called the Q band and originates from the  $S_2Q_A^-$  recombination (Rutherford et al. 1982). Besides this band an other component appears at around +50°C, which is the so called C ( $Q_A^-Tyr-D^+$ ) band. UV-A irradiation leads to rapid decrease in the amplitude of the Q band without affecting its peak temperature. In addition, the C band is lost and a small component induced at around 35°C.

Flash-induced oscillation in the B-band intensity reflects the redox cycling of the S states and of the  $Q_AQ_B$  acceptor complex. The UV-A irradiated thylakoids showed similar oscillation to the non-irradiated thylakoids, however, the increase of the B band amplitude after the 2<sup>nd</sup> flash in comparison to the 1<sup>st</sup> flash was less pronounced. Changes in the relative TL amplitude after the 1<sup>st</sup> and 2<sup>nd</sup> flash have been assigned to changes in the oxidation-state of  $Q_B$ . The simulation of the oscillatory pattern showed that the  $Q_B/Q_B^-$  ratio, which was 0.46/0.54 in the non-irradiated control increased to 0.57/0.43 after 100 min UV-A irradiation. This effect indicates that UV-A irradiation of thylakoids slightly increases the fraction of oxidized  $Q_B$  at the expense of the reduced fraction.

### Discussion

The decreased yield of  $F_{max}$  fluorescence and the slow kinetics of fluorescence rise indicate the retardation of donor side electron transfer in UV-A damaged PSII. The donor side effect of UV-A was further supported by the flash-induced oxygen evolution data. The rapid loss of the amplitude of the flash-oxygen signal could be either due to a general damage of PSII or due to specific inactivation of the water-oxidizing complex as will be discussed below. However, the modified oscillatory pattern indicates the presence of active centers whose water-oxidizing complex is modified by UV-A, and leads to an increased amount of centers in the  $S_0$  state. The gross oxidation state of the Mn cluster is lower by one oxidizing equivalent in the  $S_0$  ( $Mn^{2+}$ ,  $Mn^{3+}$ ,  $Mn^{4+}$ ,  $Mn^{4+}$ ) than in the  $S_1$  state ( $Mn^{3+}$ ,  $Mn^{3+}$ ,  $Mn^{4+}$ ,  $Mn^{4+}$ ) (Hoganson and Babcock 1997). Thus, the increased fraction of centers in the  $S_0$  state shows the reduction of one  $Mn^{3+}$  to  $Mn^{2+}$ . The exact

mechanistic background of this effect is not clear, but may indicate a structural change of PSII that makes the Mn cluster more accessible to exogenous reductants. The increased dampening of the oscillatory pattern, characterized by increased miss factor, indicates that UV-A radiation decreases the efficiency of S-state turnovers as well.

The redox cycling of the water-oxidizing complex and of the  $Q_AQ_B$  two-electron gate was also studied by TL measurements. Flash-induced oscillation of the B band retains its period-four pattern in the UV-A irradiated thylakoids. This result demonstrates that the loss of oxygen evolving capacity is not caused by specific inhibition of the  $S_2 \rightleftharpoons S_3$  or the  $S_3 \rightleftharpoons S_4 \rightleftharpoons S_0$  transitions, which would block the oscillation after the 1<sup>st</sup> or 2<sup>nd</sup> flash, respectively. However, the oscillation shows a decreased ratio of the TL amplitudes measured after the 2<sup>nd</sup> flash as compared to the 1<sup>st</sup> one in the UV-A irradiated samples. Simulation of the oscillatory pattern indicates that this change is due to the increased amount of oxidized  $Q_B$  at the expense of semi-reduced  $Q_B$ . In the  $Q_B$  binding site the oxidized form of  $Q_B$  is freely exchangeable with PQ molecules from the lipid phase of the thylakoid membrane, whereas  $Q_B^-$  is firmly bound. Thus, the shift of the  $Q_B/Q_B^-$  ratio toward  $Q_B$  in the UV-A irradiated samples could indicate a modification of the  $Q_B$  binding site which decreases the binding affinity of  $Q_B^-$  +7°C to a larger extent than that of  $Q_B$ .

The observation of a new TL component at 35°C in the presence of DCMU could support the hypothesis that UV-A induced modification may also affect inhibitor binding at the  $Q_B$  site. However, this component is unlikely to be a residual B band, which is insensitive to DCMU. Partly because its peak temperature is higher than that of the B band, and partly because its amplitude was increased with increasing UV-A irradiation time, in contrast to the decreasing tendency of the B band. Considering that the C band disappeared when the new component at 35°C was induced, the 35°C component could arise from the  $Tyr-D^+Q_A^-$  recombination, provided that the stabilization energy of this charge pair is decreased. Since the peak position of the Q band ( $S_2Q_A^-$  recombination) and the B band ( $S_2Q_B^-$  recombination) was not changed, the redox potential of the  $Q_A/Q_A^-$  ( $S_2/S_1$  and  $Q_B/Q_B^-$ ) redox couples is unaffected by UV-A. Thus, the shift of the C band from 50°C to 35°C could only be due to an UV-A induced redox potential increase of the  $Tyr-D^+/Tyr-D$  redox couple.

These results show that UV-A radiation has effect on the PSII complex, whose electron transfer is affected both at the water-oxidizing complex and at the  $Q_B$  binding site. The mechanism is similar to that induced by UV-B, although its damaging weight is smaller and the acceptor side effects are less pronounced.

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