Chapter 19
UVB and UVB/White-Light-Induced Inhibition of Thylakoid Electron Transfer Reactions Studied by Fluorescence Induction and Fluorescence Decay: Damage to Donor and Acceptor Side Components of PSII

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Contents

Abstract Approximately 1.5% of the solar radiation reaching the Earth's surface is accounted for by wavelengths in the ultraviolet-B range (280–320 nm) and its amount is increasing as a consequence of stratospheric ozone depletion linked to human activity. This radiation is known to be harmful to all biological organisms and even a modest increase in its level may induce adverse effects on the biosphere, with plants particularly sensitive. In this study we report new data on the effect of ecologically significant level of UVB light, alone or in combination with visible light, on photosynthetic electron transfer reactions in the stress-tolerant organism *Thellungiella salsuginea.* We could confirm that UVB light harms the donor side of photosystem II, but also electron transfer beyond the acceptor side of the photosystem (i.e., cytochrome b_6/f or photosystem I) is affected. In the presence of background visible light, the effect of UVB is rather different and a main target became electron transfer between Q_A and Q_B.

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19.1   Introduction

Even though light is essential for photosynthesis, when in excess, it may harm the photosynthetic apparatus itself (Li et al. 2018). Not only the amount of light, but also its quality in terms of wavelength, may have adverse effects on photosynthesis. In particular, the ultraviolet-B light component of the electromagnetic spectrum (280–315 nm) has been shown to be very dangerous to photosynthesis, as it affects a number of electron transfer steps and biochemical reactions with a very high quantum yield (Zavafer et al. 2015). These include damage to the quinone acceptors (Jansen et al. 1996), damage to the Mn cluster of the oxygen evolving complex (Barbato et al. 1995, Hakala et al. 2005), and increased degradation of reaction center D1-protein with concomitant inhibition of its synthesis (Barbato et al. 2000).

An increase in the level of ultraviolet-B light reaching the Earth's surface has been measured as a consequence of stratospheric ozone depletion (Allen et al. 1998). Since its discovery, this fact has been causing much concern as it may have strong and adverse effects on ecosystems (Caldwell et al. 2007) and have to be considered as one of main face of climate global changes. In line with this view, it seems important to understand, at any level, the effect of this light on photosynthesis. In line with this view, here we report the result from a study based on fluorescence induction and fluorescence decay after a single turnover flash, in which the effect of visible, ultraviolet-B (UVB), and a mixture of the two kinds of light (visible/UVB) is noted on the photosynthetic apparatus of *Thellungiella salsuginea*, a model organism in the field of plant environmental stress physiology. Our results indicated that the effect of ultraviolet-B light is different depending on whether visible light is present or not in the background. We found that ultraviolet-B light affects mainly the donor side of photosystem II and electron carriers beyond Q_B; however, when it was administrated together with visible light (which, per se, did not have any adverse effect), electron transfer between Q_A and Q_B was affected too.

19.2   Materials and Methods

Thellungiella salsuginea was grown as described by Goussi et al. (2018). Leaves from 4-week-old plants were used in all experiments. For irradiation, detached leaves were left to float on water and exposed to different kinds of light for either 1 or 3 h. Control leaves were kept in the dark until the end of experiments. Irradiation conditions were as follows: white light, 400 μmol·m^{-2}·s^{-1}, and UVB light (from a Vilber-Lourmat lamp), 1 μmol·m^{-2}·s^{-1}. Before acquisition of fluorescence transients,

plants were dark adapted for at least 30 min. Measurements were carried out as described by Pagliano et al. (2006) and analysis of chlorophyll transients performed as described by Strasser and Srivastava (1995). Statistical analysis was carried out by using the Origin software package, version 9.

19.3   Results

Irradiation of *Thellungiella salsuginea* leaves under our experimental conditions brought about a different effect depending on the particular light treatment. In terms of F_V/F_M, the intensity of visible white light used in this study did not significantly affect the maximal efficiency of PSII, whereas the presence of UVB, alone or in combination with white light, caused a marked decrease on F_V/F_M, depending on irradiation time (Table 19.1). This decrease, similar in both irradiation conditions (UVB, visible/UVB), was due to both an increase in F_0 and a decrease in F_M (Table 19.1 and Fig. 19.1a,b).

In Fig. 19.1a,b, chlorophyll transients (recorded after 30 min of dark adaptation) from leaves treated with visible, ultraviolet-B (UVB), or ultraviolet-B in combination with visible light (visible/UVB) for 1 h (1A) or 3 h (1B) are shown. The fluorescence F_t of the dark-adapted control leaves showed a typical OJIP transient (Strasser et al. 2004). As stated above, UVB treatment, alone or combined with background white light (visible/UVB), induced an increase in F_0; at the same time, a considerable decrease of F_M was observed. The effect was slightly more pronounced after 3 h of irradiation. At variance, irradiation with white light did not produce any major adverse effects.

To further investigate this point, transients shown in Fig. 19.1a,b were normalized at F_0 and F_M and then plotted as relative variable fluorescence, V_{OP} (Fig. 19.2a,b). While visible light did not affect the shape of the transients, UVB light produced a strong effect, which was different depending on the presence or absence of background white light. When administrated alone, ultraviolet-B light produced a general loss fluorescence (decrease of all phases, i.e., OJ, Ji, and IP); at variance, when visible light was present together with UVB light, a strong increase of the OJ and a

Table 19.1 Absolute (Fo, Fm, Fm, Fk, Fj) and relative (Fv/Fm, Fk/Fj) values of chlorophyll fluorescence from control and light-treated plants

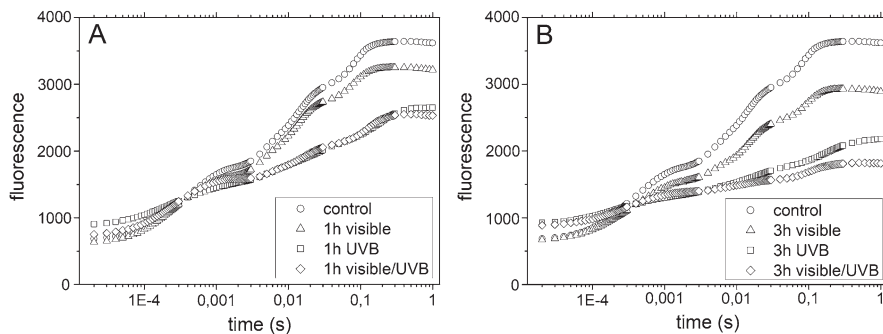|	Fo	Fm	Fv	Fv/Fm	Fk	Fj	Fk/Fj
Control	678	3642	2964	0.814	1214	1848	0.657
1 h w	623	3251	2628	0.808	1205	1720	0.700
1 h u	898	2650	1752	0.661	1254	1572	0.798
1 h uw	741	2551	1810	0.710	1245	1591	0.783
3 h w	664	2930	2266	0.779	1111	1572	0.665
3 h u	923	2178	1185	0.576	1175	1385	0.848
3 h uw	884	1822	938	0.515	1161	1397	0.831

Fig. 19.1 Unnormalized chlorophyll fluorescence transients recorded from plants treated either for 1 h (**a**) or 3 h (**b**) with white light (up triangles), UVB (squares), and with W/UVB (diamonds). Transient from dark control (circles) is also reported
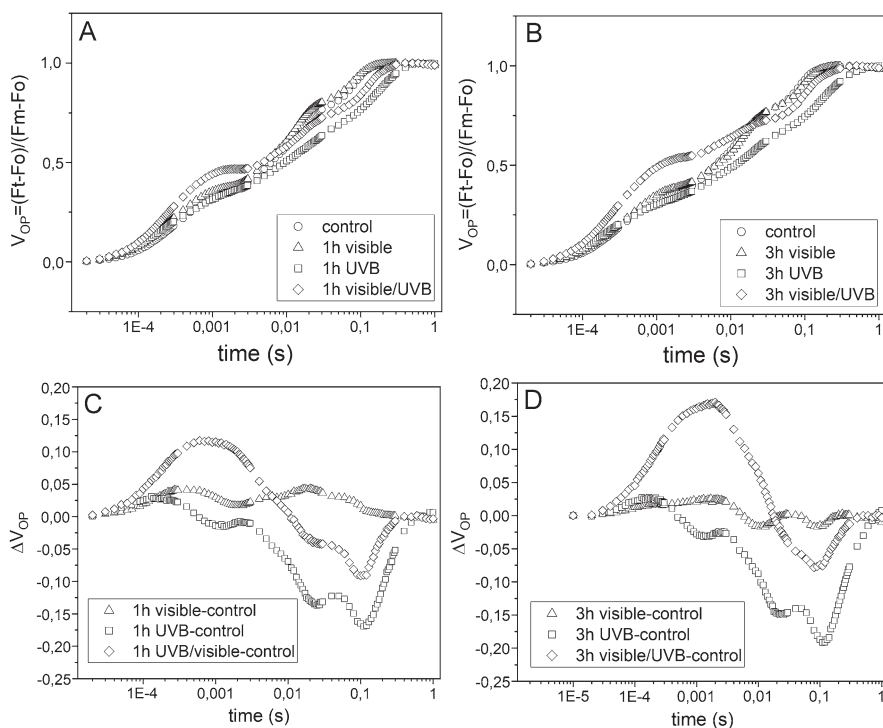

Fig. 19.2 Normalized (Fo, Fm) chlorophyll fluorescence transients (V_{OP}) from plants treated either for 1 h (**a**) or 3 h (**b**) with white light (up triangles), UVB (squares), and with W/UVB (diamonds). Transient from dark control (circles) is also reported. ΔV_{OP} was calculated as $V_{OP\,(treated)}$-$VO_{OP(control)}$; plants were treated either for 1 h (**c**) or 3 h (**d**). Triangles, white-light-treated minus dark control; squares, UVB-treated minus dark control; diamonds, white/UVB-treated minus dark control

decrease in the JI and IP phases was observed. This effect was qualitatively similar at 1 and 3 h of irradiation, but after 3 h it was more pronounced. By calculating ΔV_{OP} (Fig. 19.2c,d) it became clear that the presence of visible light (visible/UVB) brought about a strong band peaking at about 2–3 ms, whose intensity increased with irradiation time; the appearance of this band was a specific effect of irradiation with visible/UVB, as it was induced neither by UVB nor by visible light alone. In addition, irrespective of the presence of visible light, UVB light brought about the appearance of two negative bands peaking about at 20 and 100 ms, corresponding to a decrease of phases JI and IP, respectively.

To further elucidate, at least semiquantitatively, the observed differences in fluorescence kinetics, main bands occurring from O to P transients were analyzed separately. This was achieved by double normalizing curves between 0 and 300 μs (L band, between O and K steps, Fig. 19.3a,b) and between 0 and 2 ms (K band, between O and J steps, Fig. 19.4a,b). K (Fig. 19.3c,d) and L (Fig. 19.4c,d) bands were obtained, and then plotted, as the difference between treated plants with
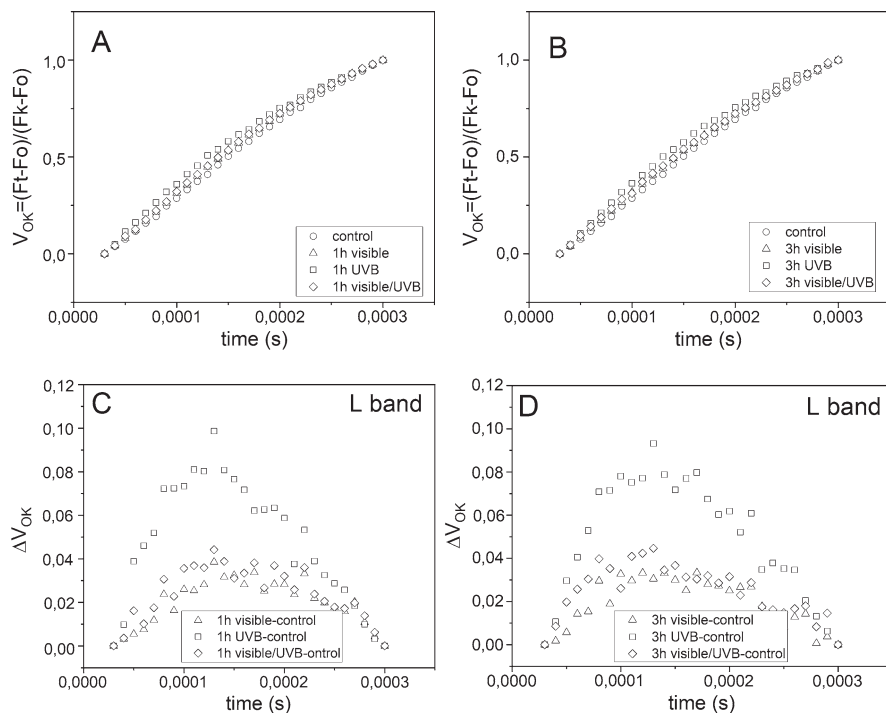

Fig. 19.3 Normalized (Fo, Fk) chlorophyll fluorescence transients (V_{OK}) from plants treated either for 1 h (**a**) or 3 h (**b**) with white light (up triangles), UVB (squares), and with W/UVB (diamonds). Transient from dark control (circles) is also reported. ΔV_{Ok} (L band) was calculated as $V_{OK\ (treated)}$-$VO_{OK\ (control)}$; plants were treated either for 1 h (**c**) or 3 h (**d**). Triangles, white-light-treated minus dark control; squares, UVB-treated minus dark control; diamonds, white/UVB-treated minus dark control
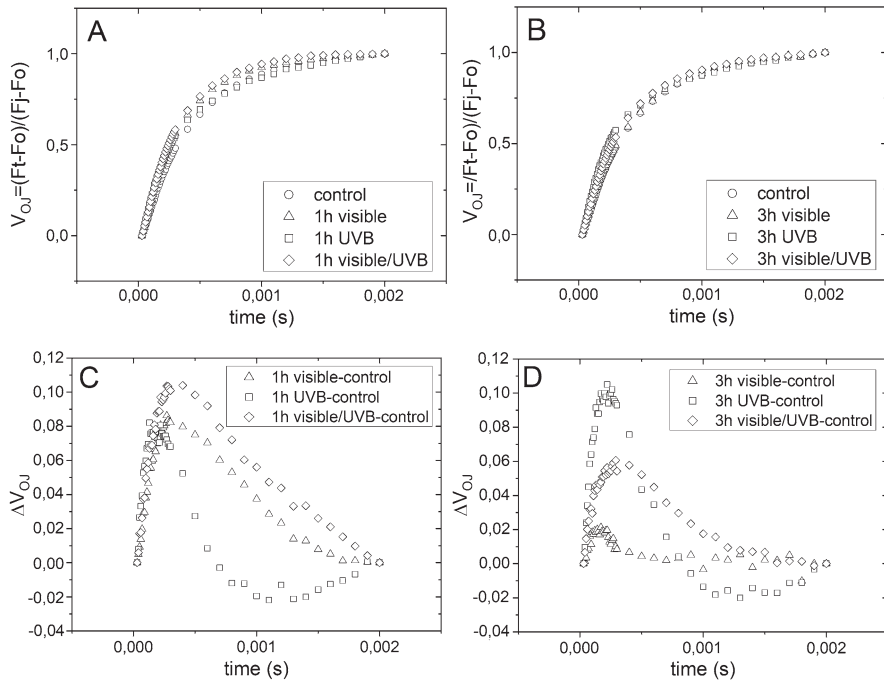
Fig. 19.4 Normalized (Fo, Fj) chlorophyll fluorescence transients (V_{Oj}) from plants treated either for 1 h (**a**) or 3 h (**b**) with white light (up triangles), UVB (squares), and with W/UVB (diamonds). Transient from dark control (circles) is also reported. ΔV_{OI} (K band) was calculated as $V_{OI\ (treated)}$-$VO_{OI\ (control)}$; plants were treated either for 1 h (**c**) or 3 h (**d**). Triangles, white-light-treated minus dark control; squares, UVB-treated minus dark control; diamonds, white/UVB-treated minus dark control

ultraviolet-B (either in the presence or absence of visible light) and dark control. After 1 h of irradiation, ΔV_{OK} revealed a positive L band, peaking between 100 and 200 µs. The maximum amplitude of the L band was after irradiation with UVB after 3 h, whereas when present together with white light, the effect of UVB was much mitigated, very similar to that observed with just white light. The kinetic difference of V_{OJ} revealed the so-called K band in treated plants, which appeared as a peak between 250 and 300 µs.

Changes in IP phase have been measured by transient normalization to the time range of 30–200 ms and expressed as $V_{IP} = (F_t - F_I) / (F_m - F_I)$ (Fig. 19.5a,b). Upon treatment with UVB light, a loss of IP phase was observed, bigger when visible light was present. Irradiation with visible light did not produce any adverse effect on this phase of the transient (Fig. 19.5a,b). Finally, fluorescence decay after a single turn-over flash (in the µs range) was investigated. Visible light did not affect fluorescence decay, whereas UVB light, particularly when visible light was present in the background, produced a strong slowing down of fluorescence decay (Fig. 19.6).
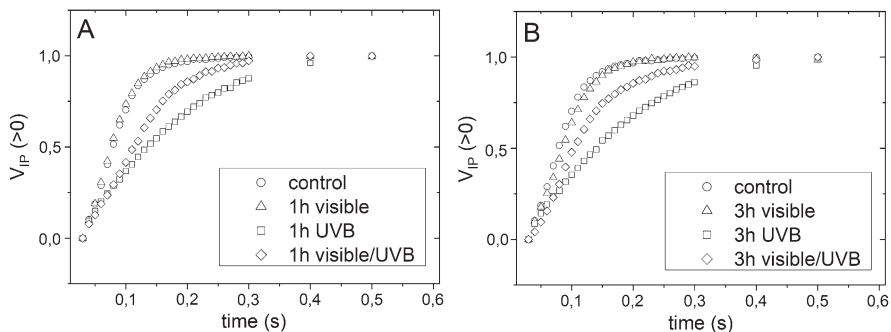
Fig. 19.5 Normalized (Fi, Fp) chlorophyll fluorescence transients (V_{IP}) from plants treated either for 1 h (**a**) or 3 h (**b**) with white light (up triangles), UVB (squares), and with W/UVB (diamonds). Transient from dark control (circles) is also reported
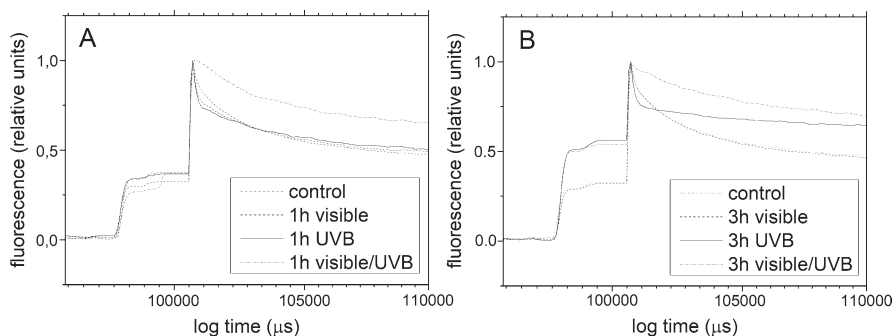

Fig. 19.6 Fluorescence decay in leaves treated for either 1 h (a) or 3 h (**b**) in plants treated with white light (dashed line), UVB light (solid line), or W/UVB light (dash dot line). Fluorescence decay from dark control is also shown (dotted line)

19.4   Discussion

The effect of UVB on photosynthetic apparatus has widely been investigated. There seems to be a number of different targets in the thylakoid membranes for this kind of radiation. Damage to the Mn cluster (Barbato et al. 1995; Hakala et al. 2005; Vass 2012; Vass et al. 1996) associated with cleavage of the D1 protein originating a 23-kDa C-terminal fragment (Barbato et al. 1995, 2000) is considered as the main target for this radiation, but additional target, such as the Q_B site (Trebst and Depka 1990; Vass et al. 1996) has been suggested. The effect of irradiation with a mixture of the two lights has been less investigated, but available data (Sicora et al. 2003) indicate that, even though administrated at the same time, the two kinds of radiations might have different and noninteracting targets. Results reported in this study suggest a slightly different view. UVB irradiation, apparently, did not affect Q_B site, as the rising in fluorescence in the 0–3 ms (OJ phase) is similar to the control. At

variance, when analysis is restricted to the first 300 μs or the Fk/Fj ratio is considered (i.e., the time required for water to diffuse to the donor side of the photosystem), UVB adversely affects the chlorophyll transient, confirming the donor side of PSII as a main target for UVB light. However, when white light is superimposed to UVB, the appearance of a strong band in the first 3 ms of the transient suggests that additional targets are hit, most likely the Q_B site. Accordingly, decay of fluorescence after a single saturating flash supports this possibility. Thus, we suggest that under mixed light conditions (at least when the light intensities here reported are used), electron transfer between Q_A and Q_B may be one main target. Clearly, if sensibility to UVB light increases when white light is present, it is likely that the actual target is a reduced or semireduced form of quinones.

The effect of UVB on the second part of the chlorophyll transient (JI, IP) instead seems to be essentially independent on the presence of visible light. In fact, a decrease in both JI and IP phases is observed. These phases are thought to describe electron transfer steps beyond quinone acceptor leading to reduction of PSI acceptors. If so, we have to conclude that cytochrome b6/f and/or PSI could be specifically targeted for damage. This possibility remains to be investigated.

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