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Damage and repair of Photosystem II under exposure to UV radiation

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Introduction

The ultraviolet component of sunlight is highly detrimental to the photosynthetic apparatus leading to impaired electron transport capacity and damage to the protein subunits. So far most of the research efforts have been devoted to understanding the mechanism of damage induced by the UV-B (280-320 nm) spectral range (Bornman 1989; Vass 1996; Jansen et al. 1998), whose intensity is increasing at the surface of earth due to the recent depletion of stratospheric ozone layer (Smith et al. 1995). The less energetic, but more intense UV-A (320-400 nm) component, which has no selective absorber in the atmosphere is also dangerous to photosynthesis (Cullen et al. 1992; Turcsányi and Vass 2000). However, the mechanism of its effect is not clarified. The first aim of our study was to get a better understanding of the damage induced by UV-A. UV-B radiation has been shown to target the water-oxidizing complex of Photosystem II (PSII) (Renger et al. 1989; Vass et al. 1996), but not clear mechanistic picture of this effect has been emerged so far. The second aim of our work was to characterize the mechanism of UV-B damage exerted on the water-oxidizing complex by selective UV-B illumination of PSII in the different S-states. Under natural conditions the UV component of sunlight is always accompanied by photosynthetically active visible light. Therefore it is very important to understand the interactions of the UV and visible spectral regions. Our previous results indicated that the combined effect of UV and visible light is probably related to the repair of photodamaged protein subunits of the PSII reaction center (Vass et al. 2000). Therefore, the third aim of our work was to study the interaction of visible and UV light in relation to regulation of protein repair.

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, 5mM $MgCl_2$, 10 mM NaCl and 40 mM Hepes (pH 7.5) at 2-3 mg mL^{-1}.

Synechocystis 6803 cells were routinely grown at 90 μE m^{-2} s^{-1} light intensity in BG11 medium at 32 °C, gently bubbled with air containing 5 % CO_2. Cells in the exponential growth phase ($A_{730\ nm}$ of 0.6-0.8) were harvested by centrifugation for 10 min at 4000 x g at room temperature. The chlorophyll *a* content was determined by methanolic extraction and cells were diluted to 6.5 μg Chl a mL^{-1} in fresh growth medium.

UV irradiation was performed under continuous stirring at room temperature in open, cylindrical glass containers. UV-B light was produced by a Vilbert-Lourmat VL-215M lamp in combination with an 0.1 mm cellulose acetate filter (Clarfoil, Courtaluds Chemicals, UK) in order to screen out any UV-C contribution, below 290 nm. The spectral distribution of the UV-B lamp has its maximum at 312 nm and the UV-B intensity at the surface of the cell suspension was 2 Wm^{-2}. A Vilbert-Lourmat VL-215A lamp, with maximal emission at 365 nm was used as UV-A light source and 13.8 Wm^{-2} intensity. In order to screen out any UV-B

or UV-C contribution from the lamp a plastic filter was used, which cut all radiation below 320 nm. As UV-C source a germicidal lamp of 1.2 Wm^{-2} intensity was applied. In some experiments the samples were illuminated with white light of 120-1500 $\mu Em^{-2}s^{-1}$ in combination with UV-B light.

Flash-induced increase and subsequent decay of chlorophyll fluorescence yield was measured by a double-modulation fluorometer (P.S.I. Instruments, Brno) (Trtilek et al. 1997) in the 150 μs to 100 s time range. Steady-state rates of oxygen evolution were measured using a Hansatech DW2 O_2 electrode at a light intensity of 1000 $\mu Em^{-2} s^{-1}$ in the presence of 0.5 mM 2,5-dimethyl-*p*-benzoquinone as electron acceptor. Typically, 2 mL of cells at 10 μg Chl *a* mL^{-1} were used in each measurement.

Results and Discussion

We have previously shown that a very useful diagnostic tool to detect lesions in the electron transport of PSII is flash-induced chlorophyll fluorescence (Vass et al. 1999). Illumination with a single saturating flash reduces the Q_A acceptor, which increases the yield of variable chlorophyll fluorescence. The decay of the increased fluorescence yield reflects the reoxidation of Q_A^-. When forward electron transfer between Q_A^- and Q_B is blocked by DCMU, the reoxidation of Q_A^- occurs via charge recombination with oxidized redox components of the PSII donor side. In functional PSII the recombination partner of Q_A^- is the S_2 state of the water-oxidizing complex, giving rise to about 1s decay time. After UV-B-induced inhibition of oxygen evolution in isolated thylakoids fast decaying phase(s) appear in the fluorescence relaxation kinetics reflecting the reoxidation of Q_A^- by intermediate electron donors (Y_Z^+ and P_{680}^+), which became stabilized by the inhibition of electron donation from the Mn cluster (Vass et al. 1999). The fast decaying fluorescence component(s) also appeared after UV-A irradiation indicating the impairment of donor-side electron transport of PSII at the level of Mn cluster. Similar phenomenon was observed after UV-C irradiation, which is more damaging than UV-A or UV-B.

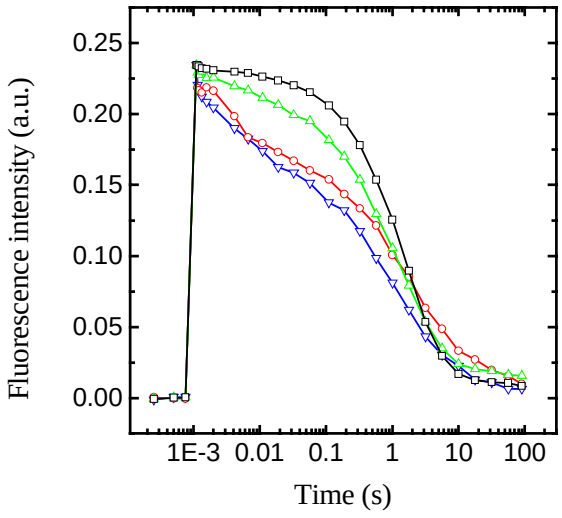

Figure 1. The effect of UV radiation on the relaxation of flash-induced chlorophyll fluorescence in the presence of DCMU. Isolated thylakoids were illuminated with UV-A (up triangles), UV-B (circles) and UV-C light (down triangles) until their oxygen evolving activity was decreased to 50 % of the untreated control (squares). The fluorescence decay was measured after a single saturating flash. The curves are normalized to the same initial amplitude.

The comparison of the curves in Fig. 1 shows that the contribution of the fast phase(s) to the fluorescence decay kinetics become more pronounced with decreasing UV wavelengths, indicating increasing damage of the water-oxidizing complex.

As it emerges from the present data and previous results, the main target site of UV radiation in PSII is the water-oxidizing complex. In order to obtain further mechanistic details of this damaging effect we studied the UV sensitivity of the water-oxidizing complex in its

Table I.

Dominating S-State	S-state distribution (%)				UV Sens.
	S_0	S_1	S_2	S_3	
S_1	37.4	54.6	5.0	3.0	0.11
S_2	8.2	39.0	46.6	6.2	0.46
S_3	7.7	12.8	39.2	40.3	0.56
S_0	35.4	9.4	16.6	38.6	<0.01

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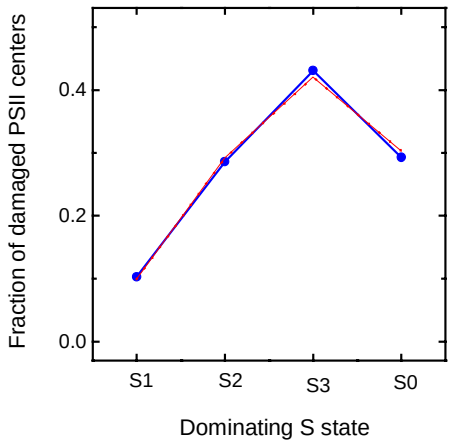

Figure 2. S-state dependent UV-B damage of PSII. Thylakoids were illuminated by Xe flashes to synchronize PSII into different S-state distributions shown in **Table I**. and were irradiated by 308 nm laser flashes. The loss of oxygen evolution was measured after 80 flash packages (circles) and plotted for the dominating S-states. The relative UV sensitivity of PSII in the different S-states was obtained by principal component analysis and listed in Table I. The dotted line shows the result of a fit by using the calculated sensitivity factors

different oxidation states, called S-states. PSII centers were synchronized into different S-states by using packages of non-damaging Xe flashes (Table I.), and then illuminated by intense UV laser flashes (λ = 308 nm). As shown in Fig. 2. the damage induced by UV-laser flashes shows a clear S-state dependence, and highest damage in the S_3 state. Evaluation of the data by principal component analysis indicated high UV sensitivity of PSII in the S_2 and S_3 states (Table I). During the advancement of the S-state cycle the oxidation state of the Mn cluster gradually increases from Mn(II)Mn(III)$Mn(IV)_2$ in S_0 to $Mn(IV)_4$ in S_3 (Debus 1992). Since Mn ions ligated by organic residues have pronounced UV absorption in the Mn (III) and Mn (IV) but not in the Mn (II) state (Bodini et al. 1976) a straightforward explanation of the S-state dependent UV damage is the increased UV absorption of the Mn cluster in the higher S-states. An alternative interpretation may be based on the UV-induced decomposition of peroxidic intermediates of the water oxidation process. UV radiation decomposes H_2O_2 and similar components, which results in highly destructive hydroxil radicals (Czapski 1984) that can be detected in UV-irradiated thylakoid preparations (Hideg and Vass 1996). The process of water oxidation has been proposed to proceed through peroxidic intermediates, leading to the formation of dioxygen released at the end of the S-state cycle (Debus 1992). These intermediates are expected to appear in the higher S-states, thus their UV-induced decomposition could also cause the S-state dependent loss of oxygen evolution. Since the UV absorption of the Mn (III) and Mn (IV) ions as well as of H_2O_2 increases with decreasing

wavelengths of UV light, both mechanisms can explain the increasing efficiency of UV damage of oxygen evolution as the irradiation protocol is changed from UV-A to UV-B and UV-C.

Sunlight damages PSII not only in the UV but also in the visible range (Aro et al. 1993). In order to characterize the interaction of UV and visible light in respect to damaging PSII different intensities of visible and UV-B light were applied separately and in combination,

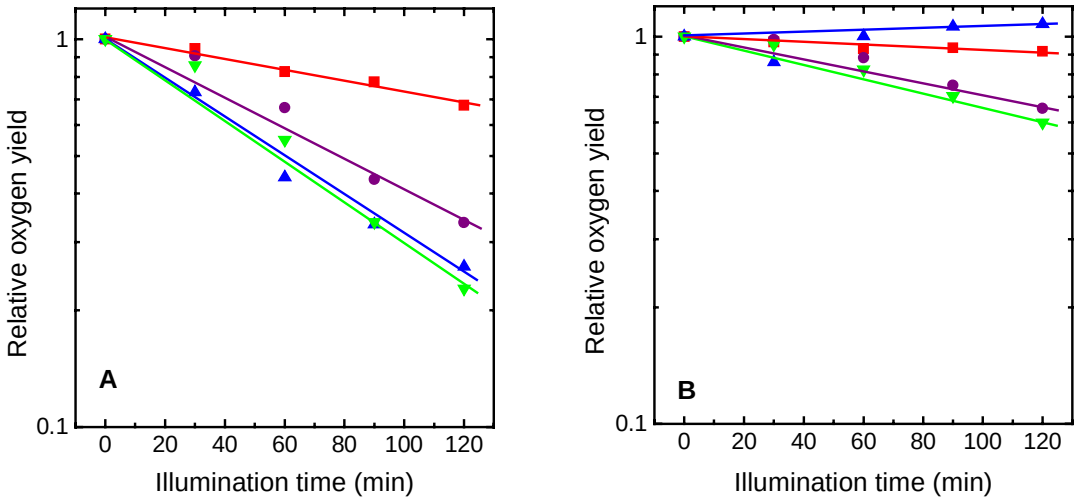

Figure 3. The interaction of UV-B and visible light in Synechocystis 6803 cells. The cells were illuminated by UV-B (circles) and by visible light (squares) separately or simultaneously (up triangles). A calculated damage curve of the combined illumination by assuming independent target sites of UV-B and visible light is also shown (down triangles). The cells were illuminated either in the presence (A) or absence (B) of the protein synthesis inhibitor lyncomycin.

in the presence and absence of the protein synthesis inhibitor lincomycin. The time course of the activity loss can be represented by straight lines in semi- logarithmic plots (Fig. 3.), showing that both UV and visible light impairs PSII by inactivating single molecular targets. When protein synthesis was inhibited by lincomycin, the combined application of UV and visible illumination resulted in larger damage than that induced by either UV or visible light alone. Importantly, the damage curve obtained for the combined illumination can be well represented by sum of the separate damage curves as shown in Fig. 3A. This phenomenon indicates that simultaneous application of UV and visible light does not enhance synergistically, or ameliorate each other's effect, and we can conclude that UV and visible light target independent damage sites in PSII. A completely different picture emerged when intact cells, which are capable of protein repair, were used for the experiments. In that case the damage induced by the separate UV and visible illuminations was less pronounced than that observed in presence lincomycin. This indicates that *de novo* synthesis of the PSII reaction center subunits can partly cope with both visible- and UV-light-induced damage. At relatively low visible light intensities (50-200 $\mu Em^{-2}s^{-1}$) an absolute protection could be observed, i.e. after combined application of the UV and visible illumination the extent damage was less than that induced by UV or visible light alone (Fig. 3B). However, at higher visible light intensities only relative protection, i.e. positive deviation from the damage predicted from the independent mechanisms, was observed (not shown). From these data we conclude that in intact cells simultaneous illumination by visible and UV light leads to a protective effect by enhancing the protein repair capacity of PSII.

In previous studies we have shown that in the cyanobacterium Synechocystis 6803 UV-B light induces a differential transcription of the psbA and psbD gene families, which encode the D1 and D2 reaction center subunits, respectively (Máté et al. 1998). Both gene families contain one gene, psbA3 and psbD2, which are lowly expressed under normal growth conditions, but their mRNA levels are largely increased under exposure to UV-B light. In order to study the regulation of the psbA3 and psbA2 genes we have constructed reporter gene mutants in which the luxAB genes, encoding prokaryotic luciferase, were fused with the psbA3 and psbA2 promoters. Whereas, the luxCDE genes, required for the production of substrates for bioluminescence were incorporated into a neutral site in the genome under

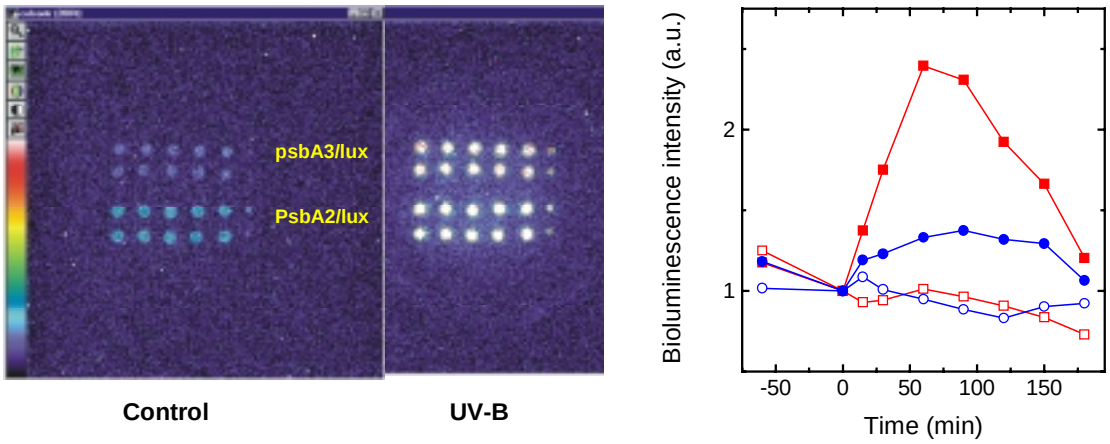

Figure 4. UV-B induced bioluminescence changes in luciferase containing Synechocystis 6803. Left panel: CCD camera recording of bioluminescence from the psbA3/lux and psbA2/lux fusion mutants before and after UV-B illumination. Right panel: The kinetics of bioluminescence in the psbA3/lux (squares) and psba2/lux (circles) mutants. The samples were exposed to UV-B light at time 0, and the controls (empty symbols) were covered by a UV-B filter.

the regulation of the highly active *psbA2* promoter. In the constructed mutants autonomous bioluminescence was observed, which could be resolved by a CCD camera system. The bioluminescence showed the expected behavior, i.e. it could be induced by UV-B light to a larger extent in the *psbA3/lux* than in the *psbA2/lux* constructs.

In conclusion, our results show that UV light damages the water-oxidizing complex not only in the UV-B, but also in the UV-A and UV-C spectral regions. The water-oxidizing complex shows an increased sensitivity in the higher S-states, which is related either to increased absorption of the Mn ions in the Mn (III) and Mn (IV) states or to free radical production from the decomposition of peroxidic intermediates of water oxidation. UV and visible light does not have any synergistic interaction at the level of damaging PSII. However, simultaneous illumination by visible and UV-B light ameliorates the damage by enhancing the capacity of protein repair. We have developed luciferase containing bioluminescent cyanobacterial mutants, which provide a versatile tool to study the regulation of light-induced *psbA* gene expression and D1 protein repair.

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