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Damage to functional components and partial degradation of Photosystem II reaction center proteins upon chloroplast exposure to ultraviolet-B radiation

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Exposure of thylakoid membranes to ultraviolet-B radiation caused inhibition of semiquinone anion formation at Q_A, inhibition of plastoquinone photoreduction, and lower rates of Photosystem II electron-transport to artificial electron acceptors. The amplitude of pheophytin photoreduction was unaffected by the UV-B treatment, suggesting lack of a UV-B adverse effect on the primary charge separation reaction between the photochemical reaction center P680 and pheophytin. Under the experimental conditions employed, approx. 50% inhibition in Q_A photoreduction and in the variable to maximal fluorescence ratio (F_v/F_{max}) was observed. However, plastoquinone photoreduction was lowered by about 65% and electron-transport measurements from H_2O to dichlorophenol indophenol were inhibited by 70–90% in the UV-B treated thylakoids. Rates of electron-transport through PS II could not be restored upon inclusion of artificial donors such as diphenyl carbazide or hydroxylamine. The results suggest a UV-B-induced damage to the primary quinone acceptor Q_A and impairment in the function of plastoquinone in the thylakoid membrane. SDS-PAGE and immunoblot analysis of UV-B-exposed thylakoids revealed the appearance of small quantities of polypeptide fragments (13, 11 and 5 kDa) from the Photosystem II reaction-center proteins. We suggest multiple independent targets of UV-B irradiance in Photosystem II and point to plastoquinone, in its many different configurations in the thylakoid membrane, as a primary UV-B photosensitizer molecule.

Introduction

Ultraviolet radiation in the 280–320 nm region (UV-B radiation) constitutes about 1.5% of the total solar energy emission [1]. In spite of the relatively low intensity, UV-B radiation may have a major impact on life on earth through a multitude of adverse effects [2–5]. Because UV-B light causes damage upon absorption in a variety of biological molecules like DNA, proteins, photosynthetic pigments and electron-transport intermediates (quinones), it has the capacity to alter metabolic pathways, the structure and development of living organisms, their genetic information, and also to alter interactions between species in individual ecosystems [5–7].

Several studies have shown that UV-B radiation adversely affects the function of Photosystem II in chloroplasts [8–11]. This is manifested as lower rates of PS II electron-transport [9], lower charge separation activity in PS II [10], and lower yield of variable fluorescence [11]. Earlier work indicated that UV-B radiation does not exert adverse effect on the concentration of quinone-herbicide (Q_B) binding sites in the thylakoid membrane [11]. However, other investigators reported loss of herbicide binding sites and damage to the PS II donor side in UV-B-treated thylakoids [12,13]. The action spectrum in the ultraviolet for the turnover of the D1/32 kDa reaction center polypeptide of PS II suggested plastoquinone as one of the photosensitizers [14].

In spite of these advances, the precise molecular targets of PS II inhibition by UV-B radiation are not

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Abbreviations: Chl, chlorophyll; PS, Photosystem; Q_A, primary quinone electron acceptor of PS II; RC, reaction center; DCMU, 3-(3,4,-dichlorophenyl)-1,1-dimethylurea; DPC, diphenyl carbazide; DCIP, dichlorophenol indophenol; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

known and the chloroplast response to UV-B damage has not yet been addressed. In the present work, electron-transport measurements, sensitive absorbance difference spectrophotometry, and SDS-PAGE and immunoblot analysis were used to investigate the molecular site(s) of damage and the integrity of the PS II-RC proteins in chloroplast thylakoids following a UV-B treatment. The results showed that UV-B treatment of thylakoids has no adverse effect on the primary charge separation reaction between P680 and pheophytin However, distinct and independent sites of damage in PS II were indicated causing: (i) inhibition in the photoreduction of the primary quinone acceptor Q_A; (ii) inhibition in plastoquinone photoreduction and lower rates of PS II electron transport and (iii) cleavage of the PS II reaction center proteins in the thylakoid membrane.

Materials and Methods

Thylakoid membranes were isolated from freshly harvested, hydroponically grown, spinach leaves (*Spinacia oleracea L.*) [15]. Samples at Chl($a + b$) = 200 μM were placed in open Petri dishes to form a 2 mm thick thylakoid suspension. To avoid thermal inactivation of PS II, and unless otherwise stated, samples were maintained at 0°C by floating the Petri dishes on an ice/water mixture. Control thylakoids were kept in the dark at 0°C. Treated thylakoids were exposed to UV-B radiation emitted by a UVP Transilluminator, model TM-36. The short-wavelength properties of the emission were defined by the transmission of a cellulose acetate filter. Thus, the optical profile of the UV-B emission was zero transmission at 275 and 400 nm, maximum transmission at 302 nm, and T(50%) at 295 and 335 nm. The intensity of this radiation was measured with a model UVX-31 radiometer sensor having a peak sensitivity in the 310–320 nm region and half-peak sensitivity at 285 and 345 nm (Ultra-Violet Products, Inc., San Gabriel, CA). The incident intensity at the sample was 30 W m^{-2}. Samples were exposed to this illumination for 90 min.

Absorbance difference measurements for the quantitation of semiquinone anion (Q_A^-) in control and treated thylakoids were performed with a laboratory-constructed difference spectrophotometer [15]. The optical pathlength of the cuvette for the measuring beam was 0.2 cm and for the actinic beam was 0.14 cm. A differential extinction coefficient of 13 mM^{-1} cm^{-1} was applied [16]. The reaction mixture contained 200 μM Chl, 50 mM Tricine buffer (pH 7.8), 5 mM $MgCl_2$, 10 mM NaCl, 20 μM DCMU, and 2.0 mM $K_3Fe(CN)_6$. The amount of photoreducible plastoquinone was determined from the light-induced absorbance change at 263 nm (ΔA_{263}) [17]. In this case, the reaction mixture contained 200 μM Chl, 50 mM Tricine buffer (pH 7.8), 5 mM $MgCl_2$, 10 mM NaCl and 0.005% Triton X-100 [18].

The concentration of the primary electron acceptor pheophytin of PS II was determined from the light-induced absorbance change at 685 nm (ΔA_{685}) [19–22]. The optical pathlength of the cuvette was 1 cm. The half-band width of the measuring beam was 1 nm. A differential extinction coefficient of 65 mM^{-1} cm^{-1} was applied [21,22]. The reaction mixture contained approx. 10 μM Chl suspended in 20 mM Tris-HCl (pH 7.8) containing 35 mM NaCl, 2 mM $MnCl_2$, 2 μM methylviologen, 2 μM indigodisulfonate and sufficient sodium dithionite to lower the redox potential to −490 mV. The light-induced absorbance-difference measurements were corrected for the effect of particle flattening [23].

Rates of electron transport were measured with a Clark-type oxygen electrode upon yellow actinic excitation (CS 3-68 Corning filter) of thylakoid membranes suspended in the presence of 10 mM NaCl, 5 mM $MgCl_2$, 50 mM Pipes (pH 7.0), 5 μM gramicidin, 200 μM dimethylbenzoquinone and 0.5 mM potassium ferricyanide. Rates of DCIP reduction were measured spectrophotometrically at 600 nm upon blue actinic excitation (CS 4-96 Corning filter) of thylakoid membranes in a reaction mixture containing 100 μM DCIP as the artificial electron acceptor. A differential extinction coefficient of 21 mM^{-1} cm^{-1} for the reduction of DCIP was used [24]. Whenever indicated, the artificial electron-donor diphenyl carbazide was added to a concentration of 500 μM.

Thylakoid membrane proteins were resolved by SDS-PAGE at 2°C in continuous 15% acrylamide linear gels containing 4 M urea by using a Tricine buffer system [25]. The samples were solubilized at room temperature in 50 mM Tris-HCl (pH 6.8) buffer containing 10% glycerol, 4% SDS, 4 M urea and 2% β-mercaptoethanol. Electrophoresis on 0.15 × 16 × 18 cm slabs was performed at a constant current of 22 mA for 44 h. Gel lanes were loaded with solubilized membranes containing 10 μg Chl ($a + b$). Gels were stained with 0.1% Coomassie brilliant blue R for protein visualization.

Identification of reaction center polypeptides was accomplished by immunoblot analysis using polyclonal antibodies. The rabbit polyclonal antibodies utilized in this work were raised in the laboratory against the isolated PS II-RC proteins (method of Nanba and Satoh [26,27]) and, therefore, they recognized epitopes on constituent PS II reaction center proteins (psbA, psbD, psbE, psbF, and psbI gene products). This was both by design and advantageous in this study as it illustrated the broad spectrum of structural changes in the PS II-RC occurring after damage by UV-B irradiance. Electrophoretic transfer of the SDS-PAGE-resolved polypeptides to nitrocellulose, and the subse-

quent incubations with the above antibodies and with alkaline-phosphatase-conjugated antibodies were performed as described in Ref. 28.

Results

Primary photochemistry

Exposure of isolated thylakoids to UV-B caused inhibition of the primary photochemical activity in PS II. This was evidenced both in measurements of fluorescence induction and in measurements of the light-induced reduction of the primary quinone acceptor Q_A. Fig. 1 shows traces of the chlorophyll *a* fluorescence induction kinetics from control and UV-B-treated thylakoids, measured in the presence of DCMU and hydroxylamine. The first illumination for the registration of the induction trace was administered to dark-adapted thylakoids. The same sample was illuminated for a second time following 1 min dark incubation. In the absence of artificial electron donors, 1 min dark incubation is sufficient for the recovery of most of the variable fluorescence. The lack of recovery suggests that functional PS II units were locked in the Q_A^- state upon the first illumination, in essence demonstrating the ability of the artificial electron donor to remove positive charges from the oxidizing side of PS II. Similar results were obtained with other artificial electron donors, i.e., diphenyl carbazide and carbonyl-cyanide *p*-trifluoromethoxyphenylhydrazone (results not shown). It is evident that UV-B-treated thylakoids have a substantially lower variable fluorescence yield. This UV-B induced quenching was not alleviated upon

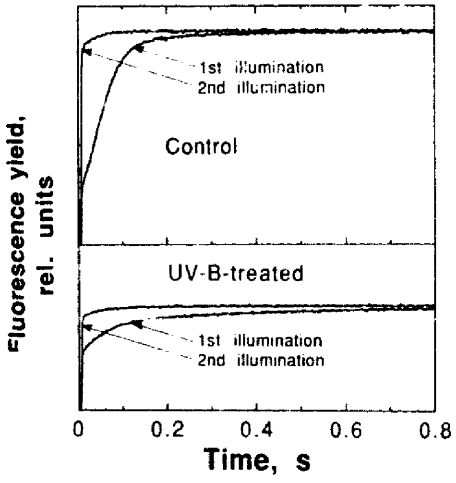

Fig. 1. Fluorescence induction kinetics with control and UV-B-treated thylakoids suspended in the presence of 20 μM DCMU and 2 mM hydroxylamine. First illumination and registration of the fluorescence induction trace was with dark-adapted thylakoids. The second illumination was administered after a 1 min dark incubation of the sample.

TABLE I

Photochemical apparatus characteristics in control and UV-B-treated thylakoids from spinach

The fluorescence yield values were normalized arbitrarily to 1.0 for the F_0 yield of the control. The Chl/Q_A ratios are given as mol/mol. Numbers reported are the average value of six independent determinations. The uncertainty in the Chl/component ratio measurements was ±50/1 (S.D.).

	Control	UV-B-treated
F_0	1.0	0.93
F_v	2.97	0.71
F_v/F_{max}	0.75	0.43
Chl/Q_A	400/1	840/1
Chl/Pheo	355/1	346/1

inclusion of a variety of artificial electron donors to the reaction mixture (Fig. 1), suggesting that a site of damage cannot be bypassed by artificial electron donors.

Fluorescence induction measurements were extended to provide an estimate of the relative concentration of Q_B-nonreducing centers in the thylakoid membrane. This quantitation is based on the measurement of the amplitude of the initial variable fluorescence yield (from F_0 to F_{pl} [29], as discussed recently [30,31]. The analysis indicated a UV-B-induced proportional decrease in the relative concentration of all PS II centers (Q_B-reducing and Q_B-nonreducing) in the thylakoid membrane. These results suggest that all PS II centers in thylakoids are about equally susceptible to UV-B damage and that UV-B does not simply convert plastoquinone-reducing centers to a Q_B-nonreducing form.

Table I presents a summary of results from related measurements. The results show similar non-variable fluorescence yields (F_0) from control and UV-B-treated thylakoids but substantially lower variable fluorescence yields (F_v) in the UV-B-treated samples. In consequence, the F_v/F_{max} ratio was lowered significantly from 0.75 in the control, to about 0.43 in UV-B-treated samples. Since the F_v/F_{max} ratio is a measure of the yield of primary photochemistry [32], the results suggest inactivation of about 50% of PS II-RCs by this UV-B treatment.

This conclusion was tested independently from direct measurements of the amplitude of the light-induced absorbance change at 320 nm (ΔA_{320}), attributed to the semiquinone anion formation upon reduction of Q_A. Fig. 2 shows traces of ΔA_{320} in control and UV-B-treated samples. It is evident that UV-B treatment resulted in significant inhibition of Q_A photoreduction. We detected the light-induced reduction of 1 Q_A per 400 Chl ($a + b$) molecules in control thylakoids. In UV-B-stressed thylakoids, the amount of Q_A that could become photochemically reduced was

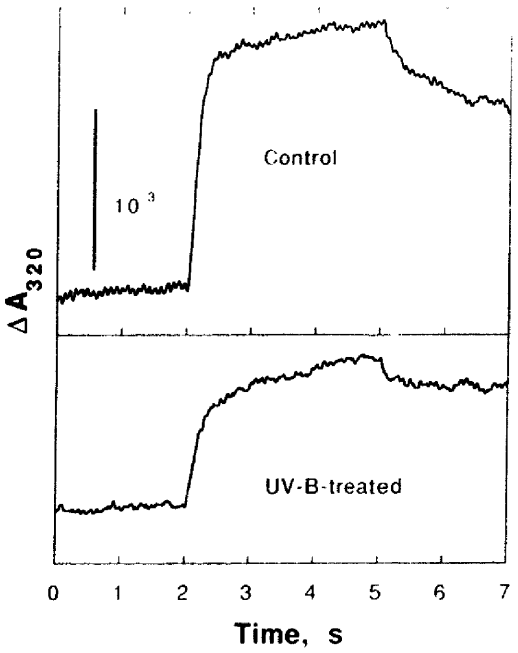

Fig. 2. Light-induced absorbance change measurements at 320 nm with control and UV-B-treated thylakoids suspended in the presence of 20 μM DCMU and 2 mM potassium ferricyanide. Actinic illumination came on at 2 s, and went off at 5 s.

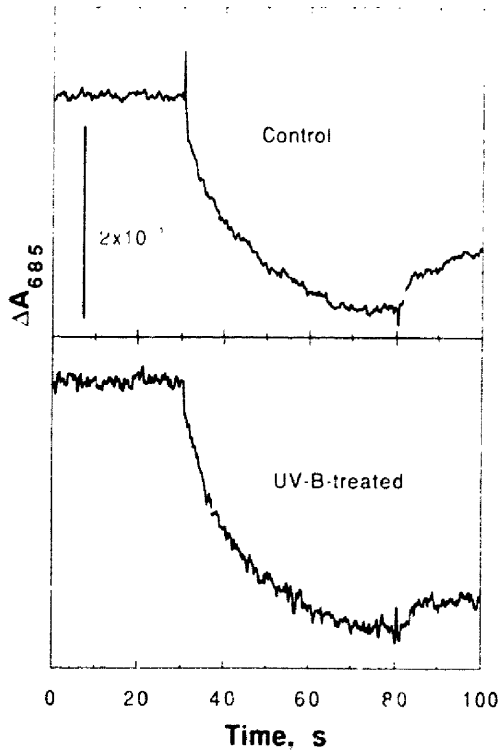

Fig. 3. Light-induced absorbance change measurements at 685 nm with control and UV-B-treated thylakoids suspended in the presence of 2 μM methylviologen, 2 μM indigosulfonate and sufficient sodium dithionite to lower the redox potential to −490 mV. Actinic illumination came on at 30 s, and went off at 80 s. Please note the significantly slower time scale in this measurement compared to those of Figs. 1 and 2.

lower, about 1 Q_A per 840 Chl molecules (Table I). In agreement with the fluorescence yield data, the inhibition of Q_A photoreduction could not be alleviated upon inclusion of an artificial electron donor, such as hydroxylamine, in the reaction mixture (results not shown). These measurements suggested UV-B-induced damage to about 50% of all PS II centers in the spinach thylakoids.

A UV-B-induced inhibition of Q_A photoreduction, as measured in this work, could come about because of damage to Q_A, or because of damage to other integral PS II-RC components such as pheophytin and/or P680. To delineate between these alternatives, we measured the amplitude of pheophytin photoreduction in control and UV-B stressed thylakoids. Fig. 3 shows traces of ΔA_{685} in control and UV-B-treated samples. The amplitude of ΔA_{685} provides a direct measure of the amount of pheophytin that can be photochemically reduced [19–22]. It is evident that illumination of control and UV-B-treated samples results in the photoreduction of approximately the same amount of pheophytin. We estimated the light-induced reduction of 1 pheophytin per 350 Chl $(a+b)$ molecules in thylakoids, independent of UV-B exposure (Table I). Fig. 4 shows the absorbance difference spectra in the red region associated with the pheophytin photoreduction. The difference spectra of control and UV-B-treated thylakoids were identical in the 680–720 nm region. In the 650–675 nm region, the amplitude of ΔA for the UV-B treated samples was consistently lower than that of the control (Fig. 4). This minor distortion in the pheophytin absorbance difference spectrum was noted earlier in measurements with isolated and purified PS II reaction center preparations [33] in which the quinone acceptor Q_A was absent.

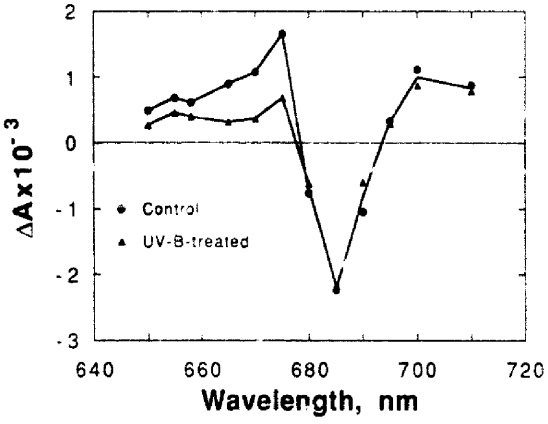

Fig. 4. Light-induced absorbance difference spectra in the red region associated with the measurements shown in Fig. 3.

The combined measurements above clearly show a UV-B-induced inhibition in the electron transfer reaction from pheophytin to Q_A and a lack of UV-B effect on the primary charge separation reaction between P680 and pheophytin. It is suggested that the primary reactants (P680 and pheophytin) in Photosystem II are not subject to damage by UV-B. However, the quinone acceptor Q_A probably is a molecular target of UV-B damage. Consistent with this interpretation is the observation of a minor distortion in the pheophytin absorbance difference spectrum (Fig. 4) which also indicates a UV-B adverse effect on Q_A.

Rates of electron transport

Linear electron-transport measurements through PS II provided further insight into the effects of UV-B damage. In order to investigate the possibility of additional UV-B-induced lesions in the vicinity of PS II, rates of oxygen evolution and DCIP reduction were measured in the presence and absence of the artificial electron donor diphenyl carbazide (DPC donation bypasses the H_2O oxidizing site [34]) both at saturating and limiting actinic intensities. Under saturating illumination, control thylakoids exhibited rates of oxygen evolution equivalent to 500 μM electron mM^{-1} Chl s^{-1} whereas that of UV-B-treated thylakoids was only 35 μM electron mM^{-1} Chl s^{-1} (93% inhibition, Table II, upper). The extent of inhibition was consistently greater than that of Q_A, suggesting additional adverse effect of UV-B at a site in the electron-transport chain near PS II. The light-saturated rates of oxygen evolution in the control (500 μM electron mM^{-1} Chl s^{-1}) remained unchanged in the presence of DPC (490 μM electron mM^{-1} Chl s^{-1}) (Table II). Such results provide justification for the use of DPC in UV-B-treated thylakoids because it shows that DPC itself does not exert an adverse effect on the function of the oxygen evolving apparatus in PS II.

To ensure that electron donation by DPC to PS II is competitive with the rate of photochemistry, we measured rates of DCIP reduction under strictly light-limiting conditions. At an actinic intensity of 200 μmol photons m^{-2} s^{-1} (Table II, lower), the rate of DCIP reduction was inhibited by about 78% in the UV-B-treated samples when compared to the control. This extent of inhibition, although less than that under saturating illumination, was still greater than the inhibition of Q_A. Interestingly, the artificial electron donor DPC restored a small portion (~ 15%) of the rate of electron-transport to DCIP (Table II, lower). Such measurements were repeated at actinic light intensities in the range of 50–500 μmol photons m^{-2} s^{-1} and with DPC concentrations in the range of 0.2–2.0 mM, yielding essentially identical results (not shown). These findings amend the results of Fig. 1 because they suggest some UV-B damage to the donor side of PS II, as evidenced from the ~ 15% restoration of the electron-transport rate by DPC [13,35]. It is clear, however, that artificial electron donors cannot alleviate the major portion of the UV-B-induced damage and that, in addition to the Q_A site, UV-B damage occurs at a site in the electron transport chain that prevents electron flow to DCIP.

We pursued the site of this inhibition further. To this end, we investigated the function of the plastoquinone pool in control and UV-B-treated thylakoids. Fig. 5 shows traces of the light-induced absorbance change at 263 nm (ΔA_{263}), reflecting the accumulation of plastohydroquinone in the thylakoid membrane. The amplitude of the absorbance change in control thylakoids indicated the photochemical reduction of approx. 12 mmol PQ per mol Chl (Chl/PQ = 85 : 1). This is consistent with measurements by other investigators [17,18]. In UV-B-treated thylakoids, the amount of photoreducible plastoquinone was lowered to about 35% of that in the control. This extent of inhibition was greater than that of Q_A but comparable to the inhibition of electron transport.

SDS-PAGE and immunoblot analysis

The consequence of UV-B-induced damage to PS II was addressed further by SDS-PAGE and immunoblot analysis of the PS II-RC polypeptides. Fig. 6 shows the Coomassie-stained polypeptide profile of control (lane 2) and UV-B-treated (lane 3) thylakoids from spinach. No significant changes in the polypeptide composition could be detected between the two samples solely on the basis of protein staining. However, immunoblot analysis with polyclonal antibodies raised against the PS II-RC proteins revealed reaction center degrada-

TABLE II

Steady-state electron-transport rates in control and UV-B-treated thylakoids from spinach

Rates of electron transport were estimated either from the rate of oxygen evolution using a Clark-type electrode, or from the rate of DCIP reduction measured as the absorbance change at 600 nm. All rate units are given in μM electron mM^{-1} Chl s^{-1}. The uncertainty in the rate measurements was ±10% of the value given (S.D.). For other experimental conditions, see Materials and Methods.

	Control	UV-B-treated
Oxygen evolution		
Light-saturated rates		
H_2O as the only electron donor (11500 μmol photon m^{-2} s^{-1})	500	35 (7%)
+ 500 μM DPC (11500 μmol photon m^{-2} s^{-1})	490	40 (8%)
DCIP reduction		
H_2O as the only electron donor (200 μmol photon m^{-2} s^{-1})	55	12 (22%)
+ 500 μM DPC (200 μmol photon m^{-2} s^{-1})	55	18 (33%)

tion products specifically in the UV-B-treated thylakoids.

Fig. 7 shows the strong cross-reaction of polyclonal antibodies with the D1/D2 32/34 kDa PS II-RC proteins (these are the psbA/psbD gene products) (lanes 1–7). In addition, there was antibody cross-reaction with minor polypeptides migrating with apparent molecular masses of 36 and 23 kDa. These may be attributed to the precursor form and primary in vivo degradation product of the rapidly-turning-over D1/32 kDa protein, respectively [36,37]. There was also antibody cross-reaction with the 9 and 4 kDa polypeptides of the cytochrome *b*-559 complex (psbE and psbF gene products, respectively).

Incubation of thylakoid membranes for 90 min in the dark at 0°C (Fig. 7, lane 1), or for 90 min in the dark at 0°C followed by 60 min at room temperature (Fig. 7, lane 2), did not elicit antibody cross reaction with additional proteins. In contrast, treatment of thylakoids with UV-B for 90 min at 0°C resulted in the appearance of additional distinct polypeptides that were recognized by the polyclonal antibodies. This is illustrated in Fig. 7 (lane 3) which shows antibody cross-reaction with several minor polypeptides in the 20–25 kDa region as well as with distinct polypeptides having apparent molecular masses of 13, 11 and 5 kDa. None of these polypeptides was present in the control samples (lanes 1 and 2). Further incubation of UV-B-treated thylakoids at room temperature in the dark (Fig. 7, lane 4, 7 min; lane 5, 15 min; lane 6, 30 min and lane 7, 60 min) did not change either the pattern or the relative amounts of the PS II-RC protein fragments. It is suggested that cleavage of the D1/D2 reaction center proteins occurred at 0°C during the UV-B exposure, and that subsequent room temperature incubation of thylakoids did not result in further processing of these partial proteolytic products.

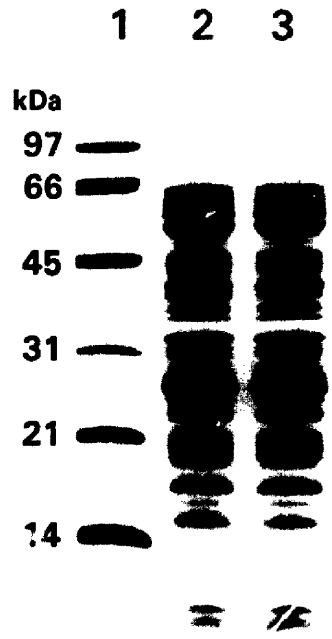

Fig. 6. Coomassie-stained polypeptide profile of control (lane 2) and UV-B stressed (lane 3) thylakoids from spinach. The UV-B treatment was administered for 90 min at 0°C. Molecular mass markers are shown in lane 1.

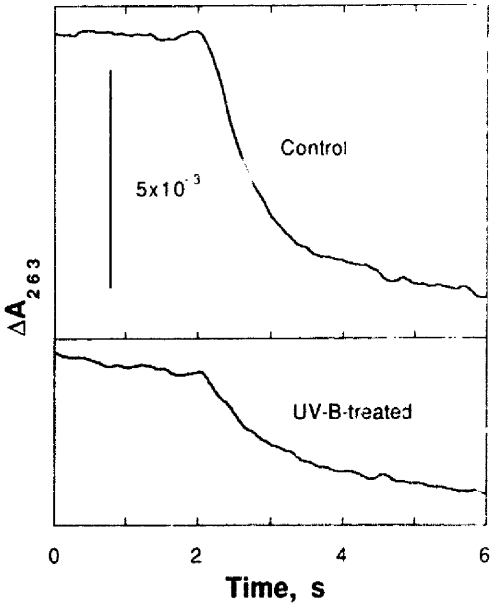

Fig. 5. Light-induced absorbance change measurements at 263 nm with control and UV-B-treated thylakoids suspended in the absence of exogenous co-factors. Actinic illumination came on at about 2 s.

Discussion

This work shows that exposure of thylakoid membranes to UV-B radiation lowers the yield of variable fluorescence, inhibits the function of the primary quinone Q_A but does not elicit any adverse effect on the primary charge separation between P680 and pheophytin. Under the experimental conditions employed in this work, only about 50% of PS II centers were inhibited in the photoreduction of Q_A (Table I). Measurements of plastoquinone photoreduction showed inhibition by about 65% after UV-B exposure, whereas measurements of PS II electron-transport activity

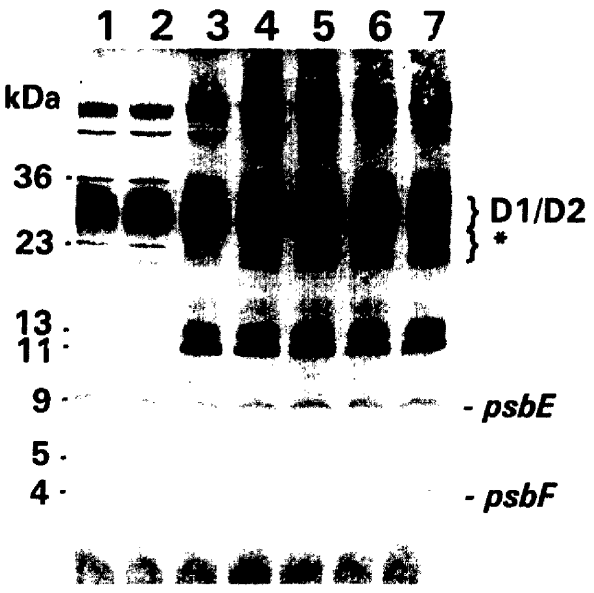

Fig. 7. Immunoblot analysis of spinach thylakoid membranes. Lanes 1, control thylakoids incubated for 90 min in the dark at 0°C; 2, control thylakoids incubated for 90 min in the dark at 0°C, followed by 60 min at room temperature; 3, thylakoids exposed to UV-B radiation for 90 min at 0°C; 4–7, thylakoids exposed to UV-B radiation for 90 min at 0°C, followed by incubation at room temperature for 7 min (lane 4), 15 min (lane 5), 30 min (lane 6), or 60 min (lane 7).

showed inhibition from 70 to 90%. In addition, SDS-PAGE and immunoblot analysis of UV-B exposed thylakoids revealed the appearance of distinct 13, 11 and 5 kDa polypeptide fragments from the PS II-RC proteins. These results provide new information on the targets of UV-B stress in chloroplast thylakoids.

Clearly, a primary site of damage by UV-B is the specialized bound quinone Q_A of PS II. We hypothesize that modification of Q_A by UV-B prevents electron transport from reduced pheophytin to Q_A, thus resulting in charge recombination reactions between $P680^+$ and $Pheo^-$ and quenching of the variable fluorescence yield F_v. Quenching of F_v due to charge recombination reactions would occur independently in those PS II centers with impaired donor sides (Table II, [13,35]).

The UV-B-induced inhibition in the photoreduction of plastoquinone cannot be explained as merely the consequence of PS II inhibition at Q_A. This is because several PS II reaction centers can feed electrons independently into a common pool of PQ molecules [38,39]. Thus, if the only site of damage-was at Q_A under our experimental conditions we should have observed photoreduction of the entire PQ pool, albeit with slower kinetics in UV-B-treated samples. The results of Fig. 5 showed the photoreduction of only 35% of the PQ pool in the UV-B-treated samples. The photoreduction kinetics were similar in control and UV-B-treated samples. We conclude that an additional primary site of damage in the thylakoid membrane is the plastoquinone pool, whose function is impaired following UV-B exposure.

The overlapping and/or additive effects of Q_A inhibition and PQ impairment will suffice to explain the disproportionate inhibition (70–90%) observed in the process of electron-transport from PS II to artificial electron acceptors (Table II). The adverse effect of UV-B on PQ function will also explain the discrepancy in the O_2 evolution (ferricyanide as the acceptor) and DCIP reduction measurements (Table II). With ferricyanide at saturating intensities, electrons must go through the PQ pool (much of which is impaired) and through the cytochrome b_6-f complex before they reach the acceptor. With DCIP at limiting intensities, two things are different: (a) the acceptor can interact directly with the PQ pool, thereby alleviating much of the need for shuttling of electrons from PS II to the cytochrome b_6-f complex; (b) the limiting intensity allows for a greater amount of time for electron transfer to the acceptor.

Since plastoquinone is an important target of UV-B stress in thylakoid membranes, it is reasonable to expect adverse effects at the Q_B-binding site, whenever the site is occupied by a PQ molecule. In this case, damage to the D1/Q_B-binding protein could occur indirectly via the bound PQ molecule which would act as the UV-B photoreceptor. This possibility is of interest in view of recent work by Greenberg et al. [14] who measured the rate of the D1/32 kDa protein degradation in vivo over a broad spectral range (UV, visible and far red) and concluded that damage and degradation of the D1/32 kDa protein of PS II was highest in the UV-B (280–320 nm) region. Their data implicated plastoquinone, in one or more of its redox states, as the photosensitizer in the UV-B [14]. Thus, mechanistically, the possibility exists of direct UV-B-induced damage to PQ molecules at the Q_B-binding site which may then act as a trigger for the D1/32 kDa protein degradation. Our results on the accumulation of partial degradation products from the PS II-RC proteins are consistent with the role played by UV-B in the turnover of the Photosystem II D1/32 kDa protein [14,40].

The UV-B-induced damage to PS II is distinct from damage by visible light, better known as photoinhibition [41,42], since the latter adversely affects both the Q_A and pheophytin photoreduction reactions [21,43]. Indeed, work by several investigators [43–46] suggested that photoinhibition by visible light impairs an intermediate on the donor side of PS II and prevents a stable charge separation, indicating damage either to the reaction center (P680) or to tyrosine (Z). It was reported that chlorophyll is the photosensitizer for

photoinhibition by visible light [14,42], whereas direct absorption of UV-B radiation by plastoquinone, in more than one of its configurations in the thylakoid membrane (e.g., Q_A, Q_B, or PQ), must be the target of the UV-B damage.

Quantitation of the UV-B effects shows that 50% inhibition in Q_A photoreduction, 65% inhibition in PQ photoreduction and 70–90% inhibition in the overall rate of PS II electron transport is accompanied by a partial degradation of about 10% of the total PS II-RC protein. The quantitative discrepancy on the damage at different sites again suggests multiple independent targets and additive effects in the thylakoid membrane. It is of interest to observe that photoinhibitory illumination (visible spectral region) of thylakoid membranes [47], and of isolated oxygen-evolving PS II-core complexes [48], results in degradation of the D1-protein, accompanied by the appearance of at least four different partial degradation products. However, in photoinhibition by visible light, proteolysis was prevented if the treatment was administered at 0°C. Low temperature incubation during UV-B exposure did not prevent protein cleavage (Fig. 7), suggesting a direct or indirect photolysis of the PS II-RC proteins by UV-B. Our results further show that room temperature incubation (up to 60 min) of the UV-B treated thylakoids does not elicit changes in the relative amounts of the primary proteolytic fragments, suggesting that no further processing of protein fragments occurs in isolated thylakoids.

There are conflicting reports in the literature on the effect and molecular targets of UV-B in thylakoid membranes. Concerning the identification of a 'universal' UV-B target for PS II [6], our work suggests independent targets in and around this multiprotein complex. In agreement with other investigators [13,14,49], we show a UV-B adverse effect on the integrity of the PS II reaction center proteins. However, we found the reducing side of PS II to be most susceptible to damage by UV-B, as evidenced by the pronounced inhibition of Q_A and PQ function. This is in contrast to reports indicating the H_2O oxidizing enzyme as the dominant target of UV-B damage [13]. This discrepancy is not understood at present. It may be the consequence of the different experimental conditions employed by the different groups, including plant material and visible/UV intensity ratios during exposure. Alternatively, it may relate to the differential sensitivity of thylakoid membrane components to UV-C and UV-B radiation [9]. It is known that most UV-B light sources emit significant amounts of UV-C [11] which may have additional adverse effects on thylakoids. In this respect, the use of cellulose acetate filters is strongly recommended [11,12] in order to eliminate UV-C components from the emission profile of ultraviolet lamps.

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