Inhibition of Photosystem II by UV-B-Radiation

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The effect of UV-B-radiation on PSII activity of spinach chloroplasts was analyzed by measuring the integrity of the herbicide-binding protein (HBP 32), by measurement of fluorescence induction in the presence of Diuron (DCMU), and by mathematical analysis of the fluorescence induction curves. It was shown that UV-B inactivates the PSII α-centers but not PSII β-centers. However, the possibility cannot be excluded that in addition the donor site of PSII near the reaction center is attacked by UV-B-radiation.

Introduction

It is well known that UV-C-radiation emitted by a mercury lamp (mostly 254 nm) inhibits photosynthetic activity of leaves and isolated chloroplasts. The action of UV-C is primarily on photosystem II, as shown by the destruction of plastoquinone/plastohydroquinone and of the water-splitting system [1–9]. This short-wave UV-C radiation, however, is not present in daylight and therefore is not relevant to damage of plant photosynthetic systems in the natural environment. UV-B (280–320 nm) present today in ambient solar radiation can also have damaging effects on the photosynthesis of UV-sensitive plants [10–12]. This is of increasing importance if destruction of the stratospheric ozone layer by air pollutants should occur and thus enhance the UV-B-radiation at the earth's surface.

It was shown that plant leaves and isolated chloroplasts irradiated with increasing amounts of UV-B show decreased activity of photosystem II (PSII), as monitored by measurements of PSII activity [13]. Furthermore, in irradiated chloroplasts the detactable functional amount of the first stable electron acceptor of PSII, X-320 ($= Q_A$), was substantially reduced, as was oxygen evolution. However, it was impossible either to restore the amplitude of the X-320 absorbance change by using the PSII electron donor couple hydroquinone/ascorbate [13] or to restore the DCPIP mediated Hill reaction with the donor DPC [9]. It was therefore concluded that UV-B inactivates the photosystem II reaction centers by transforming them into dissipative sinks. However, these results could not exclude the possibility, especially in view of the reduction of variable fluorescence, that Q_A or Q_B (second PSII electron acceptor) and its binding protein might also be attacked by UV-B-radiation. Assuming that the Q_B-binding protein is identical to the herbicide-binding protein with a molecular weight of 32–34 kD (Q_B protein, HBP 32), we investigated the intactness of the Q_B-binding protein by using the PSII herbicide atrazine, which has been shown to bind specifically at the HBP 32 [14]. Furthermore, kinetic analyses of the sigmoidal fluorescence induction curve in the presence of Diuron (DCMU) were employed to characterize the influence of UV-B on the reaction center types $PSII_\alpha$ and $PSII_\beta$, which were assumed to contribute to the biphasic fluorescence induction curve in isolated chloroplasts [15].

Materials and Methods

Broken chloroplast thylakoid membranes were isolated from young spinach leaves according to a previously published procedure [16]. The thylakoid suspensions were diluted to a chlorophyll content of 50 µg Chl/ml. Twenty ml aliquots of this solution were transferred to 10 cm ∅ glass Petri dishes, and covered with 2 mm-thick Schott WG 305 cut-off filters. The suspension was slowly stirred in a stream of nitrogen and irradiated by a UV-B lamp (Philips TL 40/12) from a distance of 30 cm for up to 1 h.

Abbreviations: DCMU, 3-(3,4-dichlorodiphenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenolindophenol; DPC, diphenylcarbazide.

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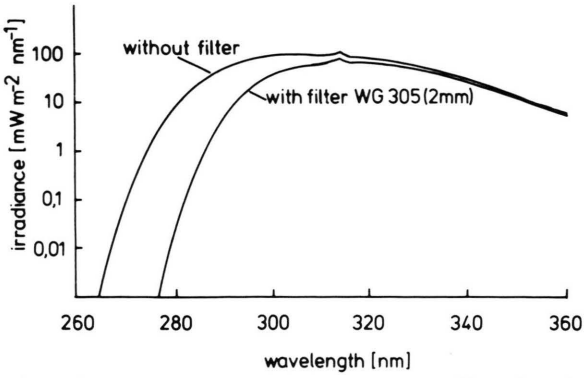

Fig. 1. Spectral energy distribution of the unfiltered and filtered (Schott WG 305, 2 mm) radiation from a UV lamp (Philips TL 40/12) at a 30 cm distance.

Table I. Relative Hill reaction rates (100% = 300 µmol/mg Chl × h), binding constants and concentration of binding sites for atrazine of spinach chloroplasts irradiated with UV-B for different periods of time.

Treatment (min UV-B)	Hill reaction	K_b [M]	Chl/ binding site	nmol binding sites/ mg Chl
0 (dark control)	100	7×10^{-8}	280	3.6
20	73	6×10^{-8}	310	3.2
40	43	6×10^{-8}	320	3.1
60	26	9×10^{-8}	310	3.2
60 (no UV)	113	8×10^{-8}	320	3.1

The spectral energy distribution of the UV radiation at the suspension surface was measured with an Optronics 742 double monochromator (Fig. 1). The UV-B irradiance was 1.4 W m^{-2}.

The Hill reaction was performed with DCPIP as electron acceptor, yielding a Hill reaction rate equivalent to 300 µmol O_2 per mg chlorophyll and hour in control chloroplasts during the experiments. The protocol for performance of the binding experiments is described in the literature [17].

Chlorophyll fluorescence was measured at a chlorophyll concentration of 2 µg/ml, using blue green actinic light from a fiberoptic illuminator (Schott KL 5000) filtered through a CS 4-96 Corning filter. Fluorescence was detected by a photodiode/amplifier combination (UDT 500) screened by a RG 665 red filter (Schott, Germany). The signal was stored in 4 K words at 12 bit resolution in a Nicolet digital oscilloscope and analyzed on line in an HP 9826 computer. The time resolution of the measurements was limited by the opening time of the electromechanical shutter to about 400 µs.

The results reported are averages of at least two measurements from three independent experiments.

Results and Discussion

Hill activity

DCPIP Hill activity of isolated spinach chloroplasts kept in the dark was equivalent to 300 µmol O_2/mg Chl × h and did not decrease during the experiments. In contrast, UV-B-irradiated chloroplasts lost 27% of their activity within 20 min and 74% within 60 min (Table I). DPC as an exogeneous electron donor was ineffective in restoring the activity of the DCPIP Hill reaction in UV-B-irradiated chloroplasts, indicating damage between the oxidizing and reducing sites of PSII, close to the reaction center (data not shown). This confirms results in UV-B-irradiated Amaranthus chloroplasts [9]. Furthermore, the donor couple hydroquinone/ascorbate was not able to restore the reduced signals of the 320 nm absorption changes assumed to be representative for the reduction of the electron acceptors Q_A and Q_B of PSII, as shown in UV-B-irradiated spinach chloroplasts [13].

Atrazine binding

The reduction of X-320 signal could be partly due to damage to the second electron acceptor Q_B and its binding protein. This binding protein is assumed to be identical to the herbicide binding protein HBP 32 with a molecular weight of 32 kD [14]. Binding of atrazine to control chloroplasts showed the usual hyperbolic saturation kinetics, which did not significantly change upon UV-B irradiation. The binding constants (K_B) during the UV-irradiation averaged 7×10^{-8} M. The concentration of binding sites was around 310 Chl/binding site or equivalent to 3.2 nmol binding sites per mg chlorophyll (Table I). Assuming that the binding sites of atrazine to HBP 32 are identical or at least very closely related, as is now commonly believed [17–19] it can be concluded that the Q_B-protein remains intact.

Fluorescence induction analysis

Variable fluorescence, defined as $F_{max} - F_0$, was shown to be reduced by UV-B irradiation in intact leaves as well as in isolated chloroplasts [12, 13]. Reduction of variable fluorescence can reflect more efficient electron transport capacity because of faster oxidation of the reduced plastoquinone (PQ) pool by external oxidants such as oxygen. To exclude this possibility, we used Diuron-inhibited chloroplasts for all fluorescence experiments (5×10^{-5} M Diuron). Diuron is now commonly assumed to inhibit the electron flow by displacing Q_B from its binding site, as was first proposed by Velthuys [18].

Chlorophyll fluorescence induction curves of Diuron-poisoned spinach chloroplasts irradiated for up to 1 h with UV-B showed no substantial change in the initial fluorescence F_0, but a decline in F_{max} (Fig. 2) and thus in F_{var} (Table II). Furthermore, the area over the induction curves is greatly increased upon UV-B irradiation (Table II). It is unlikely that this represents a true increase in the PSII acceptor pool size, since there should be only one Q_A available per PSII reaction center. Probably, new dissipative sinks are created by UV-B treatment, which prevent an efficient use of separated charges in the reduction of Q_A. This would lead to an increased area above the fluorescence induction curve, as is observed in a more detailed analysis of the induction curves (Table II). Alternatively, the decrease in F_{max} could also be due to a partial reoxidation of Q_A^- by oxygen or be induced by a deficiency of charges at the donor site.

Table II. Parameters of chlorophyll fluorescence induction of Diuron (DCMU)-poisoned spinach chloroplasts irradiated with UV-B (values in rel. units). Diuron conc.: 5×10^{-5} M. F_0 = initial fluorescence; F_{max} = maximum fl.; F_v = variable fl.; A = area over the fl. induction curves.

Treatment (min UV-B)	F_0	F_{max}	F_v/F_0	A
0	132	496	2.8	13.5
20	122	400	2.3	42
40	120	350	1.8	54
60	135	335	1.5	51

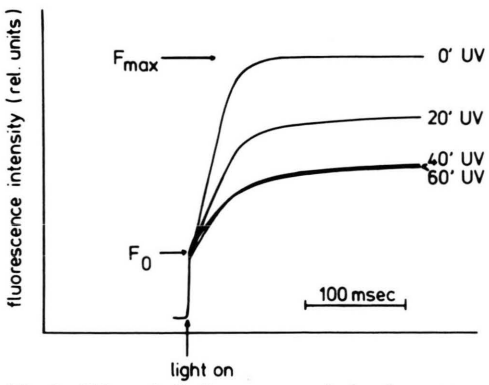

Fig. 2. Chlorophyll fluorescence induction curves of control and UV-B irradiated chloroplasts poisoned with Diuron (5×10^{-5} M).

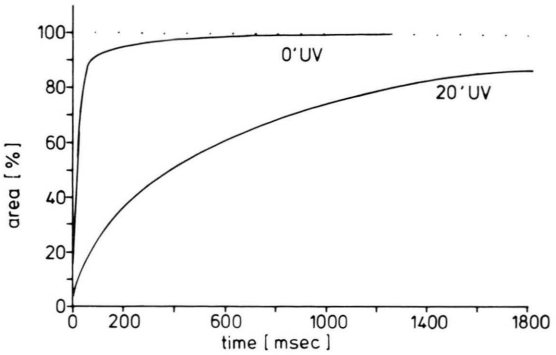

Fig. 3. Area-growth kinetics of the area over the chlorophyll fluorescence induction curves of spinach chloroplasts kept in the dark and after 20 min UV-B irradiation in the presence of Diuron (5×10^{-5} M). Area normalized to unity.

Kinetic analysis of the fluorescence induction curve shows that the growth of the area over the induction curve, which is equivalent to the degree of Q_A reduction, proceeds much slower in UV-B-treated chloroplasts as compared to the control. Of the area, 90% (equivalent to 90% of Q_A) has grown within a few ms in control chloroplasts whereas UV-treated chloroplasts showed simpler, nearly monophasic rise kinetics and accumulated 80% of the total area after 2 s (Fig. 3).

For a more detailed analysis of the area-growth kinetics in the presence of Diuron, the plot of ln(1-area) *vs* time has been used in various studies [20, 16]. In this plot the fluorescence rise curve is split into two kinetically distinct components, the so called α- and β-phases, apparently representing two different types of reaction centers: the efficient α-centers and the less-efficient β-centers [15]. Our preparation of spinach chloroplasts showed that about 90% of the Q_A reduction is mediated by the α-centers (Fig. 4). A few minutes of UV treatment (< 20 min) nearly abolished the contribution of the α-centers. The β-centers now controlled the main

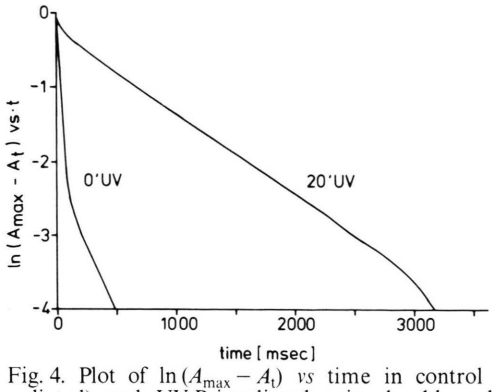

Fig. 4. Plot of $\ln(A_{max} - A_t)$ *vs* time in control (non-irradiated) and UV-B-irradiated spinach chloroplasts according to Fig. 3.

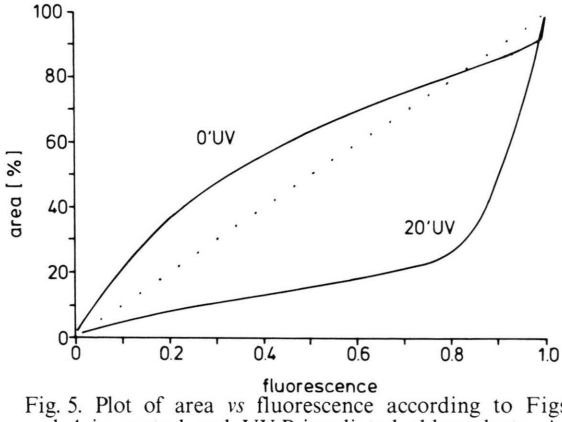

Fig. 5. Plot of area *vs* fluorescence according to Figs. 3 and 4 in control and UV-B-irradiated chloroplasts. Area and fluorescence normalized to unity.

part of the kinetics of area increase or Q_A^- accumulation, although with decreased efficiency as compared to the control.

The plot of accumulated area *vs* fluorescence shows a large positive deviation from linearity towards area accumulation in control chloroplasts (Fig. 5). This commonly observed deviation is assumed to indicate a cooperation between the PSII α-centers, which are located in a statistical pigment bed [21, 22, 25]. The much smaller negative deviation from linearity in the last phase of the Q_A reduction is assumed to be due to the contribution of the separate units of β-centers. β-Centers are assumed to be located in the stroma thylakoids, whereas α-centers are exclusively associated with the grana partition regions [23–25].

After UV-B treatment the positive deviation is entirely lost, whereas the negative deviation is dramatically increased. This is in agreement with the first order analysis of area growth (Fig. 4), which also indicates a loss of α-center activity. The plot shows, furthermore, that a large fraction of the fluorescence (~ 80%) contributes only to a small extent (~ 20%) in the accumulation of reduced Q_A, which is in contrast to the situation in untreated chloroplasts. Similar results were obtained with Mg^{2+}-depleted chloroplasts [26]. As in UV-B-treated chloroplasts, the fluorescence yield controlled by the β-centers was only slightly changed, in contrast to that of α-centers which was reduced upon Mg^{2+} depletion. Since Mg^{2+} depletion induces unstacking and decreases spill over, excitation energy is lost. Similarly, UV-B might change the stacking of thylakoids and therefore disturb the cooperation of PSII α-centers.

In conclusion: Using the PSII herbicide atrazine as an indicator for the structural integrity of the Q_B protein, it seems unlikely from our results that UV-B treatment affects the function of the secondary acceptor Q_B. UV-irradiation specifically eliminates the activity of the fast and efficient α-centers of the grana partitions within a few minutes of irradiation time (< 20 min). This effect is probably also responsible for the observed decrease of the overall electron transport rate. Whether α- and β-centers are structurally distinct [26] or whether they represent only functionally different PSII centers [27] is not yet clear. It should be noted that the effect of UV-B irradiation (inactivation of the PSII centers, and linked with this, elimination of the cooperation between the PSII centers, as concluded from study of the area *vs* fluorescence plot) is largely completed within 20 min (Fig. 4). Longer irradiation times (20–60 min) do not substantially increase these effects. However, the electron transport rate continues to decrease during the time course of our experiments (0–60 min, Table I). From this one may conclude that at least two different sites of UV action are involved in the inactivation of PSII reactions: one site probably located near the reaction center, and the other at the PSII oxidizing site.

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