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Inhibition of Chloroplasts by UV-Irradiation and Heat-Treatment

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Abstract. **The site of inhibition in UV-irradiated and heat-treated chloroplasts was examined by using artificial electron donor compounds such as *p*-phenylenediamine and hydroquinone which donated electrons specifically to photosystem II. In both cases the electron donors restored the photoreduction of nicotinamide adenine dinucleotide phosphate and the restored activity was inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethyl urea. The fluorescence of variable yield was eliminated by both inhibitory treatments and was partially restored by the electron donors in the heat-treated but not the UV-irradiated chloroplasts. The results suggest that the sites of inhibition of UV-radiation and heat treatment are in the photosynthetic electron transport chain between water and photosystem II.**

The mechanism of UV-inhibition of photosynthesis and chloroplast reactions has been the subject of a number of investigations. Bishop (1) showed that UV-radiation destroyed plastoquinone in chloroplasts and found a correlation between the degree of inhibition of the Hill reaction and the extent of destruction of plastoquinone. Shavit and Avron (2), however, were unable to demonstrate a specific restoration of activity in UV-irradiated chloroplasts by adding pure plastoquinone and concluded that the UV-radiation inactivated other components as well as plastoquinone. Jones and Kok (3) localized the UV-inhibition to a PS2[2] reaction by showing that the photoreduction of NADP with DCPIP and ascorbate as the electron donor was not inhibited by a UV-treatment which completely inhibited the water-NADP Hill reaction. They found no specific evidence to implicate plastoquinone as the site of inhibition but suggested that the reaction centers of PS2 were inactivated. The purpose of the work reported here was to explore the site of UV-inhibition by using artificial electron donor compounds which donate electrons to the photosynthetic electron transport chain on the oxidizing side of PS2.

Our work (4, 5) with chloroplasts which had been inhibited by a wash treatment with 0.8 M tris buffer, pH 8.0, showed that the site of the tris-wash inhibition was between water and PS2 and that NADP photoreduction and photophosphorylation could be restored by adding artificial electron donors which could donate electrons to PS2. The usual electron donor compounds, such as DCPIP or TMPD, which have been used to restore activity to DCMU-poisoned chloroplasts, could donate electrons to the electron transport chain both before and after PS2. PD, however, at relatively low concentration (33 μM PD plus 330 μM ascorbate) donated electrons almost exclusively to the site before PS2, as indicated by the sensitivity of PD-supported NADP photoreduction to DCMU. At higher concentration, PD donated electrons after the DCMU block as well. We have more recently examined a number of potential electron donor compounds with tris-washed chloroplasts and have found a number of compounds which can donate electrons to PS2 but are blocked by DCMU. Some, such as HQ, restored a higher rate of NADP photoreduction and showed a greater inhibition by DCMU than did PD. These electron donor systems which are specific for PS2 are useful in examining electron transport reactions in the vicinity of PS2. In the present paper we report that NADP photoreduction can be restored to UV-irradiated chloroplasts by adding HQ and ascorbate as an electron donor system and that the restored activity is almost completely inhibited by DCMU.

We also show the same restoration and DCMU sensitivity with heat-treated spinach chloroplasts. Katoh and San Pietro (6, 7) previously demonstrated that NADP photoreduction in heat-treated *Euglena* chloroplasts could be restored with ascorbate acting as an electron donor and that the restored activity was inhibited by DCMU.

Experimental Procedures

Chloroplasts were prepared by grinding 50 g of spinach leaves in a Waring Blendor for 20 sec in 150 ml of solution containing 0.4 M sucrose, 0.05 M tris-HCl (pH 7.8) and 0.01 M NaCl (abbreviated

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[2] Abbreviations: PS1 and PS2, photosystem I and photosystem II; DCPIP, 2,6-dichlorophenol-indophenol; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; PD, *p*-phenylenediamine; HQ, *p*-hydroquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

as STN solution). The supernatant from a 1-min centrifugation at 300*g* was centrifuged again at 600*g* for 7 min. The precipitate was suspended in 20 ml of STN solution and heavy particles were removed by centrifugation at 300*g* for 1 min. The remaining supernatant was centrifuged at 1500*g* for 7 min and the precipitate was resuspended in 2 ml of STN solution at 1 to 2 mg chl/ml. The heat-treated chloroplasts were heated at this stage to 50° for 3 min. Aliquots were resuspended at 10 μg chl/ml in a reaction medium consisting of 15 mM tris-HCl, 4 mM KPO_4, 4 mM $MgCl_2$, and 20 mM NaCl pH 7.8 for measurement of NADP photoreduction. The UV-irradiated chloroplasts were suspended in the reaction medium at 10 μg chl/ml before the irradiation treatment and were irradiated in a thin layer (<0.1 mm) for 10 min at a distance of 3 to 4 cm from a G. E. G8T5 8-watt germidal lamp.

NADP photoreduction was measured in a 3-ml, 1-cm cuvette with a modified Aminco-Chance Dual Wavelength Spectrophotometer with the measuring wavelengths set at 340 and 370 nm. The EMI 9524 phototube was blocked with Corning Filters 5840 and 5970. Red actinic light (3.3 × 10^4 ergs cm^{-2} sec^{-1}) from the side was obtained with a tungsten lamp and 645 nm interference filter. Fluorescence yield measurements were made with a weak, chopped monitoring beam (650 nm) and a lock-in amplifier as described previously (4, 5). The measurement indicates the change in fluorescence yield caused by the actinic beam but not the high level of fluorescence produced by the actinic beam.

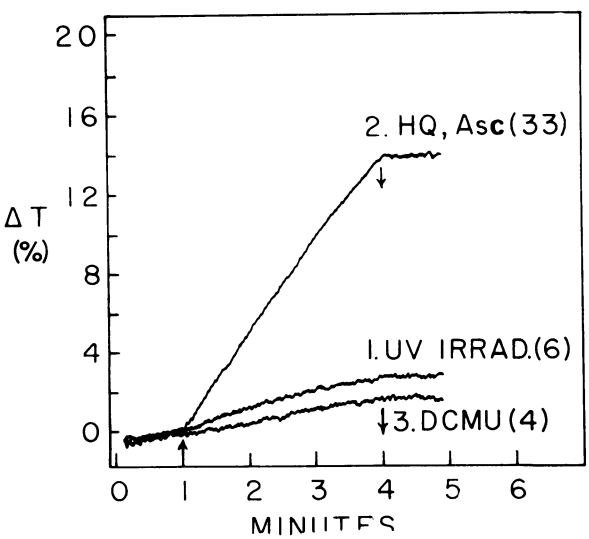

FIG. 1. Photoreduction of NADP by UV-irradiated chloroplasts (10 μg chl/ml). Curve 1) no additions. Curve 2) 200 μM HQ and 330 μM ascorbate added. Curve 3) 1 μM DCMU added after curve 2. Red actinic light (3.3 × 10^4 ergs cm^{-2} sec^{-1}) on at upward arrow, off at downward arrow.

Results

Photoreduction of NADP with UV-irradiated chloroplasts is shown in figure 1 and with heat-treated chloroplasts in figure 2. In both cases the inhibition was almost complete—the rate of photoreduction for non-inhibited chloroplasts being about 120 μmoles NADPH per mg chl per hr *vs* 6 for the UV-irradiated chloroplasts and 8 for the heat-treated chloroplasts. In both cases a substantial recovery of activity was obtained by adding 200 μM HQ and 330 μM ascorbate as an artificial electron donor system and the restored activity was inhibited by DCMU. Thus, the site of electron donation and the site of inhibition must be before the DCMU block and, therefore, presumably before PS2.

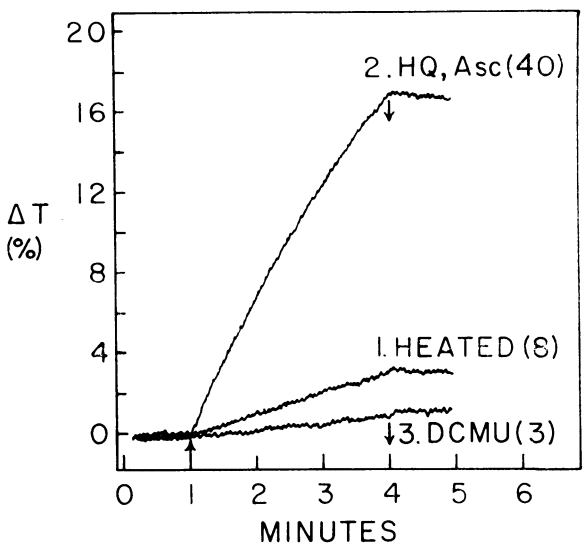

FIG. 2. Photoreduction of NADP by heat-treated chloroplasts (10 μg chl/ml). Curve 1) no additions. Curve 2) 200 μM HQ and 330 μM ascorbate added. Curve 3) 1 μM DCMU added after curve 2. Red actinic light (3.3 × 10^4 ergs cm^{-2} sec^{-1}) on at upward arrow, off at downward arrow.

The participation of PS2 in the HQ-supported photoreduction was also indicated by a rather qualitative test for the "red drop phenomenon". Figure 3B indicates the relative effectiveness of the red and far-red actinic illumination for the photoreduction of NADP in a classical PS1 reaction where DCPIP was the electron donor in the presence of DCMU. By comparison, the relative ineffectiveness of far-red light in the photoreduction when HQ was the electron donor (fig 3A) suggests the participation of PS2 in that photoreaction.

With tris-washed chloroplasts fluorescence measurements gave evidence that the site of donation was before PS2. The large increase of fluorescence yield that occurs when normal chloroplasts are illuminated has been related to the photoreduction of Q (8), the hypothetical primary electron acceptor of PS2.

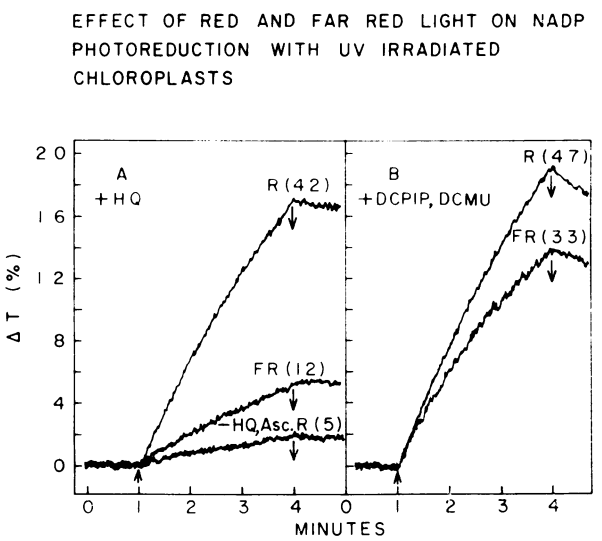

FIG. 3. Photoreduction of NADP by UV-irradiated chloroplasts (10 μg chl/ml) with red and far-red actinic light. A) In the presence of 200 μM HQ and 330 μM ascorbate and without electron donor system (-HQ, Asc). B) In the presence of 200 μM DCPIP, 1 mM ascorbate, and 1 μM DCMU. Red (3.3×10^4 ergs cm^{-2} sec^{-1}) and far-red (3.4×10^4 ergs cm^{-2} sec^{-1}) actinic light, indicated as R and FR. On at upward arrow, off at downward arrow.

Q quenches fluorescence but QH does not. The fluorescence yield of the tris-washed chloroplasts was the same as normal chloroplasts in the dark (*i.e.* in weak measuring light) but showed very little increase on irradiation. Addition of the electron donor system, however, restored the fluorescence of variable yield, presumably by restoring the source of electrons for the photoreduction of Q by PS2. The fluorescence of heat-treated chloroplasts (fig 4A) behaved much the same as that of the tris-washed chloroplasts. The heat treatment virtually eliminated the fluorescence of variable yield but an appreciable light-induced fluorescence-yield increase was restored when PD or HQ was added as an electron donor for PS2. The fluorescence of variable yield was also eliminated by the UV treatment as Kok *et al.* (9) noted previously but in this case (fig 4B) the electron donor did not restore the fluorescence yield changes. It was also noteworthy that DCMU had no effect on the fluorescence yield in the light either in the presence or absence of an electron donor. The UV-irradiation appears to impair the fluorescence of variable yield to a greater extent than the other inhibitory treatments. It was possible to obtain a high fluorescence yield (under weak measuring light) with the UV-irradiated chloroplasts by placing them in a low potential environment under strict anaerobic conditions. The fluorescence yield at low potential ($E_h < -320$ mv) was 6 to 7 times greater than that at high ($E_h > 0$ mv) and the titration curve at intermediate potentials showed the same 2 components found by Cramer and Butler (manuscript in preparation) in fluorescence titration curves with normal chloroplasts. Under aerobic conditions, however, addition of dithionite to UV-irradiated chloroplasts gives a rather small increase of fluorescence yield whereas the same treatment with normal chloroplasts gives a large increase of yield, even greater than the maximum yield obtained with DCMU.

Discussion

The site of inhibition in UV-irradiated and heat-treated chloroplasts appears to be between water and PS2 as it was with tris-washed chloroplasts. Plastoquinone is probably not the primary site of UV-inhibition, even though it is destroyed by UV-radiation (1), because it functions after PS2 (10). Huzisigi *et al.* (11) extracted a component from chloroplasts by washing with 0.8 M tris HCl pH 8.0 which increased the O_2 evolving activity of PS2 particles that had been prepared by digitonin treatment and differential centrifugation. The tris-wash treatment itself inhibits the electron transport system between water and PS2. The oxygen evolving factor (OEF), after partial purification by column chromatography, showed adsorption maxima at 215 and 265 nm, the latter band being lost on oxidation with ferricyanide. OEF, however, did not restore activity to heat-treated or tris-washed chloroplasts so that Huzisigi *et al.* (11) suggested other factors

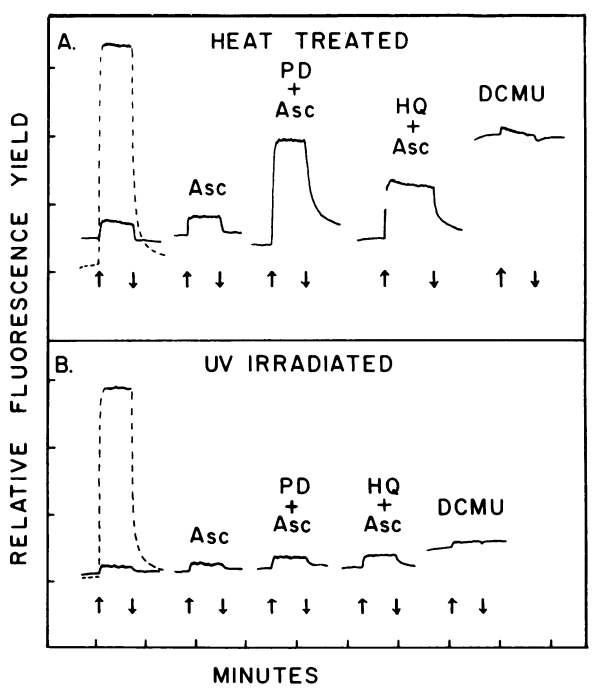

FIG. 4. Relative fluorescence yield of heat-treated (A) and UV-irradiated (B) chloroplasts, solid curves. Dash curve shows response of normal chloroplasts. 330 μM ascorbate, 33 μM PD, 200 μM HQ and 1 μM DCMU added where indicated. Red actinic light (2.4×10^4 ergs cm^{-2} sec^{-1}) on at upward arrows, off at downward arrows.

were also involved in these inhibitory treatments. The UV absorption spectrum of OEF makes it a candidate for the site of UV inhibition.

The absence of the fluorescence of variable yield in UV-irradiated chloroplasts, even in the presence of DCMU and electron donors for PS2, is anomalous particularly since PS2 appears to be functioning photochemically. Kok *et al.* (9) suggested that the PS2 traps of UV-irradiated chloroplasts were permanently open but they presumed that PS2 was inactivated by the treatment. Our results indicate PS2 can be active and not show fluorescence yield changes. It can be pointed out that fluorescence yield changes do not accompany PS1 activity but this does not account for the effect of UV radiation on PS2 fluorescence.

Duysens and Sweers (8) proposed that there was a photochemically inactive form of Q, labeled Q′, which quenched PS2 fluorescence but was not photochemically reduced to the non-quencher, QH. The photochemically active quencher, Q, was regenerated in some sort of metabolic cycle from Q′. If such a proposal is valid, the absence of fluorescence yield changes in UV-irradiated chloroplasts suggests that the regeneration of Q is blocked by the UV treatment.

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