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Photoinhibition of Photosynthesis in Broken Chloroplasts as a Function of Electron Transfer Rates during Light Treatment[1]

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ABSTRACT

Photoinhibition was studied in osmotically broken chloroplasts isolated from spinach leaves (*Spinacia oleracea* L.). Both whole chain electron transport (measured as ferricyanide-dependent O_2 evolution in the presence of NH_4Cl) and photosystem II activity (measured as O_2 evolution in the presence of either silicomolybdate plus 3-(3,4-diphenyl)-1,1 dimethylurea or parabenzoquinone) showed similar decreases in activity in response to a photoinhibitory treatment (8 minutes of high light given in the absence of an electron acceptor other than O_2). Photosystem I activity was less affected. Photoinhibition of silicomolybdate reduction was largely reversible by an 8 minute dark incubation following the light treatment. Decreasing the O_2 concentration during photoinhibition below 2% increased photoinhibition of whole chain electron transport. Addition of superoxide dismutase to the reaction medium did not affect photoinhibition. Photoinhibition of both photosystem I and photosystem II activity increased as the rate of electron transfer during the treatment increased, and was largely prevented when 3-(3,4-diphenyl)-1,1-dimethylurea was present during the photoinhibition period. Noncyclic photophosphorylation was decreased as a consequence of whole chain electron transfer photoinhibition. Since diphenyl carbazide added after light treatment did not relieve photoinhibition of dichlorophenol indophenol reduction, we conclude that the site of inhibition is located within or near the photosystem II reaction center.

Photosynthetic organisms (or organelles), when subjected to high light, exhibit decreased photosynthetic capacities. This photoinhibition is generally ascribed to excess light energy which cannot be eliminated by operation of the electron transfer chain or by other means of dissipating light energy (13). Damage caused by high light treatment appears to be located mainly within the PSII reaction center (16). However, Kyle *et al.* (11) recently showed in *Chlamydomonas reinhardii* that it was possible to induce photoinhibition of DCPIP[2] reduction (a PSII reaction in which DCPIP accepts electrons from the quinone protein QB) without inhibition of silicomolybdate reduction in the presence of DCMU (a PSII reaction in which SiMo accepts electrons from the primary acceptor QA): the primary stable acceptor of PSII might be intrinsically susceptible to light-induced damage as a consequence of its function involving a reactive semiquinone anion species. Photoinhibition would then be a consequence of the operation of whole chain electron transport.

Although it has been demonstrated in whole leaves (4, 14, 15) and in isolated intact chloroplasts (10) that maintenance of sufficient carbon metabolism is essential to prevent photoinhibition, it is not known if there is a relationship between the rate of whole chain electron transport during high-light treatment and the extent of the resulting photoinhibition. Osmotically broken chloroplasts provide a simple system in which it is easy to induce photoinhibition (8, 9, 17, 18). This system has been used to examine the site of the light-induced damage and the mechanism of photoinhibition (17, 18). It is also possible to modulate the rate of electron transport during photoinhibitory treatment in such a system. In this paper, we have used osmotically broken chloroplasts to test whether the extent of photoinhibition is related to the rates of electron transfer during a high light treatment. We have also characterized inhibition in relation to the site of high light action, its O_2 sensitivity, and its reversibility.

MATERIALS AND METHODS

Spinach plants (*Spinacia oleracea* L.) were grown in a growth cabinet. Conditions were: photoperiod, 9 h; day temperature, 22°C; night temperature, 12°C; PFD, 400 μmol quanta m^{-2} s^{-1}. Plants were grown in vermiculite in 0.5-L pots watered daily and fertilized. Intact chloroplasts were isolated from leaves as described by Heber (7). The percentage of intact chloroplasts, as determined by the ferricyanide reduction test, was usually between 70 and 85%. Experiments were performed on osmotically broken chloroplasts.

Measurement of Electron Transport. Electron transport was estimated either spectrophotometrically or polarographically: (a) spectrophotometrically: with a UNICAM spectrophotometer as either NADP or DCPIP reduction; (b) polarographically: with a temperature-controlled water jacketed Clark-type electrode assembly as either O_2 evolution or O_2 absorption. A halogen lamp (OSRAM 24 V, 150 W) was focused with a lens system on the reaction mixture contained in the electrode compartment. Maximum obtainable PFD was 5000 μmol quanta m^{-2} s^{-1}, and this was adjusted to the desired value using neutral filters. The assay medium contained 50 mM Hepes (pH 7.6), 1 mM $MgCl_2$, 1 mM EDTA. Whole chain electron transport was measured either with 3 mM Fecy or 10 μM Fd and 4 mM NADP in the presence of 5 mM NH_4Cl. PSII uncoupled electron transport was determined as silicomolybdate-dependent O_2 evolution (SiMo, 0.5 mM) in the presence of 3 μM DCMU, and parabenzoquinone-dependent O_2 evolution (PBQ, 1 mM), it was also measured at 600 nm

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[2] Abbreviations: DCPIP, dichlorophenol indophenol; Asc, ascorbate; DPC, diphenyl carbazide; Fecy, ferricyanide; Fmax, maximal fluorescence; MV, methyl viologen; PGQ, parabenzoquinone; PFD, photon flux area density; SiMo, silicomolybdate.

(DCPIP reduction). Electron donation to the oxidizing side of PSII was measured in the presence of 1 mM DPC as electron donor, and DCPIP as electron acceptor as in the method of Shneyour and Avron (19). PSI activity was assayed with 1 mM Asc, using 0.1 mM DCPIP as the electron donor in the presence of 3 μM DCMU with either 0.1 mM MV or 10 μM Fd plus 4 mM NADP as electron acceptor. NH_4Cl (5 mM) was added when indicated. ATP synthesis coupled to NADP photoreduction was measured by adding 5 mM $MgCl_2$, 4 mM NADP, 4 mM ADP, 4 mM Pi, and ^{32}P (4.5 kBq·μmol^{-1}). The reaction was stopped with cold TCA (final concentration, 2%). After centrifugation, the $AT^{32}P$ formation was determined by the method of Avron (1).

Photoinhibitory Treatment. Samples were exposed to the photoinhibitory treatment in the cuvette of the O_2 electrode, and consisted of an 8-min illumination (preceded by 1 min in darkness) of osmotically broken chloroplasts at the stated PFD and gas compositions. At the end of this period, the appropriate reagents were added, and chloroplast activities were determined. The control treatment consisted of a 9-min period of darkness under the same conditions. The effect of the 8-min preillumination on various activities was calculated using the ratio:

$$([AO - A]/AO) \times 100$$

where AO and A are the activities measured after the control treatment and after the photoinhibitory treatment, respectively. Measurements were usually made at saturating PFD (2500 or 3500 μmol quanta m^{-2} s^{-1}). All experiments were at 25°C in the presence of catalase (350 units/ml).

Chemicals. Ferredoxin was isolated from fresh spinach leaves according to the method of Mayhew (12), and NADP, catalase, DCPIP, MV, and Asc were purchased from Sigma Chemical Co.

RESULTS

Characterization of Photoinhibition. Table I shows the extent of photoinhibition (expressed as percentage of inhibition of control activity) measured at saturating light either with Fecy plus NH_4Cl (whole chain electron transport), parabenzoquinone (PBQ; PSII activity) or silicomolybdate (SiMo) plus DCMU (PSII activity), added at the end of the preincubation period in a medium equilibrated with 21% O_2. The observed inhibitions were very similar, though the absolute rates measured with SiMo in the presence of DCMU were usually about one-third lower than those measured with FeCy or PBQ (see 6, 11). The inhibition of PSI activity, measured as methyl viologen-dependent O_2 absorption in the presence of Asc, DCPIP, and DCMU was comparatively low. PSII-fluorescence measurements showed that Fmax measured in liquid nitrogen at 685 nm was reduced by about 20%, while constant level of fluorescence was increased slightly (data not shown). Table I also shows that when DCPIP and Asc were added to the reaction medium just after the photoinhibitory treatment the photoinhibition of NADP photoreduction in the presence of Fd and NH_4Cl (whole chain electron transport) was largely suppressed. The small inhibition that remained was presumably due to decreased PSI activity. In contrast, when DPC was added just after the preillumination period, photoinhibition of PSII, measured as DCPIP reduction, was only partially suppressed. The values of photoinhibition of NADP and DCPIP photoreduction are higher than those reported for the other reactions because of higher PFD (5000 μmol quanta m^{-2} s^{-1}, as opposed to 3500 μmol quanta m^{-2} s^{-1}) during treatment.

Preillumination of broken chloroplasts inhibited noncyclic electron transport to NADP in the presence of Fd and ADP to the same extent as it inhibited photophosphorylation; the ATP/2e-ratio remained constant (Table II).

Addition of superoxide dismutase during the preincubation period had no effect on the subsequent rate of Fecy reduction, even when photoinhibition was increased by various additions during photoinhibition treatment (data not shown). Moreover, the photoinhibition of Fecy-dependent O_2 evolution, measured in a medium containing 5% O_2, was increased when the O_2 concentration during preincubation was lowered below 2% (Fig. 1).

Figure 2 illustrates the effect of the dark period duration following the photoinhibitory treatment on the subsequent photoinhibition of SiMo-dependent O_2 evolution in the presence of DCMU. The actual rates of O_2 evolution measured after the dark period following preincubation period in the light or in the dark (control treatment) are also shown. The photoinhibition induced by 8-min high light treatment was largely reversed by 8 min of darkness; Fecy and PBQ gave similar results (data not shown). However, when inhibition was increased above 60% by various additions during photoinhibitory treatment, inhibition was only partially reversed after 8 min in darkness.

Effect of the Rate of Electron Flow during Photoinhibitory Treatment on the Extent of Photoinhibition. Table III shows the extent of PSII inhibition, measured as PBQ-dependent O_2 evolution, when electron transfer during the photoinhibitory treatment was varied by addition of NH_4Cl and/or of Fd and NADP.

Table I. *Effect of an 8-Min Preillumination Period (Photoinhibitory Treatment) on Various Subsequent Chloroplast Activities*

The control treatment consisted of an 8-min incubation period in the dark. The conditions during treatment were: photon flux density, 3500 μmol m^{-2} s^{-1} (reactions 1–4) or 5000 μmol m^{-2} s^{-1} (reaction 5 → 8); temperature, 25°C; medium equilibrated with 21% O_2. The actual rate of O_2 evolution (±SD, n = 4) and the rate of NADP or DCPIP reduction (mean of two measurements) are shown together with the percentage of inhibition of the measured activities. 3 mM Fecy; 1 mM PBQ; 5 mM NH_4Cl; 0.5 mM SiMo; 3 μM DCMU; 0.1 mM DCPIP; 1 mM Asc; 0.1 mM MV; 4 mM NADP; 10 μM Fd; 1 mM DPC.

Reaction	Addition during Measurement	Rate of Electron Transfer		Inhibition
		Control	Treated	
		microequivalents mg^{-1} Chl h^{-1}		%
H_2O → Fecy	NH_4Cl	267 ± 13	175 ± 8	34.4
H_2O → PBQ	None	273 ± 18	169 ± 15	38.0
H_2O → SiMo	DCMU	180 ± 21	117 ± 12	35.8
DCPIP → MV	DCMU, Asc, NH_4Cl	232 ± 9	217 ± 7	6.5
H_2O → NADP	Fd, NH_4Cl	96	28	70.8
DCPIP → NADP	Fd, NH_4Cl, Asc	110	82	25.4
H_2O → DCPIP	NH_4Cl	171	34	80.2
DPC → DCPIP	NH_4Cl	150	57	62.0

Table II. *Effect of an 8-Min Photoinhibition Treatment on the Subsequent NADP Reduction and ATP Synthesis*

PFD during photoinhibition treatment was 3500 μmol m^{-2} s^{-1}. Control treatment consisted of an 8-min incubation in the dark. Measurements were performed under PFD of 2000 μmol m^{-2} s^{-1}. Temperature was 25°C and the medium equilibrated with 21% O_2. Assay medium contained 330 units catalase/ml. Chl concentration was 15 μg/ml. The data are the mean of two measurements. The actual rates of reduction and synthesis are shown together with the P/2e$^-$ ratio.

	Reaction Rate		
	NADP reduction	ATP synthesis	P/2 e$^-$
	μmol mg^{-1} Chl·h^{-1}		*ratio*
Control	76	108	1.4
Treated	60	77	1.3

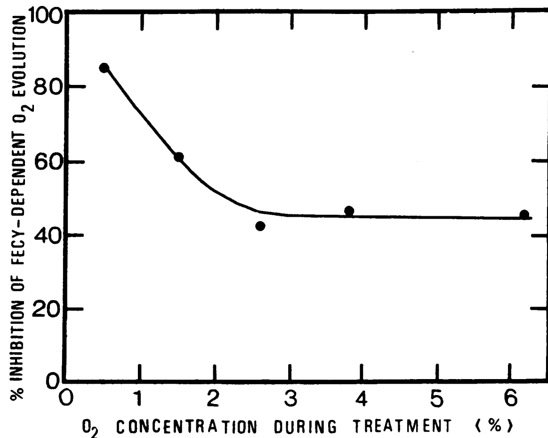

FIG. 1. Relationship between percentage of inhibition of Fecy-dependent O_2 evolution induced by an 8-min photoinhibitory treatment and O_2 concentration prevailing in the medium during treatment. PFD during treatment was 3500 μmol quanta m^{-2} s^{-1}. In order to avoid the inhibitory effects of low O_2 concentrations on electron transfer during measurement of photoinhibition, the electrode cuvette was opened 30 s at the end of the treatment in order to increase O_2 concentration in the assay medium to about 5%. Temperature was 25°C. Control rate of O_2 evolution: 220 μmol mg^{-1} Chl h^{-1}.

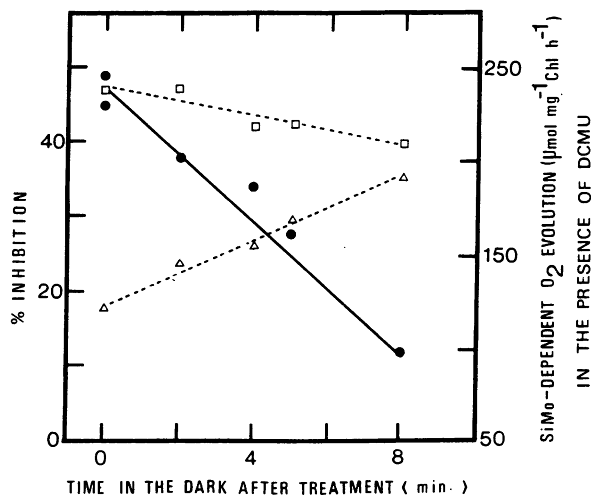

FIG. 2. Relationship between percentage inhibition of SiMo-dependent O_2 evolution in the presence of DCMU and the length of the dark period following an 8-min photoinhibitory treatment (●). PFD during treatment was 3500 μmol quanta m^{-2} s^{-1}. Actual rates of SiMo-dependent O_2 evolution as a function of the duration of the dark period following photoinhibitory treatment are also shown; (□), control; (△), treated. The medium was equilibrated with 21% O_2 at 25°C.

The rates of NADP-dependent O_2 evolution at the beginning and at the end of the treatment are shown. The rate of O_2 uptake was measured only at the beginning of the preincubation period, on separate chloroplast samples (because the photoinhibitory treatment was always performed in the presence of catalase). The highest photoinhibition occurred when the initial rate of O_2 evolution was the highest. Figure 3 shows a time course of photoinhibition both with NH_4Cl and with NH_4Cl, Fd, and NADP in the reaction medium during the preincubation period. Photoinhibition was always higher in the presence of NH_4Cl, Fd, and NADP during preincubation, increasing rapidly with the length of preillumination and reaching a plateau after 5 min.

The use of SiMo as the electron acceptor for PSII makes it possible to add DCMU during the preincubation period to suppress electron flow, and then to study the photoinhibition of PSII. It is also possible to study PSI photoinhibition under these same conditions because, for this assay, MV, Asc, and DCPIP are already used in the presence of DCMU. Tables IV and V show the results of experiments done in the presence or in the absence of 3 μM DCMU when various additions were made during preincubation to increase photoinhibition (see also Table III). Measurements of PSII and PSI activities were performed at both saturating (3500 μmol quanta m^{-2} s^{-1}) and limiting (1500 and 340 μmol quanta m^{-2} s^{-1}, respectively) light. Increasing electron flow during the photoinhibitory treatment increased the subsequent photoinhibition of both PSII and PSI activities. The extent of photoinhibition was always higher under limiting light. When incubations were carried out in the presence of DCMU, the resulting photoinhibition of PSII (Table IV) was greatly reduced and that of PSI completely prevented (Table V). The control rate of SiMo-dependent O_2 evolution in the presence of DCMU was unaffected by the presence or absence of DCMU during preincubation.

Table III. *Effect of Different Additions during the Photoinhibition Treatment on the Initial and Final Rate of O_2 Absorption or Evolution during Treatment and on Subsequent PBQ Dependent-O_2 Evolution*

Photoinhibitory treatment (8-min illumination period under 3500 μmol m^{-2} s^{-1}) was given either with no addition or in the presence of the compounds listed. Measurements were performed under a PFD of 2000 μmol m^{-2} s^{-1}. As the assay medium contained 300 units of catalase per ml, the rate of O_2 uptake at the beginning of the treatment was estimated on separate chloroplast samples. Control rate of PBQ-dependent O_2 evolution: 270 μmol mg^{-1} Chl h^{-1}.

Additions during Treatment	Initial Rate	Final Rate	PBQ-Dependent O_2 Evolution	Inhibition
	μmol mg^{-1} Chl·h^{-1}			%
None[a]	14		176	35.1
NH_4Cl, 5 mM[a]	47		168	38.4
Fd, 10 μM + 4 mM NADP[b]	40	40	115	57.4
Fd, 10 μM + 4 mM NADP + 5 mM NH_4Cl[b]	193	46	63	76.8

[a] Rate of O_2 absorption. [b] Rate of O_2 evolution.

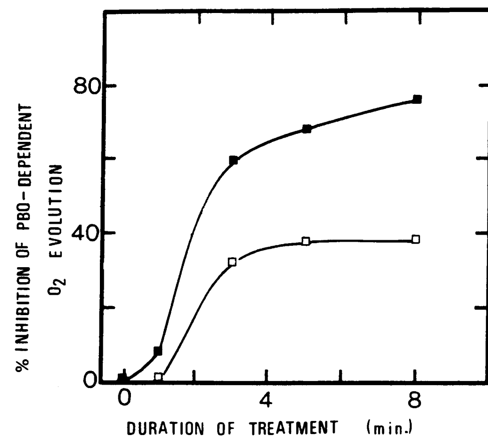

FIG. 3. Time course of percentage of inhibition of PBQ-dependent O_2 evolution. (□), Photoinhibitory treatment in the presence of 5 mM NH_4Cl; (■), photoinhibitory treatment in the presence of Fd (10 μM), NADP (4 mM), and NH_4Cl (5 mM). PFD during treatment was 3500 μmol quanta m^{-2} s^{-1}. The buffer was equilibrated with 21% O_2 at 25°C. Control rate of O_2 evolution: 270 μmol mg^{-1} Chl h^{-1}.

Table IV. *Effect of DCMU (2 μM) during Photoinhibitory Treatment on the Percentage Inhibition of PSII Activity Assayed with SiMo (500 μM) in the Presence of DCMU*

Photoinhibitory treatment was as in Table III. Measurements of PSII activity were done either at saturating light (PFD: 3500 μmol m^{-2} s^{-1}) or limiting light (PFD: 1300 μmol m^{-2} s^{-1}). The assay medium contained 330 units catalase/ml. Chl concentration was 18 μg/ml. Temperature was 25°C and the medium was equilibrated with 21% O_2. The results are the mean of two independent measurements. Control rate of SiMo-dependent O_2 evolution (±SD, $n = 12$): 190 ± 17 μmol mg^{-1} Chl h^{-1}.

Additions during Treatment	% Inhibition of SiMo-Dependent O_2 Evolution in the Presence of DCMU			
	PFD during measurement 3500 μmol m^{-2} s^{-1}		PFD during measurement 1300 μmol m^{-2} s^{-1}	
	− DCMU during treatment	+ DCMU during treatment	− DCMU during treatment	+ DCMU during treatment
None	17	0	30	0
NH_4Cl, 5 mM	48	17	82	34
NH_4Cl, 5 mM + 10 μM Fd + 4 mM NADP	76	15	94	36

Table V. *Effect of DCMU (2 μM) during Photoinhibitory Treatment on the Percentage Inhibition of PSI Activity Assayed with Methyl Viologen (0.1 mM) plus Ascorbate and DCPIP in the Presence of DCMU*

Photoinhibitory treatment was as in Table III. Measurements of PSI activity were done either at saturating (PFD: 3500 μmol m^{-2} s^{-1}) or limiting light (PFD: 380 μmol m^{-2} s^{-1}). Experimental conditions were as in Table IV. The results are the mean of two independent measurements. Control rate of methyl viologen-dependent O_2 absorption (±SD, $n = 12$): 210 ± 23 μmol mg^{-1} Chl h^{-1}.

Additions during treatment	% Inhibition of MV-Dependent O_2 Absorption in the Presence of DCMU			
	PFD during measurement 3500 μmol m^{-2} s^{-1}		PFD during measurement 380 μmol m^{-2} s^{-1}	
	− DCMU during treatment	+ DCMU during treatment	− DCMU during treatment	+ DCMU during treatment
None	0	0	15	0
NH_4Cl, 5 mM	13	0	51	0
NH_4Cl, 5 mM + 10 μM Fd + 4 mM NADP	10	0	48	0

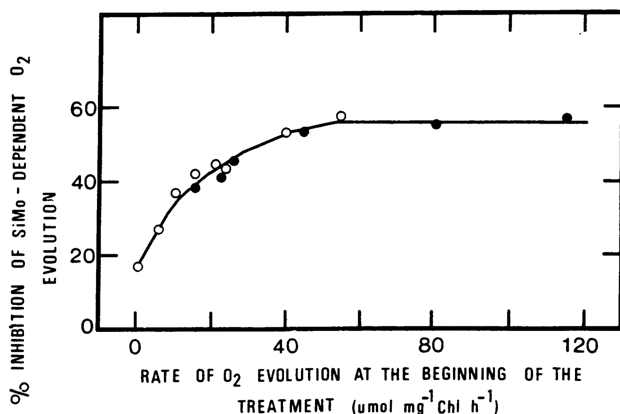

FIG. 4. Relationship between percentage of inhibition of SiMo-dependent O_2 evolution in the presence of DCMU and the initial rate of O_2 evolution during photoinhibitory treatment. (●, ○), Two independent experiments. Photoinhibitory treatment was given in the presence of NADP (4 mM), NH_4Cl (5 mM), and various concentrations of Fd (0.3-7 μM). The zero of O_2 during treatment was obtained by adding DCMU to the assay medium in the presence of NADP (4 mM), NH_4Cl (5 mM), and Fd (7 μM). The assay medium was equilibrated with 21% O_2 at 25°C. PFD during treatment: 3500 μmol quanta m^{-2} s^{-1}. Control rate of SiMo-dependent O_2 evolution: 112 μmol mg^{-1} Chl h^{-1}.

Figure 4 shows the results of two independent experiments in which different rates of electron transfer during the photoinhibitory treatment were obtained by varying the Fd concentration in the presence of 5 mM NH_4Cl and 4 mM NADP. Total absence of electron flow was obtained by adding 3 μM DCMU together with the highest amount of 7 μM Fd in the presence of NH_4Cl and NADP. The rate of electron transfer during the treatment was estimated by the measurement of the initial rate of O_2 evolution. The resulting inhibition of SiMo-dependent O_2 evolution in the presence of DCMU increased with the initial rate of O_2 evolution during high light treatment. It is known that increasing Fd concentration also increases O_2 uptake, but that in the presence of Fd, NADP is reduced in preference to O_2. It therefore follows that the lowest initial rates indicated in Figure 5 are an underestimation of the actual rate of electron flow, and that, in this region, the shape of the true curve is somewhat different from Figure 4. Nevertheless, photoinhibition evidently became constant when the initial rate of O_2 evolution during treatment reached 60 μmol mg^{-1} Chl h^{-1}. It should be noted that the CO_2 fixation rate (measured at saturating level of light and of bicarbonate) of the intact chloroplasts which were used in this study was about 85 μmol CO_2 mg^{-1} Chl h^{-1}, a rate close to the 60 μmol O_2 mg^{-1} Chl h^{-1} above.

Figure 5, A and B, shows that increasing PFD during photoinhibitory treatment increased the subsequent inhibition of PBQ-dependent O_2 evolution measured at saturation. Treatments were given in the presence of either NH_4Cl (Fig. 5A) or NH_4Cl, Fd,

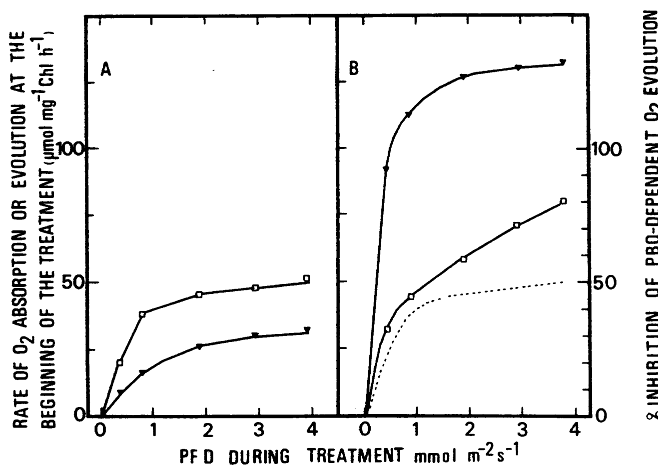

FIG. 5. Effect of photon flux density during photoinhibitory treatment on the initial rate of O_2 absorption (▼, A) or evolution (▼, B) during treatment and on the subsequent inhibition of PBQ-dependent O_2 evolution (□). A, NH_4Cl (5 mM) during treatment; B, NH_4Cl (5 mM), NADP (4 mM), and Fd (10 μM) during treatment. The assay buffer was equilibrated with 21% O_2 at 25°C. To facilitate comparison between the effects of PFD during treatment and the resultant percentage of inhibition of PBQ-dependent O_2 evolution, the results of A have been drawn on B (- - -).

and NADP (Fig. 5B). The rates of electron transfer during these treatments were estimated either as the initial rate of O_2 uptake (on separate chloroplast samples, Fig. 5A) or as the initial rate of O_2 evolution (Fig. 5B). Both the initial O_2 uptake and O_2 evolution during preincubation in the light increased with increasing PFD until a plateau was reached. Photoinhibition was always higher when the treatment was given in the presence of Fd, NADP, and NH_4Cl (Fig. 5B), though the estimated rate of electron transfer during treatment was always greater in that case (the broken line on Fig. 5B enables a direct comparison between photoinhibition measured in the two experimental conditions). In addition, it should be noted that the effect of higher electron flow during treatment is greater under high light than under low light.

DISCUSSION AND CONCLUSION

As already noted by several authors (8, 9, 17, 18), preillumination of osmotically broken chloroplasts in the absence of electron acceptors other than O_2 causes a decrease of whole chain electron transport capacity; PSII activity is more affected than PSI activity. In the experiments reported here, the photoinhibition treatment was performed in the presence of catalase to avoid the damage that might be caused by H_2O_2 produced by the Mehler reaction during preillumination (3). In order to avoid extensive damage to the membranes, the PFD and the Chl concentration during the experiments were usually adjusted to obtain an inhibition of about 40% in a medium equilibrated with 21% O_2. There was no photobleaching, since there was no change in either Chl concentration or the Chl *a*/Chl *b* ratio.

In contrast to other results (3), addition of superoxide dismutase during the treatment did not decrease inhibition (data not shown). Because photoinhibition increased when the O_2 concentration during treatment was below 2% (Fig. 1), as already noted by Trebst (21), it is unlikely that the photoinhibition observed in our work involved damage caused by products of O_2 radicals. Light-inhibition of PSII activity was largely reversible in the dark (Fig. 2), suggesting that rather than membrane destruction, photoinhibition involves a temporary inhibition of photosystem activity. Although polyribosomes have been shown to be bound to isolated thylakoids (2), it is unlikely that the above reversibility was due to protein synthesis; addition of soluble factors would be necessary for protein synthesis to occur in thylakoids. Further investigation is necessary to explain this reversibility.

The damage induced by preillumination occurred mainly at the level of PSII rather than PSI; Fmax measured in liquid N_2 at 685 nm was lowered by preillumination as already shown, and SiMo-dependent O_2 evolution in the presence of DCMU was inhibited. This inhibition was of the same magnitude as the inhibition of whole chain electron transport (measured by Fecy-dependent O_2 evolution in the presence of NH_4Cl) and PSII activity (measured by PBQ-dependent O_2 evolution) (Table I). The inhibition described here is obviously different from that observed by Kyle *et al.* (11) in *Chlamydomonas*, where high light treatment led to an inhibition of photoreduction of acceptors acting at the plastoquinone QB sites: inhibition of SiMo reduction in the presence of DCMU was not observed in contrast to our results. Thylakoids isolated from photoinhibited pea and spinach leaves also show the same inhibition of SiMo-dependent O_2 evolution in the presence of DCMU, and of Fecy-dependent O_2 evolution in the presence of NH_4Cl (Cornic, unpublished data). Photoinhibition in algae may therefore be qualitatively different from photoinhibition in higher plants.

As our data show, photoinhibitory damage was mainly localized at or near PSII reaction centers. The water-splitting reaction was not affected since the addition of DPC during assay only slightly reduced the inhibition of DCPIP reduction (Table I). PSI activity was only slightly affected by high light treatment, and it appears that the electron transport chain between plastoquinone and the site of NADP reduction was not affected; addition of ascorbate and DCPIP during the measurement after treatment almost completely restored the rate of NADP reduction (Table I).

As already noted by Barenyi and Krause (3) and in contrast to the results of Forti and Jagendorf (5), photophosphorylation was decreased as a consequence of whole chain electron transport inhibition, since the P/2e-ratio of thylakoids was independent of whether or not they had been photoinhibited (Table II). However, we were able to reproduce the results of Forti and Jagendorf when the photoinhibition treatment was done under higher PFD (data not shown).

PSII activity was not protected by increasing the rate of electron removal during the photoinhibitory treatment. On the contrary, PSII photoinhibition increased as the electron transport rate increased during high light treatment (Tables III and IV; Fig. 4). Accordingly, when DCMU was added during the photoinhibitory period, photoinhibition of PSII activity was dramatically decreased. Similar results were obtained for PSI activity (Table V). Satoh has also found that DCMU protected PSI activity against high light treatment, but in contrast to our results, he found that DCMU enhanced photoinhibition of PSII. The reason for this difference is not known. However, whatever the reason, this effect of DCMU suggests that lipid peroxidation is not responsible for photoinhibition since it has been shown that addition of DCMU to thylakoid preparations during high light treatment increased lipid peroxidation in broken chloroplasts (20). The reversibility of PSII inhibition shown in Figure 2 is also in agreement with this idea.

The results shown in Figure 1 appear to be in contradiction with those showing a relationship between the rate of electron transfer during treatment and the subsequent photoinhibition. Decreasing the O_2 concentration below 2% during treatment decreased the rate of O_2 uptake by the Mehler reaction; nevertheless, it increased the extent of the resulting photoinhibition measured at about 5% O_2. Photoinhibition observed at low O_2 could be qualitatively different from that occurring at normal O_2 concentrations. An increase of PSI photoinhibition at low O_2 as

observed by Satoh (18) could explain the O_2 sensitivity of whole chain electron transport photoinhibition since PSII photoinhibition has been shown to be rather insensitive to O_2 (16).

The observations reported in this paper are not in agreement with the hypothesis that drainage of light energy by electron transport activity protects against photoinhibition (13). Photoinhibitions of PSII and PSI activities induced by high light treatment of osmotically broken chloroplasts are positively related to operation of whole chain electron transport. However, the comparison between Figure 5B and Figure 4 suggests that there is also a direct effect of light on photoinhibition of PSII activity, independent of electron transfer rate. When the electron transfer rate was varied by varying electron acceptor concentrations, at constant PFD (3500 μmol quanta m^{-2} s^{-1}), photoinhibition of PSII reached a plateau when the initial rate of O_2 evolution approximated 60 μmol O_2 mg^{-1} Chl h^{-1} (Fig. 4). When the electron transfer rate was increased by increasing PFD during the photoinhibitory treatment, photoinhibition steadily increased with increasing PFD, even when the initial electron transfer rate was as high as 120 μmol O_2 evolved mg^{-1} Chl h^{-1}. In that case, not only the electron transfer rate was varied, but also the amount of light energy reaching the reaction centers. Since SiMo-dependent O_2 evolution in the presence of DCMU was photoinhibited, the mechanism of that inhibition must be different from that proposed by Kyle *et al.* (11) on *Chlamydomonas*.

It is known that in chloroplasts and in leaves, protection against high light is afforded by a minimal level of photosynthetic carbon metabolism (16). This might involve other mechanisms than a quantitative drainage of light energy trapped in the reaction centers.

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