Photoinhibition of Photosynthesis in Broken Chloroplasts as a Function of Electron Transfer Rates during Light Treatment1

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GABRIEL CORNIC AND MYROSŁAWA MIGINIAC-MASLOW*
Laboratoire du Phytotron, Centre National de la Recherche Scientifique, Gif-sur-Yvette, 91190 France; and Laboratoire de Photosynthèse et Métabolisme (LA 40), Bât. 430, Université de Paris-Sud, 91405, Orsay, France

ABSTRACT
Photoinhibition was studied in osmotically broken chloroplasts isolated from spinach leaves (Spinacia oleracea L.). Both whole chain electron transport (measured as ferricyanide-dependent O2 evolution in the presence of NH4Cl) and photosystem II activity (measured as O2 evolution in the presence of either silicomolybdate plus 3(3,4-diphenyl)-1,1-dimethylurea or parabenzquinone) 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 O2). 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 O2 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 transport 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.

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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 PSI reaction center (16). However, Kyle et al. (11) recently showed in Chlamydomonas reinhardii that it was possible to induce photoinhibition of DCPIP2 reduction (a PSI reaction in which DCPIP accepts electrons from the quinone protein QB) without inhibition of silicomolybdate reduction in the presence of DCMU (a PSI reaction in which SiMo accepts electrons from the primary acceptor QA); the primary stable acceptor of PSI

1 Supported by a Centre National de la Recherche Scientifique-Action thématique programmé grant ‘Conversion de l'énergie dans les membranes biologiques.’

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

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 O2 evolution or O2 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 MgCl2, 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 NH4Cl. PSII uncoupled electron transport was determined as silicomolybdate-dependent O2 evolution (SiMo, 0.5 mM) in the presence of 3 μM DCMU, and parabenzquinone-dependent O2 evolution (PBQ, 1 mM), it was also measured at 600 nm
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(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. NH₄Cl (5 mM) was added when indicated. ATP synthesis coupled to NADP photoreduction was measured by adding 5 mM MgCl₂, 4 mM NADP, 4 mM ADP, 4 mM Pi, and 32P (4.5 kBq, μmol⁻¹). The reaction was stopped with cold TCA (final concentration, 2%). After centrifugation, the ATP₂P formation was determined by the method of Avron (1).

**Photoinhibitory Treatment.** Samples were exposed to the photoinhibitory treatment in the cuvette of the O₂ 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:

\[
\frac{(A - O) \times 100}{A} \times 100
\]

where \( A \) and \( O \) 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 NH₄Cl (whole chain electron transport), parabenzoquinone (PBQ; PSI II activity) or silicomolybdate (SiMo) plus DCMU (PSI activity), added at the end of the preincubation period in a medium equilibrated with 21% O₂. 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 O₂ absorption in the presence of Asc, DCPIP, and DCMU was comparatively low. PSI-II-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 FeCy and NH₄Cl (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 PSI, 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) during treatment.

Preillumination of broken chloroplasts induced noncyclic electron transport to NADP in the presence of FeCy 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 period (data not shown). Moreover, the photoinhibition of FeCy-dependent O₂ evolution, measured in a medium containing 5% O₂, was increased when the O₂ 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 O₂ evolution in the presence of DCMU. The actual rates of O₂ 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 PSI inhibition, measured as NADP-dependent O₂ evolution, when electron transfer during the photoinhibitory treatment was varied by addition of NH₄Cl and/or of FeCy 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% O₂. The actual rate of O₂ 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 NH₄Cl; 0.5 mM SiMo; 3 mM DCMU; 0.1 mM DCPIP; 1 mM Asc; 0.1 mM MV; 4 mM NADP; 10 mM Fd; 1 mM DPC.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Addition during Measurement</th>
<th>Rate of Electron Transfer</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>μeq Chl h⁻¹ (%)</td>
<td></td>
</tr>
<tr>
<td>H₂O → Fecy</td>
<td>NH₄Cl</td>
<td>267 ± 13 175 ± 8</td>
<td>34.4</td>
</tr>
<tr>
<td>H₂O → PBQ</td>
<td>None</td>
<td>273 ± 18 169 ± 15</td>
<td>38.0</td>
</tr>
<tr>
<td>H₂O → SiMo</td>
<td>DCMU</td>
<td>180 ± 21 117 ± 12</td>
<td>35.8</td>
</tr>
<tr>
<td>DCPIP → MV</td>
<td>DCMU, Asc</td>
<td>232 ± 9 217 ± 7</td>
<td>6.5</td>
</tr>
<tr>
<td>H₂O → NADP</td>
<td>Fd, NH₄Cl</td>
<td>96 ± 28</td>
<td>70.8</td>
</tr>
<tr>
<td>DCPIP → NADP</td>
<td>Fd, NH₄Cl, Asc</td>
<td>110 ± 82</td>
<td>25.4</td>
</tr>
<tr>
<td>H₂O → DCPIP</td>
<td>NH₄Cl</td>
<td>171 ± 34</td>
<td>80.2</td>
</tr>
<tr>
<td>DPC → DCPIP</td>
<td>NH₄Cl</td>
<td>150 ± 57</td>
<td>62.0</td>
</tr>
</tbody>
</table>

**Table II. Effect of an 8-Min Photoinhibitory 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% O₂. 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.

<table>
<thead>
<tr>
<th>Reaction Rate</th>
<th>NADP reduction μmol mg⁻¹ Chl h⁻¹</th>
<th>ATP synthesis μmol mg⁻¹ Chl h⁻¹</th>
<th>P/2e ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76</td>
<td>108</td>
<td>1.4</td>
</tr>
<tr>
<td>Treated</td>
<td>60</td>
<td>77</td>
<td>1.3</td>
</tr>
</tbody>
</table>
and evolution was measured both photoinhibition and 
The rates separate of the reaction medium during treatment was 3500 \mu mol quanta m^{-2} s^{-1}. In order to avoid the inhibit the 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 \mu mol mg^{-1} Chl h^{-1}.

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 PSI makes it possible to add DCMU during the preincubation period to suppress electron flow, and then to study the photoinhibition of PSI. 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 \mu M DCMU when various additions were made during preincubation to increase photoinhibition (see also Table III). Measurements of PSI and PSII activities were performed at both saturating (3500 \mu mol quanta m^{-2} s^{-1}) and limiting (1500 and 340 \mu mol quanta m^{-2} s^{-1}, respectively) light. Increasing electron flow during the photoinhibitory treatment increased the subsequent photoinhibition of both PSI and PSII 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 PSI (Table IV) was greatly reduced and that of PSII 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

<table>
<thead>
<tr>
<th>Additions during Treatment</th>
<th>Initial Rate</th>
<th>Final Rate</th>
<th>PBQ-Dependent O_2 Evolution</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chl h^{-1}</td>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>None^a</td>
<td>14</td>
<td>176</td>
<td>35.1</td>
<td></td>
</tr>
<tr>
<td>NH_4Cl, 5 mM^a</td>
<td>47</td>
<td>168</td>
<td>38.4</td>
<td></td>
</tr>
<tr>
<td>Fd, 10 \mu M + 4 mm NADP^b</td>
<td>40</td>
<td>40</td>
<td>115</td>
<td>57.4</td>
</tr>
<tr>
<td>Fd, 10 \mu M + 4 mm NADP</td>
<td>193</td>
<td>46</td>
<td>63</td>
<td>76.8</td>
</tr>
</tbody>
</table>

^a Rate of O_2 absorption.  
^b Rate of O_2 evolution.
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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% O₂. The results are the mean of two independent measurements. Control rate of SiMo-dependent O₂ evolution (±SD, n = 12): 190 ± 17 μmol mg⁻¹ Chl h⁻¹.

<table>
<thead>
<tr>
<th>Additions during Treatment</th>
<th>PFD during measurement 3500 μmol m⁻² s⁻¹</th>
<th>PFD during measurement 1300 μmol m⁻² s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DCMU during treatment</td>
<td>+ DCMU during treatment</td>
</tr>
<tr>
<td>None</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>NH₄Cl, 5 mM</td>
<td>48</td>
<td>17</td>
</tr>
<tr>
<td>NH₄Cl, 5 mM + 10 μM Fd + 4 mM NADP</td>
<td>76</td>
<td>15</td>
</tr>
</tbody>
</table>

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 O₂ absorption (±SD, n = 12): 210 ± 23 μmol mg⁻¹ Chl h⁻¹.

<table>
<thead>
<tr>
<th>Additions during treatment</th>
<th>PFD during measurement 3500 μmol m⁻² s⁻¹</th>
<th>PFD during measurement 380 μmol m⁻² s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DCMU during treatment</td>
<td>+ DCMU during treatment</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NH₄Cl, 5 mM</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>NH₄Cl, 5 mM + 10 μM Fd + 4 mm NADP</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

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 NH₄Cl 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 NH₄Cl and NADP. The rate of electron transfer during the treatment was estimated by the measurement of the initial rate of O₂ evolution. The resulting inhibition of SiMo-dependent O₂ evolution in the presence of DCMU increased with the initial rate of O₂ evolution during high light treatment. It is known that increasing Fd concentration also increases O₂ uptake, but that in the presence of Fd, NADP is reduced in preference to O₂. 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 O₂ evolution during treatment reached 60 μmol mg⁻¹ Chl h⁻¹. It should be noted that the CO₂ 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 CO₂ mg⁻¹ Chl h⁻¹, a rate close to the 60 μmol O₂ mg⁻¹ Chl h⁻¹ above.

Figure 5, A and B, shows that increasing PFD during photo-inhibitory treatment increased the subsequent inhibition of PBQ-dependent O₂ evolution measured at saturation. Treatments were given in the presence of either NH₄Cl (Fig. 5A) or NH₄Cl, Fd,
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\(_4\)Cl (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; PSI activity is more affected than PSII 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\(_2\)O\(_2\) 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 PSI 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\(_4\)Cl) and PSI 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 Q\(_B\) 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\(_4\)Cl (Corin, 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 PSI 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, PSI 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 PSI 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 PSI. 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

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**Figure 5**

Effect of photon flux density during photoinhibitory treatment on the initial rate of O\(_2\) absorption (\(\nabla\), A) or evolution (\(\nabla\), B) during treatment and on the subsequent inhibition of PBQ-dependent O\(_2\) evolution (\(\ominus\)). A, NH\(_4\)Cl (5 mm) during treatment; B, NH\(_4\)Cl (5 mm), NADP (4 mm), and Fd (10 \(\mu\)m) 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 (---).
observed by Satoh (18) could explain the \( \text{O}_2 \) sensitivity of whole chain electron transport photoinhibition since PSII photoinhibition has been shown to be rather insensitive to \( \text{O}_2 \) (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 PSI and PSII 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 PSI activity, independent of electron transfer rate. When the electron transfer rate was varied by varying electron acceptor concentrations, at constant PFD (3500 \( \mu \text{mol quanta m}^{-2} \text{s}^{-1} \)), photoinhibition of PSII reached a plateau when the initial rate of \( \text{O}_2 \) evolution approximated 60 \( \mu \text{mol} \text{O}_2 \text{mg}^{-1} \text{Chl h}^{-1} \) (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 \( \mu \text{mol} \text{O}_2 \text{evolved mg}^{-1} \text{Chl h}^{-1} \). 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 \( \text{O}_2 \) 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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