Stimulation of Photosystem I Electron Transport by High Concentration of 3-(3,4-Dichlorophenyl)-1,1-dimethyl Urea in Uncoupled Chloroplasts

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ABSTRACT

The light saturated rate of photosystem I-dependent electron transport (ascorbate/dichlorophenol-indophenol → methyl viologen in presence of 1 micromolar 3-[3,4-dichlorophenyl]-1,1-dimethyl urea [DCMU]) was increased by a high concentration of DCMU added to broken and uncoupled chloroplasts isolated from pea (Pisum sativum). At 50 micromolar DCMU, the increase was around 50%. No stimulation was observed under limiting intensity of illumination, indicating that the relative quantum yield of electron transport was not affected by high DCMU. The light-saturated rate in coupled (to proton gradient formation) chloroplasts was unchanged by 50 micromolar DCMU, suggesting that the rate-limitation imposed by energy coupling was not affected. Using N,N,N',N'-tetramethyl-p-phenylene diamine as electron donor, essentially no DCMU stimulation of the rate was observed, indicating further that the electron donation at a site close to P700 was not affected by high DCMU. It is concluded that DCMU, in the range of 10 to 50 micromolar, affected the thylakoid membranes in such a way that the rate constant of electron donation by dichlorophenol-indophenol at the site prior to the site of energy coupling increased. Further observations that DCMU at 100 micromolar stimulated the rate in coupled chloroplasts indicated an additional DCMU action, presumably by uncoupling the chloroplasts from phosphorylation, as suggested by Izawa (Shibata et al., eds, Comprehensive Biochemistry and Biophysics of Photosynthesis, University Press, State College, Pennsylvania, pp 140–147, 1968). A scheme has been proposed for multiple sites of DCMU action on the electron transport system in chloroplasts.

DCMU is best known as an inhibitor of photosynthetic electron transport between Q, the primary electron acceptor of PSII, and plastoquinone (7). It has been reported that DCMU has additional effects on PSII, namely, a direct inhibition of the reaction center (6) (or a site between water oxidation and the reaction center [17]) and an acceleration of deactivation of certain intermediate states of the water-splitting enzyme system (16). All of these effects of DCMU are completed at concentrations below 5 μM. The PSI activities are insensitive to DCMU at concentrations sufficient to saturate its effects on PSII activities (see Ref. 5). At high concentrations (>100/μM) of DCMU, however, PMS, or pyocyanine-mediated cyclic photophosphorylation, is inhibited (3, 8). High DCMU (>100 μM) increased the rate of PSI electron transport (ascorbate/DCIP → MV), the magnitude of increase being apparently parallel to that of the inhibition of cyclic photophosphorylation (12). The result has been interpreted in terms of an uncoupling effect of high DCMU (>100 μM) on PSI. This report describes a stimulation of the rate of PSI electron transport by DCMU (<100 μM), which is not due to uncoupling but probably due to increasing the rate constant of electron donation from DCIP to the electron transport chain at a site prior to the site of energy coupling.

MATERIALS AND METHODS

Class II chloroplasts were isolated from peas (Pisum sativum) as described previously (4). The chloroplasts were washed in 10 mM NaCl and resuspended in 20 mM Tris-HCl (pH 7.8), 400 mM sorbitol, 20 mM NaCl, and 3 mM MgCl2. Chlor concentration was measured according to Arnon (1). PSI electron transport was measured by continuous recording of O2 uptake with a Clark type electrode (Yellow Springs Instruments Company, Yellow Springs, Ohio). The reaction mixture was magnetically stirred and thermoregulated by circulating water at 20°C. White actinic light of intensity 105 erg cm−2 s−1 was passed through 20 cm of water before illuminating on the sample. DCIP, TMPD, and DAD were purchased from BDH chemicals, Poole, England, and MV and gramicidin were from Sigma. The Hill reaction inhibitors were generously supplied by Dr C. J. Arntzen, Michigan State University, E. Lansing, MI. All other chemicals were of reagent grade.

RESULTS

The effect of DCMU on the rate of PSI electron transport (DCIPH2 → MV) under saturating intensity of illumination is illustrated in Figure 1. One μM DCMU was added in the control to block PSII electron transport, and 5 mM NH4Cl were added to uncouple the chloroplasts. No O2 evolution was detected in these chloroplasts in the presence of 1 μM DCMU using DCIP as electron acceptor (data not shown). The rate increased with increasing concentration of DCMU. At 50 μM DCMU, the increase was about 50%. The rate increased further to about 75% at 100 μM DCMU, showing a break in the curve near 50 μM. DCMU was not used beyond 100 μM because of its solubility difficulties.

Figure 2 shows the effects of 50 μM DCMU on the rate of PSI electron transport as a function of light intensity. At weak illumination, DCMU had no effect on the rate, while the light-saturated rate increased by about 50% with DCMU. The inset (Fig. 2) shows the data (mean with sd) from six experiments using light intensities, namely 0.4% limiting light and 100% saturating light. These observations suggest that the quantum yield was not affected by high DCMU, but the overall rate-limiting step (biochemical) was affected.

Table I illustrates the stimulation of the rate of PSI electron transport.
FIG. 1. The rate of PSI electron transport (DCIPH$_2$ → MV) as a function of DCMU concentration with pea chloroplasts. The control reaction mixture (2 ml) contained 20 mM Tris-HCl (pH 7.8), 50 mM sorbitol, 20 mM NaCl, 3 mM MgCl$_2$, 50 μM DCIP, 2 mM ascorbate, 1 μM DCMU, 5 mM NH$_4$Cl, 0.5 mM MV, and chloroplast of 10 to 20 μg Chl equivalent. Maximum ethanol concentration was 0.5%, which had no effect on the control rate. The control rate varied between 300 and 400 μeq/mg Chl-h. White light of intensity 10$^4$ erg·cm$^{-2}$·s$^{-1}$ was used. The data were obtained by averaging four experiments.

FIG. 2. Effects of 50 μM DCMU on the rate of PSI electron transport (DCIPH$_2$ → MV) as a function of light intensity. The inset shows the data (mean with sd) from six experiments, using two intensities as indicated. Other experimental conditions were the same as in Figure 1.

transport by different Hill reaction inhibitors. Each of them shows approximately similar stimulation to that of DCMU at 50 μM, suggesting that the observed stimulation is not DCMU specific. The data in Table II compare the effects of DCMU on PSI electron transport in the presence and absence of uncouplers. No stimulation in the rate was observed by 50 μM DCMU in the absence of uncouplers. At 100 μM DCMU, there was an increase of about 17% in these chloroplasts. In the presence of uncouplers, the rate increased by 50 μM DCMU between 25 to 40%. With NH$_4$Cl and gramicidin, the rate increased by 35% and, with methylamine, by 28%. Table III summarizes the results of a number of parallel experiments to compare the effects of 50 μM DCMU on PSI electron transport using DCIPH$_2$ and TMPD/ascorbate as electron donors. With TMPD/ascorbate as a donor, 50 μM had very little or no effect, as compared to DCIPH$_2$ as donor. No stimulation was observed, either, with DAD/ascorbate as electron donor (data not shown).

Table I. Stimulation of the Rate of PSI Electron Transport by Different Hill Reaction Inhibitors

<table>
<thead>
<tr>
<th>Hill Reaction Inhibitors</th>
<th>DCIPH$_2$ → MV</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCMU</td>
<td>50 μM</td>
</tr>
<tr>
<td>Monuron$^a$</td>
<td>47 ± 5</td>
</tr>
<tr>
<td>Nebruron$^b$</td>
<td>43 ± 5</td>
</tr>
<tr>
<td>Atrazine$^c$</td>
<td>48 ± 7</td>
</tr>
<tr>
<td>Ametryne$^d$</td>
<td>51 ± 10</td>
</tr>
</tbody>
</table>

$^a$ 3-(p-Chlorophenyl)-1, 1-dimethyl-urea.
$^b$ 1-α-Butyl-3-(3,4-dichlorophenyl)-1-urea.
$^c$ 2-Chloro-4-ethylamino-6-isopropylamino-5-triazine.
$^d$ 2-(Ethylamino)-4-(isopropylamino)-6-(methylthio)-5-triazine.

Table II. Effects of DCMU on the Rate of PSI Electron Transport (DCIPH$_2$ → MV)

Experimental conditions were the same as in Figure 1, except that different uncouplers were used as indicated. SD was calculated from the data of six experiments. The actual control rate in absence of the added uncoupler varied between 150 and 200 μeq/mg Chl-h. The data are reported as percentage of control.

<table>
<thead>
<tr>
<th>Uncoupler</th>
<th>Control</th>
<th>50 μM DCMU</th>
<th>100 μM DCMU</th>
</tr>
</thead>
<tbody>
<tr>
<td>No uncoupler added</td>
<td>100</td>
<td>99 ± 12</td>
<td>117 ± 6</td>
</tr>
<tr>
<td>+ NH$_4$Cl, 5 mM</td>
<td>208 ± 44</td>
<td>287 ± 68</td>
<td></td>
</tr>
<tr>
<td>+ Gramicidin, 2 μM</td>
<td>300 ± 17</td>
<td>402 ± 20</td>
<td></td>
</tr>
<tr>
<td>+ Methylamine, 10 mM</td>
<td>240 ± 16</td>
<td>307 ± 18</td>
<td></td>
</tr>
</tbody>
</table>

Table III. Stimulation of the Rate of PSI Electron Transport by 50 μM DCMU

Experimental conditions were the same as in Figure 1. TMPD was added to 50 μM in the reaction mixture. The control rates (μeq/mg Chl-h) with DCIPH$_2$ and TMPD varied between 300 and 400, 400 and 600, respectively.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>DCIPH$_2$ → MV</th>
<th>TMPD → MV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>162</td>
<td>116</td>
</tr>
<tr>
<td>2</td>
<td>127</td>
<td>102</td>
</tr>
<tr>
<td>3</td>
<td>160</td>
<td>109</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>117</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>95</td>
</tr>
</tbody>
</table>

DISCUSSION

The data presented in this paper show that DCMU at high concentrations stimulated the rate of PSI electron transport. No effect was observed at limiting light intensities, indicating that the relative quantum yield (i.e. the efficiency of light utilization) was not affected and that the overall rate-limiting step (biochemical) was affected. The observed stimulatory effect of DCMU at 50 μM cannot be due to uncoupling of the thylakoids from proton-gradient formation because (a) the chloroplasts were uncoupled by addition of 5 mM NH$_4$Cl and (b) no change in the rate was noted when no uncoupler was added (Table II).

DCIP is known to donate electrons at two sites in the electron transport chain between PSII and PSI (Ref. 11, for review): one site (site I) is at or close to P700, and the other (site II) is between plastoquinone and Cyt f. It has been suggested (2, 9) that one of the energy coupling sites is located between these two sites of...
electron donation. It is also known that the TMPD/ascorbate donor system is not coupled to ATP formation (10, 18), presumably donating electrons at or near site I of DCIPH 

The two observations, (a) that 50 \mu M DCMU had no effect on the rate in chloroplasts with DCIPH as donor and (b) that in chloroplasts with TMPD as donor, 50 \mu M DCMU had essentially no effect, indicated clearly that site I was not affected by DCMU.

From the above arguments, the reasonable conclusions are that site II (the primary site of electron donation by DCIPH) (11) imposes a rate limitation in uncoupled chloroplast to the reaction DCIPH → MV (14) and that DCMU increased the rate constant of this step. Because, in uncoupled chloroplast, the rate-limitation is imposed by the energy coupling itself with the formation of proton gradient, 50 \mu M DCMU had no effect in coupled chloroplasts.

Izawa (12) reported earlier that DCMU at high concentration (150 \mu M) stimulated the rate of electron transport of DCIPH → MV reaction by 35% in coupled chloroplasts and inhibited the rate of photophosphorylation (PMS-mediated) by 36%. The rate of electron transport in TMPD → MV was little affected by DCMU, even at 500 \mu M. The relative quantum yield of electron transport of these reactions was not affected, even by 500 \mu M DCMU. Izawa (12) interpreted these results as an uncoupling effect of DCMU at concentration >100 \mu M. Unfortunately, no data was shown by Izawa (12) in which DCMU was less than 150 \mu M, and, in our experiments, we avoided the use of DCMU above 100 \mu M (with maximum ethanol concentration of 0.5%), because of solubility problems. Therefore, direct comparison between these results was not possible. Our observations have shown clearly that the stimulation of the rate of electron transport of PSI at 50 \mu M DCMU was not due to uncoupling. In fact, no stimulation of the rate was observed in the absence of uncouplers at 50 \mu M DCMU. The stimulation (~17%) observed at 100 \mu M DCMU in these chloroplasts (Table II) may be due to uncoupling. In that case, the effect of DCMU at higher concentrations (>100 \mu M) is an additional effect to that observed at concentrations below 100 \mu M. The break in the curve of stimulation as a function of DCMU concentration (Fig. 1) near 50 \mu M indicated further that the stimulation at ≥100 \mu M DCMU was a separate event from that at 50 \mu M DCMU.

As a practical point, it may be noted that the experiments have been done in the presence of ascorbate and MV, but the contribution of superoxide to the rate of O₂ uptake has not been determined. Assuming that the fraction of the rate contributed by the superoxide reaction with ascorbate is the same in the high-DCMU sample as it is in the control, the corrected magnitude of the stimulation of the PSI rate by high DCMU would be higher than that estimated in this report.

From our observation and the discussion above, we propose a scheme for multiple effects of DCMU in the various concentrations on the electron transport system of chloroplast (Fig. 3). A recent study (15) has shown that a specific polypeptide associated with PSI complex contains the binding site for DCMU, resulting in an inhibition of electron transport between Q and plastoquinone. Stimulation of PSI rate by 50 \mu M DCMU may result from direct binding of DCMU to another site which is closer to PSI and which has a different binding constant. Alternatively, DCMU at high concentration alters the membrane organization in such a way that the primary site or DCIPH₂ donation (site II, Fig. 3) is affected. The scheme proposed (Fig. 3) does not exclude either of these possibilities.

The observations reported here suggest that the site of DCMU action in PSI stimulation is not identical to the commonly known site of DCMU inhibition of electron transport between Q and plastoquinone, because (a) the concentration required was markedly higher and (b) the same concentration of other known PSII inhibitors showed similar stimulation of PSI rate in spite of their differential concentration requirement for PSII inhibition of electron transport. It might be worthwhile to investigate the binding site for DCMU at concentrations between 10 and 50 \mu M in photosynthetic membranes to elucidate its action in this concentration range. This work carries a lesson that care should be taken in choosing the concentration of DCMU and interpreting the observations, depending on the concentration of DCMU (as well as other Hill reaction inhibitors) used.

**LITERATURE CITED**

3. Asahi T, AT Jagendorf 1963 A spinach enzyme functioning to reverse the inhibition of cyclic electron flow by p-chlorophenyl-1,1-dimethyl urea at high concentrations. Arch Biochem Biophys 100: 531-537

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**FIG. 3.** Scheme to illustrate multiple effects of DCMU on the electron transport system in chloroplasts.