CHLOROPLAST STRUCTURE AND FUNCTION
IN ac-20, A MUTANT STRAIN OF CHLAMYDOMonas REINHARDI

II. Photosynthetic Electron Transport

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ABSTRACT
Photosynthetic electron transport is markedly affected in mixotrophic cells of ac-20 because they lack the capacity to form the wild-type level of cytochrome 559, as well as Q, the quencher of fluorescence of photochemical system II. The other components of the electron-transport chain, as well as reactions dependent upon photochemical system I, are unaffected in the mutant strain. These observations are discussed in terms of the previously reported effects of the ac-20 mutation on CO₂ fixation and ribulose-1,5-diphosphate carboxylase activity.

INTRODUCTION
In the preceding paper (19) it was shown that ac-20, a mutant strain of Chlamydomonas reinhardi, lacks the wild-type capacity to fix carbon dioxide by photosynthesis, that it lacks the wild-type level of RuDP carboxylase, an enzyme essential for photosynthetic carbon dioxide fixation, and that compared with phototrophically-grown cells, mixotrophically-grown cells have a lower rate of carbon dioxide fixation as well as a lower level of the carboxylation enzyme. In this paper we describe studies of the mutant strain's photosynthetic electron-transport chain. It is shown that in mixotrophic ac-20, the rate of electron flow from water through PS II and PS I to NADP is slower than in wild type. Cytochrome 559, a component known to be associated with PS II (15), and Q, the quencher of fluorescence of PS II, are both diminished in amount or activity in the mutant strain, whereas the other known components of the transport chain exhibit normal activities or are present in normal amounts. The extent of photosynthetic electron transport and the levels of cytochrome 559 and Q are both affected by mixotrophic versus phototrophic growth conditions, in a manner similar to that described for CO₂ fixation and RuDP carboxylase.

MATERIALS AND METHODS
Organisms and Culture Conditions
The wild-type strain (137c) and the mutant strain, ac-20, of C. reinhardi were used in the experiments described here. They were cultured under the conditions described in the previous paper (19). Transfer experiments were carried out as described in that paper.
Preparation of Chloroplast Fragments

Chloroplast fragments for the study of photosynthetic electron transport were prepared by the ultrasonic disruption of cells (15) and for the study of cyclic and noncyclic photosynthetic phosphorylation by disrupting the cells by grinding them in sand (9).

Chlorophyll Content and Cell Number

The chlorophyll content of chloroplast fragments and of whole cells was determined by a modification (1) of the method of Mackinney (16). Cell numbers were obtained with the aid of a hemacytometer.

Reactions of the Photosynthetic Electron-Transport Chain

The Hill reaction with either DPIP or NADP as the electron acceptor and the photoreduction of NADP with the DPIP-ascorbate couple were measured in chloroplast fragments as described elsewhere (6, 9).

Photosynthetic Phosphorylation

Cyclic photosynthetic phosphorylation with PMS as the electron carrier, and noncyclic photosynthetic phosphorylation with ferricyanide as the electron acceptor, were measured in chloroplast fragments as previously described (7, 9).

Fluorescence

The fluorescence of PS II was measured in the manner described by Yamashita and Butler (20).

Components of the Photosynthetic Electron-Transport Chain

Total carotenoids were determined as described by Krinsky and Levine (12). Ferredoxin was assayed according to methods previously described (18), using the crude supernates from a 140,000 g centrifugation of sonically disrupted cells. A known number of cells having a known amount of chlorophyll per cell was used. Chloroplast fragments prepared from wild-type cells were used in the assay and purified ferredoxin-NADP reductase prepared from wild-type cells was present in excess. The amount of ferredoxin (units) in the crude extracts was expressed in terms of the quantity of protein necessary to photoreduce 1 µmole of NADP.

Ferredoxin-NADP reductase in the crude preparations was assayed according to the procedure of Avron and Jagendorf (2), where one unit is the amount of protein in the crude extract that catalyzes the reduction of 1 µmole of DPIP.

Plastocyanin content was not determined directly, but its presence was ascertained by measuring the photoreduction of NADP with DPIP-ascorbate.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth condition</th>
<th>NADP-Hill reaction</th>
<th>DPIP-Hill reaction</th>
<th>NADP photoreduction with DPIP-ascorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>Phototrophic</td>
<td>60.0</td>
<td>250</td>
<td>50.5 228  23.0 97.0</td>
</tr>
<tr>
<td></td>
<td>Mixotrophic</td>
<td>61.0</td>
<td>149</td>
<td>61.0 174  18.0 51.0</td>
</tr>
<tr>
<td>ac-20</td>
<td>Phototrophic</td>
<td>6.8</td>
<td>57</td>
<td>21.5 180  10.0 81.0</td>
</tr>
<tr>
<td></td>
<td>Mixotrophic</td>
<td>1.2</td>
<td>5</td>
<td>11.0 45   8.0 31.0</td>
</tr>
</tbody>
</table>

For the Hill reaction with DPIP, the cuvette in the sample compartment of the spectrophotometer contained chloroplast fragments (10 µg chlorophyll) prepared by the sonication of cells, and the following in µmoles: potassium phosphate, pH 7.0, 20; KCl, 40; MgCl₂, 5.0; and DPIP, 0.1. The final volume was 2.0 ml. The DPIP was omitted from the control cuvette in the reference compartment.

For the Hill reaction with NADP, the cuvette contained chloroplast fragments (10-15 µg chlorophyll) prepared by the sonication of cells, and the following in µmoles: potassium phosphate, pH 7.0, 20; KCl, 40; MgCl₂, 5; NADP, 0.5; and ferredoxin prepared from wild-type C. reinhardi, 0.005. Half a unit of ferredoxin-NADP reductase, prepared from wild-type C. reinhardi, was also added. The final volume was 2.0 ml. Ferredoxin, ferredoxin-NADP reductase, and NADP were omitted from the control cuvette.

For the photoreduction of NADP from the DPIP-ascorbate couple, the reaction mixture contained, in addition to the components for the NADP Hill reaction, the following in µmoles: DPIP, 0.1; sodium ascorbate, pH 7.0, 10; and DCMU, 0.2. The control cuvette contained everything but ferredoxin, ferredoxin-NADP reductase, and NADP.

The reactions were run at 25°C.
couple. This reaction in \textit{C. reinhardi} is known to require the presence of plastocyanin (9).

Total quinones including plastoquinones and ubiquinone were determined from heptane extracts of cells (3).

The content of cytochromes 553, 559, and 564 was determined spectrophotometrically with an Aminco-Chance double beam spectrophotometer (American Instrument Co., Inc., Silver Spring, Md.). Both light-induced and chemically-induced absorbance changes at their respective \(\alpha\) bands were measured (10, 15). An extinction coefficient, reduced-minus-oxidized, of 20 cm\(^2\)/µmole cytochrome was assumed (18). P-700 was determined spectrophotometrically (7).

**RESULTS**

**Reactions of the Photosynthetic Electron-Transport Chain**

The Hill reaction, in which several different oxidants (or electron acceptors) can be photo-

<table>
<thead>
<tr>
<th>Component</th>
<th>(a-20)</th>
<th>Wild type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll (a) (µg/10(^6) cells)</td>
<td>1.20</td>
<td>2.88</td>
</tr>
<tr>
<td>Chlorophyll (b) (µg/10(^6) cells)</td>
<td>0.80</td>
<td>1.23</td>
</tr>
<tr>
<td>P-700 (mole P700/moles chlorophyll)</td>
<td>1:2580</td>
<td>1:2490</td>
</tr>
<tr>
<td>Carotenoid (µg/10(^6) cells)</td>
<td>0.29</td>
<td>0.62</td>
</tr>
<tr>
<td>Ferredoxin (units)</td>
<td>0.83</td>
<td>1.24</td>
</tr>
<tr>
<td>Ferredoxin-NADP reductase (units)</td>
<td>5.06</td>
<td>3.38</td>
</tr>
</tbody>
</table>

The reactions were run at 25°C in 25-ml Erlenmeyer flasks. The reaction mixture (2 ml) contained chloroplast fragments (40-80 µg chlorophyll) prepared from cells disrupted by grinding with sand, and the following in µmoles: glycylglycine buffer, pH 8.0, 40; MgCl\(_2\), 10; ADP, pH 7.3, 5; AMP, pH 7.5, 5; and potassium phosphate buffer, pH 8.0, 10, containing 0.5-1.0 µCi \(^{32}\)P. For cyclic photosynthetic phosphorylation, the mixtures contained 0.057 µmoles of phenazine methosulfate and 0.02 µmoles of DCMU. For noncyclic photosynthetic phosphorylation, 2 µmoles of potassium ferricyanide were added. All reaction flasks were flushed continuously with nitrogen throughout the experiment. Reactions were terminated after 3 min of illumination (30,000 lux) by turning off the lights and by adding 0.2 ml of 20% trichloroacetic acid to each flask. Dark controls were run in flasks covered with black tape.
A B

WILD TYPE ac-20

\[ \Delta A = 1 \times 10^{-3} \]

\[ \text{1 MIN} \]

\[ \begin{array}{c}
\text{720} \\
\text{650}
\end{array} \]

\[ \begin{array}{c}
\text{720} \\
\text{650}
\end{array} \]

\[ \begin{array}{c}
\text{720} \\
\text{650}
\end{array} \]

\[ \begin{array}{c}
\text{650} \\
\text{720}
\end{array} \]

Figure 1 Light-induced absorbance changes in chloroplast fragments of mixotrophically-grown wild type and ac-20. The preparations contained 1 µmole of ascorbate to reduce cytochromes 553 and 559. In A and C, the measuring wavelength was 553 nm, and in B and D it was 559 nm. The reference wavelength was 542 nm. The wavelengths of actinic light were 650 and 720 nm. Arrows pointing upward indicate actinic light ON; arrows pointing downward indicate actinic light OFF. Chloroplast fragments equivalent to a chlorophyll concentration of 100 µg/ml were present in a volume of 1.6 ml. The temperature was 25°C. Parts A and B show for wild type the light-induced oxidation of cytochromes 553 and 559, respectively. Part C shows the light-induced oxidation of cytochrome 553 in chloroplast fragments of ac-20. Part D shows that in ac-20 there is no light-induced oxidation of cytochrome 559.

(10, 14), this result indicates that the photosynthetic electron-transport chain is intact between plastocyanin and NADP, and that the reduced activity of some component or components lying between water and plastocyanin limits the NADP Hill reaction in the mutant strain.

Photosynthetic Phosphorylation

Measurements of cyclic and noncyclic photosynthetic phosphorylation agree with the findings described in the preceding paragraphs. Cyclic photosynthetic phosphorylation, dependent upon PS I and not PS II, is normal in the mutant strain, whereas noncyclic photosynthetic phosphorylation, which depends on both photochemical systems, is markedly reduced (Table II).

Components of the Photosynthetic Electron-Transport Chain

The results of an analysis comparing the components of the photosynthetic electron-transport chain of mixotrophic wild type and mixotrophic ac-20 are given in Table III. Also shown are the chlorophyll and carotenoid content of the mutant strain. It is seen in Table III and in Figs. 1 and 2 that cytochrome 559 is not detected in ac-20, either by light- or chemically-induced absorbance changes whereas, with the exception to be noted below, known components are present in amounts essentially equivalent to those found for the wild-type strain.

Fluorescence Properties

The levels of fluorescence in cells of the wild-type and the mutant strain are compared in Fig. 3. It can be seen that the initial level of fluorescence (i.e., the fluorescence detected by the weak measuring beam of 69 ergs/cm² per sec) is higher in mixotrophically-grown cells of the mutant strain than in mixotrophically-grown cells of the wild-type strain. The fluorescence yield in the mutant cells is not, however, appreciably increased upon
Reduced-minus-oxidized differencespectrum

**Figure 2.** Reduced-minus-oxidized difference spectrum for chloroplast fragments of mixotrophically-grown ac-20. The reducing agent was ascorbate, and the oxidizing agent was potassium ferricyanide. The difference in absorbance of a reduced and of an oxidized sample was determined at the wavelengths indicated on the abscissa. The reference wavelength was 542 nm. Chlorophyll concentration in a 1.6 ml suspension of chloroplast fragments was 100 µg/ml, and the temperature was maintained at 25°C. Note that the peaks representing the α-bands of cytochromes 550 (presumably the mitochondrial cytochrome c) and 553 (the chloroplast c-type cytochrome) are present and prominent, whereas there is no peak at 559 nm, the position of the α-band of the chloroplast cytochrome 559.

illumination with the relatively intense actinic light (4.2 × 10⁴ ergs/cm² per sec), in contrast to wild type. It can also be seen that the addition of DCMU does not have an appreciable effect on the

over-all fluorescence yield of mixotrophic ac-20. Results of this sort have been interpreted (5, 13, 17) to mean that Q₁, the quencher of fluorescence of PS II, is either missing or inactive. It is of interest that other mutant strains of Chlamydomonas that lack cytochrome 559 also appear to lack Q₁ (13).

Phototrophically-grown cells of ac-20 have fluorescence properties that more closely resemble those of mixotrophically- or phototrophically-grown cells of the wild-type strain (Fig. 3); the yield is increased upon actinic illumination, although it does not reach the wild-type level, and it can be further increased upon the addition of DCMU. Thus the fluorescence data also indicate that photosynthetic electron transport is not greatly impaired in phototrophically-grown cells of ac-20.

**Transfer Experiments**

The change in the level of RuDP carboxylase that occurs when mixotrophic cells of ac-20 are transferred to phototrophic growth conditions (19) is paralleled by changes in photosynthetic electron transport. Fig. 4 gives the results of light-to-light and light-to-dark transfer experiments in which the course of NADP photoreduction was followed. Similar results were obtained in experiments that followed the course of photoreduction of DPIP. It will be noted that in comparison with the recovery of RuDP carboxylase activity and CO₂ fixation (19), the recovery of electron transport in a light-to-light transfer occurs after a shorter lag, and the rate of recovery appears to be more rapid.

The recovery of photosynthetic electron transport in transfer experiments is inhibited by antibiotics that block protein synthesis (experiments to be reported elsewhere). Thus, like the recovery of RuDP carboxylase activity, the recovery of photosynthetic electron transport appears to require protein synthesis and is not a simple activation process.

**DISCUSSION**

The rate of photosynthetic CO₂ fixation is reduced in ac-20 because of a deficiency of RuDP carboxylase (19). The data presented in this paper reveal that the rate of photosynthetic electron transport is also reduced and that this is because of a deficiency of cytochrome 559 and, most likely, of Q. The limiting step in photosynthesis by mixotrophic cells of ac-20, however, is probably in the fixation of CO₂, since RuDP carboxylase activity
FIGURE 3 Fluorescence yield of chloroplast fragments of mixotrophically- and phototrophically-grown ac-20 and wild type. The fluorescence was induced by 650 nm actinic light (4.2 X 10⁴ ergs/cm² per sec). Arrow pointing upward indicates actinic light ON; arrow pointing downward indicates actinic light OFF. The chloroplast fragments were contained in 1 ml, and the chlorophyll concentration was 48 µg/ml. The temperature was 23°C.

FIGURE 4 The recovery of the NADP Hill reaction in a transfer experiment. At time 0, mixotrophically-grown cells were transferred to minimal medium and placed in the light (light-to-light transfer) or the dark (light-to-dark transfer). The results of the light-to-light transfer (open circles) show that a lag of 4 hr precedes the onset of Hill reaction recovery. The results of the light-to-dark transfer (filled circles) show no Hill reaction recovery after 12 hr of incubation in the dark. However, activity recovers immediately when the cells are placed in the light (arrow).

The effect of acetate on the rate of photosynthetic electron transport and on the level of cytochrome 559 is analogous to its effect on the rate of CO₂ fixation and RuDP carboxylase activity, in that the transfer of mixotrophic cells to minimal medium results in an increase in the rate of transport and in the level of cytochrome 559.

Clearly, cells of ac-20 possess at least three anomalies that affect their photosynthetic capacity; as will be shown (8), the organization of chloroplast membranes is also affected, and there is also a deficiency in chloroplast ribosomes.

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REFERENCES