The Photosynthetic Electron Transport Chain of Chlamydomonas reinhardi

I. TRIPHOSPHOPYRIDINE NUCLEOTIDE PHOTOREDUCTION IN WILD-TYPE AND MUTANT STRAINS*

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Reduced pyridine nucleotide and adenosine triphosphate are sources of reducing power and energy for the cellular metabolism and growth of photosynthetic algae and higher plants. Both are considered to be primary products of photosynthesis in these organisms (1) and are generated within chloroplast lamellae by a light-induced electron transfer chain. At least two separate light-dependent reactions are involved (2-8).

The nature of the pathway of electron transfer in chloroplast lamellae remains one of the central problems in understanding the mechanism of photosynthesis. We have chosen to investigate this pathway, and its components, by utilizing mutant strains of the unicellular green algae Chlamydomonas reinhardi that are unable to fix CO₂ by photosynthesis (9-11). Mutant strains of unicellular green algae having impaired photosynthesis, other than chlorophyll-deficient strains, were first reported for Chlorella vulgaris (12) and subsequently for Chlamydomonas reinhardi (9). Recently, a mutant strain of Scenedesmus with impaired photosynthesis has been obtained (13). One important feature of the mutant strains of C. reinhardi, in contrast to those found in Chlorella and Scenedesmus, is that they can be investigated in an organism having both a sexual cycle and well known genetics (14, 15). This makes it possible to examine the interesting and fundamental questions of the manner in which the genome is organized to control photosynthesis.

This paper is concerned with an enzymatic study of the pathway of TPN photoreduction in wild-type C. reinhardi and three mutant strains. Genetic studies will appear elsewhere. The way of TPN photoreduction in wild-type C. reinhardi and three mutant strains having impaired photosynthesis was used in the experiments described here and in the following paper (16). The mutant strains were obtained from wild-type by induction with ultraviolet light followed by screening for their loss of ability to fix carbon dioxide (17). They will not grow in minimal medium (18) unless the latter is supplemented with sodium acetate. Hence, they are known as acetate-requiring strains. The three strains used in these investigations are ac-21, ac-115, and ac-141. They are unable to fix carbon dioxide in the light at rates comparable to wild-type (10).

Both wild-type and mutant C. reinhardi were grown at 25℃ in liquid shake cultures of high salt medium (19) supplemented with sodium acetate. Daylight fluorescent lamps provided a light intensity of 4000 lux at the level of the cultures. Cells were harvested from cultures that were in the logarithmic stage of growth.

Peas (Pisum sativum var. Laxton’s Progress) were grown in a greenhouse. Spinach (Spinacea oleracea) was grown in the field.

Preparations—Chloroplast fragments from spinach or pea leaves were prepared according to the method used for spinach by San Pietro and Lang (20). Chloroplast fragments from C. reinhardi were prepared by a similar method (10).

Purified spinach PPN reductase (chloroplast ferrirdoxin (21)) was prepared by the procedure of San Pietro (22). Partially purified enzyme was obtained from C. reinhardi by applying the same procedure as far as the precipitation in the 75% acetone.

Glutathione reductase was obtained from wheat germ by a method of Barnett et al. (23).

The copper protein, plastocyanin, was extracted from C. reinhardi according to the procedure of Katoh (24).

General Assay Procedures—Light dependent and light-independent reactions were measured with a Cary model 14 recording spectrophotometer as previously described (25). Reactions took place in 0.8-ml volumes except where noted. The temperature was 23℃.

Chlorophyll determinations were made according to Arnon’s modification (26) of the procedure of MacKinney (27).

Protein was determined by the biuret reaction (28).

Photoreduction of TPN by Chloroplast Fragments from C. reinhardi were used in the experiments described here and in the following paper (16). The mutant strains were obtained from wild-type by induction with ultraviolet light followed by screening for their loss of ability to fix carbon dioxide (17). They will not grow in minimal medium (18) unless the latter is supplemented with sodium acetate. Hence, they are known as acetate-requiring strains. The three strains used in these investigations are ac-21, ac-115, and ac-141. They are unable to fix carbon dioxide in the light at rates comparable to wild-type (10).

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C. reinhardi—The photoreduction of TPN by chloroplast fragments obtained from either wild-type or mutant C. reinhardi was measured in the presence of an excess of purified chloroplast PPN reductase from spinach or with PPN reductase from the different mutant strains. The rate of TPNH production in the light was determined by measuring the rate of absorbance increase at 340 nm (20).

Oxygen is normally evolved during the photoreduction of TPN by chloroplast fragments. In the absence of the oxygen-evolving reaction, TPN can still be reduced by illuminated chloroplast fragments if suitable electron donors are supplied (20). In the experiments reported here the electron donors were a catalytic amount of reduced DCI and a substrate amount of ascorbate.

Assay for C. reinhardi PPN Reductase—The activity of PPN reductase from wild-type and mutant C. reinhardi was measured as described above for the photoreduction of TPN except that pea chloroplast fragments were used, and PPN reductase obtained from the various strains of C. reinhardi was added in limiting amounts.

Pyridine Nucleotide Transhydrogenase—Pyridine nucleotide transhydrogenase from chloroplasts was assayed according to the procedure of Keister, San Pietro, and Stolzenbach (30). Since the partially purified PPN reductase from C. reinhardi, prepared as described above, contains the pyridine nucleotide transhydrogenase, it was used as the source of the enzyme. The acceptor for the transhydrogenase reaction was 3-acetylpyridine adenine dinucleotide obtained through the kindness of Dr. Nathan Kaplan.

Hill Reaction—The Hill reaction activity of chloroplast fragments was measured with one of the following electron acceptors: potassium ferricyanide, DCI, beef heart cytochrome c, or plastocyanin prepared from wild-type C. reinhardi. The reduction of these compounds in the light was measured by following the absorbance changes at 420, 600, 550, and 597 nm, respectively. In experiments in which it was necessary to inhibit the Hill reaction, 0.2 μmole of α-phenoanthroline was included in the reaction mixture.

DPNH- and TPNH-linked Cytochrome c Reductases—Cytochrome c reductases linked to either DPNH or TPNH oxidation were measured by following the reduction of cytochrome c at 550 nm.

Cytochrome c Oxidases—The activity of cytochrome c oxidase was assayed by following the light-independent oxidation of reduced cytochrome c at 550 nm. Cytochrome c photo-oxidase activity (31) was measured similarly but in the light. Potassium cyanide was added to inhibit the light-independent oxidation of cytochrome c, and α-phenoanthroline was added to inhibit the light-dependent reduction of cytochrome c.

RESULTS

Photoreduction of TPN—Illuminated chloroplast fragments from wild-type C. reinhardi can reduce TPN in the presence of added spinach PPN reductase (Table I). The reduction was not detectable if PPN reductase was omitted from the reaction mixture. PPN reductase from wild-type C. reinhardi could be substituted for spinach PPN reductase.

Chloroplast fragments from the three mutant strains were ineffective in TPN photoreduction in the presence of PPN reductase from either spinach or the wild-type strain (Table I).

Chloroplast fragments from each of these strains were unable to photoreduce TPN in the presence of PPN reductase isolated from the same mutant strain.

Chloroplast PPN Reductase and Transhydrogenase Activity—Although the majority of the proteins involved in the light-dependent reduction of TPN are bound in the chloroplast lamellae, at least two of these proteins, PPN reductase and pyridine nucleotide transhydrogenase, can be extracted and assayed individually. Extracts of wild-type and the mutant strains were examined for these proteins. All strains contained PPN reductase (Table II). Therefore, the inability of ac-21, ac-115, and ac-141 to yield TPNH by photoreduction of TPN cannot be ascribed to the loss of ability to synthesize PPN reductase. These mutant strains, as well as wild-type, also possessed pyridine nucleotide transhydrogenase (Table II). The differences in specific activities of PPN reductase and transhydrogenase between the strains tested are not necessarily significant, for in some cases assays were done with enzyme preparations which had been frozen and thawed, which resulted in losses of activity.

Hill Reaction—Since chloroplast fragments from the mutant strains were unable to photoreduce TPN, their capacity to photosynthesize was assayed by following the reduction of PPN reductase, which was added in limiting amounts.

<table>
<thead>
<tr>
<th>Source of chloroplast fragments</th>
<th>TPN reductase by PPN reductase from</th>
<th>Spinach</th>
<th>Chlamydomonas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>0.47</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>ac-21</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ac-115</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ac-141</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE II

PPN reductase and pyridine nucleotide transhydrogenase activity of wild-type and mutant C. reinhardi

The reaction mixture for the measurement of PPN reductase activity was the same as in Table I, except that spinach chloroplast fragments were used (equivalent to 17 μg of chlorophyll) and partially purified PPN reductase was added in limiting amounts. The reaction mixture for the measurement of pyridine nucleotide transhydrogenase activity contained transhydrogenase present in partially purified PPN reductase preparations and the following (in micromoles): Tris, pH 8.7, 50; TPNH, 0.2; and 3-acetylpyridine adenine dinucleotide, 0.1 (test cuvette only).

<table>
<thead>
<tr>
<th>Source of PPN reductase or transhydrogenase</th>
<th>PPN reductase activity</th>
<th>Transhydrogenase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>ac-21</td>
<td>0.24</td>
<td>0.23</td>
</tr>
<tr>
<td>ac-115</td>
<td>0.27</td>
<td>0.10</td>
</tr>
<tr>
<td>ac-141</td>
<td>0.06</td>
<td>0.03</td>
</tr>
</tbody>
</table>
**Table III**

**Hill reaction rates of wild-type and mutant C. reinhardi**

The test and control cuvettes consisted of micromoles of phosphate buffer, pH 6.6; 10 mM KCl, 5 mM; and chloroplast fragments containing 2 to 4 μg of chlorophyll in the reaction with DCI and 12 to 17 μg of chlorophyll in reactions with the other Hill oxidants. The test cuvette also contained one of the following: K$_3$Fe(CN)$_6$, 0.61 μmole; 2,6-dichlorophenolindophenol, 0.05 μmole; beef heart cytochrome c, 0.042 μmole; or plastocyanin purified from wild-type C. reinhardi, 0.0071 μg atom of Cu$^{++}$. No stimulation of ferricyanide reduction may occur with well-reduced PPN reductase has no effect if either 2,3,6-trichlorophenolindophenol or DCI is substituted for cytochrome c, although some stimulation of ferricyanide reduction may occur with well-washed spinach chloroplasts.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Rate of reduction</th>
<th>Rate of reduction</th>
<th>Plastocyanin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K$_3$Fe(CN)$_6$</td>
<td>DCI</td>
<td>Cytochrome c</td>
</tr>
<tr>
<td>Wild-type</td>
<td>5.4</td>
<td>0.92</td>
<td>0.48</td>
</tr>
<tr>
<td>ac-21</td>
<td>1.9</td>
<td>0.91</td>
<td>0.11</td>
</tr>
<tr>
<td>ac-115</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ac-141</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table IV**

**Effect of PPN reductase on Hill reaction of wild-type and ac-21**

Reaction mixtures were identical with those used in assaying the Hill reaction (Table III). To measure the effect of PPN reductase, an excess of purified spinach PPN reductase (30 μg) was added to the reaction mixture.

<table>
<thead>
<tr>
<th>Rate of reduction</th>
<th>Wild-type</th>
<th>ac-21</th>
<th>Wild-type</th>
<th>ac-21</th>
<th>Wild-type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K$_3$Fe(CN)$_6$</td>
<td>DCI</td>
<td>Cytochrome c</td>
<td>Plastocyanin</td>
<td></td>
</tr>
<tr>
<td>With PPN reductase</td>
<td>4.85</td>
<td>1.20</td>
<td>1.80</td>
<td>0.63</td>
<td>2.88</td>
</tr>
<tr>
<td>Without PPN reductase</td>
<td>4.85</td>
<td>1.21</td>
<td>1.81</td>
<td>0.60</td>
<td>0.50</td>
</tr>
</tbody>
</table>

reduce various Hill reaction oxidants was tested. Initial investigations had revealed that whole cells of wild-type and ac-21 could not carry out the Hill reaction when measured manometrically with p-benzoquinone (10). Illuminated cells of ac-115 and ac-141 did not evolve oxygen. These observations were corroborated and extended by studying Hill reaction rates with isolated chloroplast fragments. K$_3$Fe(CN)$_6$, DCI, and the proteins cytochrome c and plastocyanin were reduced by illuminated chloroplast fragments prepared from either wild-type or ac-21 (Table III). The reactions were completely inhibited by including 0.2 μmole of o-phenanthroline in each reaction mixture. Chloroplast fragments from ac-115 or ac-141 were ineffective in reducing any of these Hill reaction oxidants.

The Hill reaction of spinach chloroplasts with cytochrome c as the oxidant is stimulated by PPN reductase (32, 33). PPN reductase has no effect if either 2,3,6-trichlorophenolindophenol (22) or K$_3$Fe(CN)$_6$ (34) is substituted for cytochrome c, although some stimulation of ferricyanide reduction may occur with well-washed spinach chloroplasts. In addition, PPN reductase increased the rate of plastocyanin reduction, but to a much smaller extent. Ferricyanide and DCI reduction were essentially unaffected by PPN reductase. In contrast to wild type, the Hill reaction of ac-21 was not stimulated by PPN reductase. Although illuminated chloroplast fragments from ac-21 reduced cytochrome c, the rate of reduction was not increased by the addition of PPN reductase.

**Fig. 1.** TPN photoreduction by chloroplast fragments from C. reinhardi, wild-type and ac-141. The curves were obtained by continuous recording with a model 14 Cary spectrophotometer. Curve 1, TPNH formation by spinach PPN reductase and chloroplast fragments from wild type. The reaction mixture is given in Table I. Curve 2, as for Curve 1, except that 0.2 μmole of o-phenanthroline was included in the reaction mixture. Curve 3, as for Curve 1, except that the chloroplast fragments were from ac-141. Curve 4, TPNH formation by spinach PPN reductase and chloroplast fragments from wild type in the presence of ascorbate and DCI. Oxygen evolution was inhibited by o-phenanthroline. The reaction mixture is given in Table V. Curve 5, as for Curve 4, except that the chloroplast fragments were from ac-141. At the points indicated by arrows, 2.5 μmole of oxidized glutathione and an excess of glutathione reductase were added to the reaction mixtures.
ascorbate and reduced DCl as electron donors. That the increase in absorbance at 340 mp was due to TPNH formation was confirmed by the subsequent addition of oxidized glutathione and glutathione reductase. The reduction of TPN coupled to O2 evolution by illuminated wild-type chloroplast fragments is shown in Curve 1 of the figure. Curve 2 shows that TPN photoreduction does not proceed in the presence of the Hill reaction inhibitor o-phenanthroline. Curve 3 shows that there is no TPN photoreduction by chloroplast fragments of ac-141. This same result was obtained with ac-21 and ac-116 (Table I). TPN photoreduction from ascorbate and reduced DCl, in the presence of o-phenanthroline, is shown for wild-type chloroplast fragments in Curve 4 of Fig. 1. The reaction proceeded at a rate equal to about one-third of that obtained in the presence of Hill reaction activity, but it was preceded by a lag of about 6 minutes. A similar result was obtained with ac-21 (Table V). Thus, chloroplast fragments of both wild-type and ac-21 are capable of TPN photoreduction in the absence of O2 evolution when supplied with suitable electron donors. Curve 5 of Fig. 1 illustrates that ac-141 chloroplast fragments can also photoreduce TPN from ascorbate and reduced DCl. However, the lag was smaller and the rate of TPN reduction was approximately twice that of either wild-type or ac-21 (Table V). The presence of o-phenanthroline had no effect on this reaction. Essentially the same result was obtained with chloroplast fragments from ac-116.

Cytochrome c Reductases and Oxidases—Both DPNH- and TPNH-linked cytochrome c reductases are present in chloroplast fragments from wild-type and the mutant strains of C. reinhardtii.

**Table V**

<table>
<thead>
<tr>
<th>Strain</th>
<th>TPN reduction (μmol TPNH/min/mg chlorophyll)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>0.13</td>
</tr>
<tr>
<td>ac-21</td>
<td>0.15</td>
</tr>
<tr>
<td>ac-116</td>
<td>0.23</td>
</tr>
<tr>
<td>ac-141</td>
<td>0.37</td>
</tr>
</tbody>
</table>

**Table VI**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Cytochrome c reductase activity (μmol cytochrome c/min/mg chlorophyll)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPNH</td>
</tr>
<tr>
<td>Wild-type</td>
<td>0.024</td>
</tr>
<tr>
<td>ac-21</td>
<td>0.094</td>
</tr>
<tr>
<td>ac-116</td>
<td>0.090</td>
</tr>
<tr>
<td>ac-141</td>
<td>0.171</td>
</tr>
</tbody>
</table>

**Table VII**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Light-dependent activity</th>
<th>Light-independent activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(μmol cytochrome c/min/mg chlorophyll)</td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>0.06</td>
<td>0.75</td>
</tr>
<tr>
<td>ac-21</td>
<td>0.08</td>
<td>0.46</td>
</tr>
<tr>
<td>ac-116</td>
<td>0.72</td>
<td>0.79</td>
</tr>
<tr>
<td>ac-141</td>
<td>0.15</td>
<td>0.46</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The investigation of photosynthesis by a variety of approaches has led to the formulation of several pathways to explain the light-dependent sequence of reactions leading to the formation of reduced pyridine nucleotide and ATP (1-8). Although photosynthesis by wild-type C. reinhardtii is basically identical with that of higher plants, the use of mutant strains as described in this paper is a new approach. It is hoped that it will provide some of the additional information necessary for distinguishing between the various pathways that have been proposed.

A number of biochemical pathways, such as arginine biosynthesis in Neurospora, have been elucidated through the use of mutant strains unable to carry out different steps in the pathway. This technique may also be used to infer, at least in part, the sequence of steps in the pathway of TPN reduction in photosynthesis. There is, however, an important distinction between the usual biosynthetic pathway and the pathway of electron transfer in photosynthesis. In a biosynthetic pathway where compound a is converted to a compound b and b to c, etc., the compounds are usually structurally related (ornithine, citrulline, and arginine, for example). In photosynthesis, on the other hand, the reactions leading to TPN reduction are transfer reactions and the components of the pathway need not be structurally related (plastoquinone and cytochrome f, for example). A genetic block in the pathway of TPN photoreduction could be due to several reasons; namely, a loss of ability to carry out one of several transfer reactions between components of the pathway, the loss of ability to synthesize a component of the pathway, or the loss of ability to organize a structurally normal chloroplast. Regardless of the specific nature of the genetic block, it is possible to obtain information indicating which step in TPN photoreduction is blocked in a given mutant strain.
With this information it should be possible to reconstruct at least part of the pathway of TPN photoreduction.

Chloroplast fragments isolated from wild-type Chlamydomonas reinhardtii can photoreduce TPN provided that PPN reductase is present in the reaction mixture. A common feature of the three mutants used in these studies (ac-21, ac-115, and ac-141) is that they are unable to carry out this reaction (Table I). The inability to reduce TPN in the light cannot be ascribed to a deficiency of either PPN reductase or pyridine nucleotide transhydrogenase, two proteins that are essential for TPN photoreduction (20, 37). In addition to these, several enzymes involved in the oxidation or reduction of cytochromes may function in light-dependent electron transfer. A TPNH-linked cytochrome c reductase is present in chloroplasts (38, 39). Both DPNH- and TPNH-linked cytochrome c reductases have been found in the photosynthetic bacteria, Rhodospirillum rubrum (31). In addition, the light-dependent oxidation of cytochrome c can be brought about by bacterial chromatophores (31) as well as by spinach chloroplast fragments (40). All these activities are present in chloroplast fragments from both wild-type and mutant strains (Tables VI and VII).

Particular attention was focused on the Hill reaction, since preliminary experiments (10) had shown that whole cells of ac-21 evolved oxygen in the light in the presence of p-benzoquinone whereas ac-115 and ac-141 did not. A variety of Hill reaction oxidants have been employed in the experiments reported here and, as the data in Table III reveal, only chloroplast fragments from wild-type and ac-21 carry out the Hill reaction. Since the Hill reaction involves the oxidation of water coupled with the formation of an unknown photoreductant, ac-115 and ac-141 appear to be blocked in the formation of the photoreductant. On the other hand, although TPN photoreduction cannot be obtained with ac-21, the production of the photoreductant and its oxidation by compounds such as ferricyanide, DCI, and cytochrome c is not impaired. It is significant that whereas chloroplast fragments from ac-21 reduce cytochrome c in light, in contrast to wild-type, the rate of reduction was not increased by the addition of PPN reductase (Table IV). A 5-fold increase in cytochrome c reduction occurred with chloroplast fragments from wild-type cells in the presence of PPN reductase. Recent studies with chloroplasts (21) and anaerobic bacteria (41, 42) have shown that a new group of iron-containing proteins, including PPN reductase, may function in several different systems as intermediate electron carriers in TPN reduction. Tagawa and Arnon (41) demonstrated a direct reduction of chloroplast PPN reductase by illuminated chloroplasts. With chloroplast fragments of ac-21 it appears that the photoreductant produced during the oxygen-evolving step cannot be utilized for the reduction of PPN reductase and the subsequent reduction of TPN.

It has been shown, by means of higher plant chloroplasts, that the photoreductant generated during the oxygen-evolving step may be replaced by either reduced DCI (36) or N-methylphenazonium methosulfate (43) as the electron donor for TPN reduction. The DCI or N-methylphenazonium methosulfate were maintained in the reduced state by ascorbate. A similar reaction was demonstrated with wild-type Chlamydomonas reinhardtii chloroplast fragments when O_2 evolution was inhibited by o-phenanthroline (Table V and Fig. 1). Chloroplast fragments from either ac-115 or ac-141 also carry out a reduction of TPN from DCI and ascorbate. It is interesting that the rate of TPN reduction of ac-115 and ac-141 was about double that found for wild-type when compared on a chlorophyll basis. These observations support the suggestion that gene mutation in ac-115 and ac-141 has resulted in blocks that affect the production of the photoreductant.

The mutant strains ac-115 and ac-141 are, in some respects, similar to Mn++-deficient photosynthetic cells, or to normal cells treated with Hill reaction inhibitors such as o-phenanthroline and 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Mn++ deficiency in Chlorella leads to a loss of Hill reaction activity (44), and chloroplasts prepared from Mn++-deficient higher plants do not show a Hill reaction with dyes (45), or with TPN in the presence of added PPN reductase (46). Since photoreduction from hydrogen in algal cells is not affected by an Mn++ deficiency (47, 48), presumably chloroplasts isolated from such cells are still capable of photoreducing TPN in the presence of ascorbate and DCI. The lack of Hill reaction activity in ac-115 and ac-141 is not due to the inability of the cells to incorporate Mn++, since preliminary analyses have shown that the Mn++ content of these strains is similar to that of wild-type.

The results obtained with wild-type and mutant Chlamydomonas reinhardtii support the current concept that there are at least two distinct light-dependent reactions in photosynthesis (1–8). The first reaction results in the formation of a photoreductant and the oxidation of water. The second reaction results in the formation of a photo-oxidant and the reduction of TPN. The photoreductant is oxidized by the photo-oxidant via light-independent exergonic reactions. The two mutant strains ac-115 and ac-141 are genetically blocked either in the first light-dependent reaction or in the coupled dark reactions leading to O_2 evolution. A less likely alternative is a block which renders the photoreductant of the first light reaction unavailable for oxidation either by the photo-oxidant in vivo, or in vitro by added Hill oxidants. In ac-21 the photoreductant is produced, but appears to be unavailable for TPN reduction. Since photoreduction of TPN with this mutant can take place in the presence of ascorbate and DCI, the second light-dependent step is also operative. The genetic block appears to lie in the dark reactions between the oxidation of the photoreductant and the second light-dependent reaction.

A recent investigation of electron spin resonance spectra in the wild-type and mutant strains (49) has provided additional evidence to support the contention that ac-21 is blocked in some light-independent step, whereas ac-115 and ac-141 are blocked in the light-dependent step coupled to O_2 evolution. Whole cells of the wild-type strain generate two different electron spin resonance signals, both of which appear in response to light. One of them, a broad response of complex structure, is not elicited from cells of ac-115 and ac-141. On the other hand, the two electron spin resonance signals characteristic of wild-type are generated in ac-21. These observations are in accord with the biochemical data presented here. That is, it has been shown that both ac-115 and ac-141 have lost one of two light-dependent reactions necessary for TPN photoreduction whereas both reactions are present in ac-21. It appears likely, therefore, that ac-21 has lost the ability to carry out a light-independent rather than a light-dependent step in the pathway of TPN reduction.

**SUMMARY**

The photosynthetic electron transport chain of Chlamydomonas reinhardtii chloroplasts was studied in the wild-type strain and three mutant strains having impaired photosynthesis. Triphos-
plastosemic nucleotide photoreduction, the Hill reaction, and the activity of several enzymes that are considered to function in the photosynthetic electron transport chain were investigated.

TPN was reduced by illuminated chloroplast fragments isolated from the wild-type strain. Chloroplast fragments isolated from three mutant strains failed to photoreduce TPN. The chloroplasts from two of the mutant strains lacked the Hill reaction. However, TPN photoreduction did occur in the presence of the electron donors 2,6-dichlorophenolindophenol and ascorbate. Chloroplast fragments prepared from the third mutant strain, although capable of carrying out the Hill reaction, did not photoreduce TPN except in the presence of added electron donors.

These results are discussed in relation to the current concept that there are at least two distinct light-dependent reactions in the photosynthetic electron transport chain.

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