Photolability of Photosynthesis in Two Separate Mutants of Scenedesmus obliquus

PREFERENTIAL INACTIVATION OF PHOTOSYSTEM I

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ABSTRACT

Two separate mutants of the green alga, Scenedesmus obliquus, are described in which photosynthesis is sensitive to moderate intensities of white light (100 mw cm⁻²). Heterotrophic cultures of both mutants lose photosynthetic activity when exposed to white light; the site of at least the initial phase of this inactivation is within photosystem I. Although all whole cell and cell-free reactions typical of photosystem I examined are inhibited by irradiation, the principal component of photosystem I affected is P-700. In light-sensitive-4 the inactivation of P-700 activity is restored during the subsequent dark period. This recovery is prevented by various antibiotics and by anaerobic conditions. In light-sensitive-41 P-700 activity is recovered only after a complete cell division and new growth. Irradiation periods which inhibit photosynthesis in both mutants are without effect upon the activity or presence of ferredoxin, ferredoxin-NADP⁺ oxidoreductase, plastocyanin, cytochrome f (552), cytochrome b-562 or cytochrome b-559.

Prolonged irradiation of cells of light-sensitive-41 causes the disappearance of photosystem II activity, α-tocopherol, and plastquinone. Some decrease of both the chlorophylls and carotenoids occurs but there is no preferential deletion of any particular carotenoid.

That the photosynthetic apparatus of green plants is injured by intense white light (concentrated sunlight) has been known since the early experiments of Pringsheim (33) and Ewart (12). Although the work of these earlier researchers was qualitative, and confounded by both heating and photochemical effects, subsequent research confirmed that irradiation with wavelengths of light absorbed preferentially by Chl caused inhibition of photosynthesis and bleaching of the pigment itself in some, but not all, instances (11, 16). Emerson (11) suggested that the inactivation of photosynthesis was caused by the preferential loss of activity of an enzyme associated with CO₂ assimilation but later studies by Franck and French (14), Myers and Burr (30), and Kok (23) showed that the quantum yield of photosynthesis was also affected by intense irradiation. In general, this type of inhibition of photosynthesis was irreversible, particularly when pigment bleaching accompanied the inactivation. Similar photoinactivation studies on isolated chloroplasts by Avron (1), Kok et al. (25), Jones and Kok (20, 21), and Forti and Jagendorf (13) demonstrated that Hill reaction activity was also adversely affected. This inhibition differed from that seen with intact green plant tissue since the effect required only light and not the combination of light plus O₂. Moreover, PSII activity was more strongly affected than was PSI. Earlier Kok (23) had suggested somewhat reluctantly that photo-inhibition involved a primary destruction of the photosynthetic pigment complex and secondary phototoxicative reactions. Satoh (34–36) extended the isolated chloroplast studies and found that both PSI and PSII were inhibited by light; the inactivation of PSI required both light and O₂ whereas PSII inhibition required only light. In our attempts to isolate specific photosynthetic mutant phenotypes of Scenedesmus obliquus, several LS¹ types have been found. These include various pigment mutants unable to synthesize carotenoids, photosynthetic electron transport mutants which bleach when grown mixotrophically but show normal pigmentation when grown heterotrophically (6), and mutants which only grow heterotrophically, have near normal pigmentation but possess a photosynthetic activity sensitive to light. The initial phenotype is similar to the mutants developed in Chlorella by Claes (10) and later characterized as LS phenotypes by Kandler and Schotz (22). The second LS phenotype is normally characterized by a deficiency of Cyt f and of cyclic photophosphorylation; the inability of this phenotype to form ATP photosynthetically apparently limits the rate of glucose uptake necessary to sustain growth in the light. The last group has been utilized in the current study and the behavior of two selected mutants, LS-4 and LS-41, is described. The purpose of the present investigation was to evaluate the underlying cause of the induced light lability in the two mutants, and also to examine further the basic mechanism of photoinactivation of the photosynthetic process.

MATERIALS AND METHODS

CULTURE OF ALGAL STRAINS

The techniques employed for maintenance of the normal and mutant strains of Scenedesmus have been described previously (4, 31). Cultures were normally harvested for use by centrifugation after 2 days of growth; at this time heterotrophic cultures were still within the logarithmic growth phase and had maximum photosynthetic capacity.

ISOLATION OF MUTANTS

Mutations were induced by either chemical or x-ray treatment (5) and the mutants lacking photosynthetic capacity selected by the ¹⁴CO₂ technique (27) or by the fluorescence selection technique

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4 Abbreviations: DCPIP, 2,6-dichlorophenol indophenol; LS: light-sensitive; WT: wild-type.
(3). Among the numerous mutants gained by these techniques, several have shown erratic photosynthetic behavior depending upon the conditions of growth and the presence of light. Such strains, although possessing normal pigmentation, we now know have a photosensitive site within the photosynthetic machinery. More precise selection of this type of mutant is possible by a combination of the fluorescence selection technique and comparison of the abilities of algal clones to grow under autotrophic, heterotrophic, or mixotrophic conditions by replicate plating techniques. Selection of strains capable of growth only under heterotrophic conditions results in the isolation of at least three types of mutants; these are autotrophic mutants, low fluorescence photosynthetic mutants, and LS photosynthetic mutants. Individual determination of the mutant type is subsequently required.

HIGH INTENSITY IRRADIATION CONDITIONS

Irradiation was performed with a light source equipped with a 1,000-w Sylvania tungsten-halogen lamp (DXN). The light beam was focused and collimated to produce a nearly uniform field of light. Light intensities were regulated with calibrated copper-wire screens and measured with a YSI model 65 radiometer. All algal cells were obtained from 2-day-old heterotrophic cultures; cells were collected by centrifugation, washed once in phosphate buffer, and divided into aliquots for subsequent treatment and analysis. The algal cells were incubated in a Koller culture vessel of 3-cm depth, held in a constant temperature bath at 25°C; 2 μl of packed cell volume/ml were resuspended in 0.05 M K2H2PO4 buffer (pH 6.5) and normally bubbled with 3% CO2 in air (exceptions will be noted). At prescribed times 10-ml samples were removed, centrifuged at 2,000g, the pelleted cells resuspended in 2 ml of either the phosphate buffer or of normal inorganic growth medium and photosynthetic capacity monitored as described below.

PHOTOSYNTHESIS, PHOTOREDUCTION, AND p-BENZOQUINONE HILL REACTION

Photoreduction and the p-benzoquinone Hill reaction were performed using the methods described by Bishop (5). Slight modification was made in the assay for Hill reaction activity by including the following in 2 ml of the assay solution: 10 mM MgCl2, 20 mM KCl and 23 mM p-benzoquinone. Rates of photosynthesis and respiration were determined with the Clark electrode in association with a Gilson model KM oxygraph. Photosynthesis was measured for 5 min at a light intensity of 15 mw cm2.

FLUORESCENCE

Variable yield fluorescence and low temperature fluorescence emission spectra were measured according to Senger and Bishop (37) and Bishop (5).

PLASTOQUINONE, VITAMIN E, CAROTENOID AND CHL ANALYSES

Holden’s (17) method of Chl determination, based upon the extinction coefficients of Chl a and b in methanol as determined by MacKinney (28) was used.

Quinone and carotenoid separation and identification were performed with methods similar to those described by Bishop and Wong (8, 9). Quantitation of the plastoquinone A content of the normal and mutant cells was performed by a single plate chromatography of an aliquot of a methanol extract of whole cells. This procedure, employing silica gel G as adsorbent and benzene-heptane (85:15, v/v) as solvent, replaced the column and TLC procedure previously employed. The area on the plate containing plastoquinone was scraped from the plate, the plastoquinone eluted from the adsorbent with chloroform, and the sample evaporated to dryness. The sample was redissolved in methanol and the amount of plastoquinone determined spectrophotometrically.

The area on the plate corresponding to α-tocopherol (RF = 0.4) was removed by the method indicated above and rechromatographed with the same TLC system. After elution of the purified sample from the adsorbent, its concentration was determined spectrophotometrically at λ = 292 nm using an E1%1 cmα = 75 in absolute ethanol, as determined in this laboratory with authentic α-tocopherol.

Individual carotenoids were purified by the method developed for Scenedesmus by Bishop and Williams (in preparation) and their concentrations determined spectrophotometrically using an average value for E1%1 cm of 2,500 (λ = 450 nm) in absolute ethanol.

ANAEROBIC GLUCOSE PHOTOASSIMILATION

Anaerobic glucose photoassimilation was measured using a modification of the technique reported by Pratt and Bishop (32). Glucose concentration was measured with the Nelson method on an aliquot of the supernatant following centrifugation of the cell suspension. The amount of glucose consumed during anaerobic irradiation was calculated from the difference between the amounts of glucose remaining in the darkened control vessel and in the illuminated vessels.

HYDROGEN PHOTOPRODUCTION

The production of H2 by anaerobically adapted cells was measured with the Clark electrode adapted for H2 detection (41) as modified by Jones and Bishop (19) for the simultaneous measurement of O2 and H2 production by green algae.

LIGHT-INDUCED ABSORBANCE CHANGES

518 Nm Absorbance Change. An Amino-Chance (DW-2) spectrophotometer was used to measure the 518 nm A change. Cells (15 μl of packed cell volume/sample) were resuspended in 3 ml of 0.05 M K2H2PO4 buffer (pH 6.5) and placed in the sample cuvette. A measuring wavelength of 518 nm was used with the isosbestic reference wavelength set at 540 nm. A Balzer K-4 broad band interference filter and a Corning green 4-77 filter were used to exclude wavelengths of light generated by the actinic light beam. A high pressure Hg arc lamp (150 w) served as the actinic light source; its output was filtered through a Corning 1R filter (No. 1-69) and a Schott interference filter transmitting light as 663 nm with a half-bandwidth of 11 nm. The light beam was collimated and focused upon the reaction cuvette with appropriate lenses; light intensity at the surface of the cuvette was 250 μw cm2.

P-700. Light-induced changes in P-700 were measured according to the method of Kok (24) but with a phosphoroscope design similar to that described by Murata and Takamiya (29).

CHLOROPLAST ISOLATION AND REACTIONS

Chloroplasts were isolated from Scenedesmus using the techniques developed by Berzborn and Bishop (4). Analyses were made either on chloroplasts prepared by the technique described earlier or on partially purified samples of the reaction center complex of PSI prepared according to the procedures of Thornber (40).

Methyl viologen reduction measurements, using either water or DCPIP-ascorbate as electron sources, were modified from conditions originally described by Kok et al. (26) and Izawa et al. (18). For methyl viologen photoreduction with water as the electron source, the standard reaction buffer (20 mM Tricine-KOH [pH 7.5], 30 mM KCl, 0.4 mM sucrose, and 1% [w/v] BSA) was made 0.1 mM methyl viologen and 0.3 mM NaCN with chloroplasts (90 μg of Chl in a final volume of 2 ml. A second control
reaction contained 2 μM DCMU as well. Reactions were also tested which included saturating levels (0.03 m) of purified Scenedesmus plastocyanin. Methyl viologen photoreduction, utilizing DCPIP-ascorbate as an electron source, was carried out by making 2 ml of the standard buffer (see above) to 0.1 mm methyl viologen, 0.3 mm NaH₂O₂, 0.2 mm DCPIP, 20 mm Na-ascorbate, 2 μM DCMU, and 0.03 μM plastocyanin. Sufficient chloroplasts were added to provide 90 μg of Chl. Both photoreductions were measured as O₂ uptake with the Clark electrode when illuminated with red light (λ = 680 nm) of an intensity of 25 mw cm⁻².

RESTORATION OF PHOTOSYNTHETIC CAPACITY

Recovery of photosynthetic capacity was assayed for either in the original irradiation flask or in Erlenmeyer flasks (125 ml) maintained in a Gilson respirometer at 25 °C; aeration with 3% CO₂ in air was continued during the dark incubation. Photosynthetic activity was measured every 30 min.

To determine the role of protein synthesis during the recovery phase of photosynthesis, varying levels of the antibiotics cycloheximide (0–7.0 μg/ml), chloramphenicol (0–20 μg/ml), and lincomycin (0–20 μg/ml) were included in separate reaction flasks.

CYTOCHROME ANALYSIS

Analyses of Cyt f, Cyt b-559 (high and low potential forms), and Cyt b-563 were made on both chloroplast fragments and the supernatant fractions obtained during their preparation. Determination of the concentration of individual Cyt was made spectrophotometrically according to the procedure previously outlined for Scenedesmus (9). Soluble forms of Cyt f and plastocyanin were analyzed following chromatographic separation and purification (31).

RESULTS AND DISCUSSION

GENERAL PHOTOSYNTHETIC RESPONSE

The influence of high intensity white light irradiation upon the photosynthetic capacities of heterotrophic cultures of WT Scenedesmus and mutants LS-4 and LS-41 is shown in Figure 1. WT cultures generally show an increase in their photosynthetic ability which is both time- and light intensity-dependent, while cells of LS-4 and LS-41 reveal a marked decline in such activity. Prolonged irradiation of the mutants, particularly LS-41, causes a loss of Chl and carotenoids but only after the first 100 min of irradiation wherein the major inhibition of photosynthesis has already occurred. The data of Figure 1 also show that LS-41 is more light-sensitive than LS-4 and, furthermore, that the photoinactivation of photosynthesis in LS-4 is reversed in a subsequent dark period. This latter point differentiates the two mutants and will be examined in more detail later.

The intensity of light required to initiate inactivation in LS-41 begins at a value greater than 1.5 mw cm⁻² and saturation is attained only at values greater than 150 mw cm⁻² (Fig. 2). At values equal to or less than 1.5 mw cm⁻² photosynthesis is stimulated in LS-41 as is also observed with WT cells. Higher intensities are required to elicit an equal inhibition of photosynthesis by LS-4. Also, when cells of either LS-41 or LS-4 are illuminated at one intensity (150 mw cm⁻²) but in the absence of O₂, inhibition of photosynthesis is curtailed. Typical inhibition occurs when the gas phase is 3% CO₂ in air but initial activation of photosynthesis, prior to inactivation, is observed when the gas phase is 3% CO₂ in N₂ (Fig. 3). It is also evident no inactivation occurs when the gas phase is pure N₂. The delayed inactivation seen when the gas phase contains CO₂ and N₂ results from accumulation of the O₂ generated photosynthetically. The data summarized in Figures 2 and 3 demonstrate the photooxidative nature (photodynamic) of the inhibition to photosynthesis in the two phenotypes. This same type of inhibition of photosynthesis occurs with the WT cells of Scenedesmus but only at light intensities approaching 750 mw cm⁻² (data not presented). Our findings confirm the more general concept of the photooxidative inhibition of photosynthesis in intact algal cells.

As indicated earlier loss or modification of pigments occurs during the photoinactivation period to a greater extent in LS-41 than in LS-4 (Table I). The most dramatic change noted, and apparently unrelated to the light sensitivity of the mutant strains, occurs in LS-4 and the WT. This is the disappearance of α-
carotene and the corresponding accumulation of lutein, the dihydroxy derivative of α-carotene. A corresponding change in the cellular content of β-carotene and zeaxanthin is not observed in the WT or LS-4 but appreciable loss of β-carotene does occur during irradiation of LS-41. The light-sensitized conversion of α-carotene to lutein appears to be a normal event in *Scenedesmus* and may explain why α-carotene is found in only trace amounts in autotrophic cultures of other Chlorophyceae. The loss of Chl and various carotenoids examined in LS-41, but not in LS-4, further differentiates these two LS phenotypes. The general lower pigment content of LS-41 suggests also that even the dark mechanisms for biosynthesis of the various photosynthetic pigments are labile; this apparent liability of the total photosynthetic machinery is expressed in changes in activities of PSII and content of electron transport components of this photosystem (Table II).

To examine the specific site of light inhibition of photosynthesis in the two mutants a number of partial reactions of photosynthesis using intact irradiated and nonirradiated cells were examined. The reactions studied included photoreduction, the p-benzoquinone Hill reaction, anaerobic assimilation of glucose, and the 518 nm light-induced Δ change. The reactions were analyzed to determine the activity of PSI, PSII, and photosphorylation, respectively, and the influence of irradiation upon them. Like photosynthesis, photoreduction is rapidly inhibited by light in both LS-4 and LS-41; the rate of inhibition of the two reactions appears quite similar (Fig. 4, upper and lower). The rate of the quinone Hill reaction in cells of LS-41 decreases during the irradiation period but is much less sensitive than either total photosynthesis or photoreduction. In LS-4 irradiation enhances the rate of the quinone Hill reaction throughout the period of illumination in a manner comparable to that seen for cells of the WT. This higher sensitivity of a PSII-type reaction in LS-41 to light inhibition further distinguishes the two mutants; the possible cause of the inhibition of PSII is discussed in a later section.

Because photosynthesis and photoreduction are similarly inhibited in both mutants it appears that the mode of inactivation involves either the mechanism of CO$_2$ fixation or of PSI. Several lines of evidence suggest the inhibition occurs within PSI. First, the variable yield fluorescence of both LS-4 and LS-41 shows an immediate loss of the quenching of fluorescence produced by PSI light (712 nm) without a corresponding inhibition of the enhancement of fluorescence by PSII light (650 nm). Because the fluorescence of WT cells is quenched by strong white light irradiation the meaning of the changes in the variable yield fluorescence of the mutants is difficult to interpret; however, the general effect is that electron transport between the two photosystems appears to be affected by the irradiation (data not presented). Second, the anaerobic uptake of glucose, which has been interpreted by Tanner et al. (39) to be an indicator of cyclic photophosphorylation activity in algae, and to be an exclusive PSI reaction, is also inhibited by irradiation but at rates greater than that for either photosynthesis or photoreduction in LS-4 (Fig. 5). Comparable data, excluding dark reactivation, were also obtained for LS-41 (not shown). Third, observations on the light-induced Δ change at 518 nm [a phenomenon associated with PSI and the capacity of the chloroplast to perform photophosphorylation (32)] and on photosynthetic evolution by anaerobically adapted algal cells [a

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**Table I.** Carotenoid levels of *Scenedesmus* wild-type and mutants LS-4 and LS-41 before and after irradiation.

Carotenoid levels of the major components of 500 μl PCV of wild-type or mutant cells expressed as a mole ratio of chlorophyll per carotenoid. Figures in parentheses represent the actual amount of the individual carotenoid (in μMoles). Cells of each strain were grown heterotrophically for two days. Treated samples were subjected to five hours of irradiation in white light with an incident intensity of 150 μW cm$^{-2}$.

<table>
<thead>
<tr>
<th>Experimental</th>
<th>Chlorophyll (μMoles)</th>
<th>Chlorophyll</th>
<th>Chlorophyll</th>
<th>Chlorophyll</th>
<th>Chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α-carotene</td>
<td>β-carotene</td>
<td>lutein</td>
<td>zeaxanthin</td>
<td></td>
</tr>
<tr>
<td>ScD$_4$ W.T.</td>
<td>untreated</td>
<td>2.92</td>
<td>57.2(0.051)</td>
<td>44.1(0.066)</td>
<td>18.2(0.16)</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>2.97</td>
<td>220.2(0.012)</td>
<td>58.3(0.031)</td>
<td>7.2(0.41)</td>
</tr>
<tr>
<td>ScD$_4$-LS-4</td>
<td>untreated</td>
<td>3.24</td>
<td>72.1(0.065)</td>
<td>95.2(0.034)</td>
<td>7.0(0.45)</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>3.17</td>
<td>389.0(0.008)</td>
<td>63.2(0.050)</td>
<td>4.2(0.75)</td>
</tr>
<tr>
<td>ScD$_4$-LS-41</td>
<td>untreated</td>
<td>3.27</td>
<td>78.4(0.003)</td>
<td>64.0(0.037)</td>
<td>15.8(0.15)</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>3.24</td>
<td>258.3(0.003)</td>
<td>99.6(0.013)</td>
<td>15.2(0.08)</td>
</tr>
</tbody>
</table>

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**Table II.** Mole ratios of plastoquinone and α-tocopherol in *Scenedesmus* wild-type and mutants LS-4 and LS-41.

Plastoquinone A and α-tocopherol are presented in both μMoles per 2 ml PCV and as a mole ratio of chlorophyll per component. Untreated cells were grown heterotrophically for two days, while treated cells were grown in a light manner and then subjected to a five hour irradiation period in a white light field of 150 μW cm$^{-2}$.

<table>
<thead>
<tr>
<th>Experimental</th>
<th>Chlorophyll</th>
<th>Plastoquinone</th>
<th>α-tocopherol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μMoles</td>
<td>Plastoquinone/PP</td>
<td>α-tocopherol/α-carotene</td>
</tr>
<tr>
<td>ScD$_4$ W.T.</td>
<td>untreated</td>
<td>15.66</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>17.08</td>
<td>0.14</td>
</tr>
<tr>
<td>ScD$_4$-LS-4</td>
<td>untreated</td>
<td>11.28</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>11.11</td>
<td>0.09</td>
</tr>
<tr>
<td>ScD$_4$-LS-41</td>
<td>untreated</td>
<td>8.17</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>6.7</td>
<td>0.00</td>
</tr>
</tbody>
</table>

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**Fig. 4.** Upper: comparison of effect of high intensity irradiation on photosynthesis (Δ—Δ), photoreduction (□—□), and p-benzoquinone Hill reaction (O—O) expressed as per cent peak activity in cells of LS-41. Lower: same as in upper figure except experiments made on cells of LS-41.
system also dependent upon PSI activity (7)], reveal an inhibition during irradiation with kinetics similar to those for the loss of activity of photoreduction, photosynthesis, and anaerobic glucose photoassimilation (Figs. 5 and 6). The data are convincing for both mutants that light impairs photosynthesis by initially altering either the reaction centers, the electron transport system or photophosphorylation or a combination of these. Differentiation among these possibilities requires thorough examination of the electron transport components and the activities related to the individual reaction centers.

ELECTRON TRANSPORT COMPONENTS OF LS-4 AND LS-41

NADP+ Oxidoreductase, Ferredoxin and Plastocyanin. These three chloroplast components were extracted and purified from normal and irradiated cells of WT, LS-4, and LS-41. Irradiation periods which caused complete inhibition of photosynthesis, photoreduction, and other associated PSI-type reactions did not decrease either the activity or amount of these components (data not presented).

P-700. Spectrophotometric analysis of P-700 in isolated chloroplast particles prepared from irradiated and control cells shows that the amount detected by light-induced oxidation of P-700 decreases sharply during a 30-min irradiation period. The relative activity of P-700 in LS-41 and LS-4 decreases to only 10 and 25%, respectively, of the control values within the period of illumination (Fig. 7). Estimation of the half-times of either the oxidation or reduction of P-700 shows that these values remain unaffected by the irradiation. Examination of the chloroplast membrane polypeptides by SDS-polyacrylamide gel electrophoresis of samples prepared from control and irradiated samples of WT LS-4 and LS-41 did not show a loss of the 91 kilodalton band associated with P-700 in Scenedesmus. In other photosynthetic mutants, i.e. strain PS-8, which is known to lack P-700 activity, this polypeptide is not detectable by SDS-polyacrylamide gel electrophoresis. It is assumed, consequently, that the loss of P-700 activity during the irradiation periods results from either a loss of the chromophore of the PSI reaction center or of a dissociation of the pigment-protein complex sufficient to render it inactive. The degree of dissociation induced during the irradiation period may determine the subsequent extent of dark reactivation.

Although observations on chloroplast particles may not correlate precisely with intact cell evaluations of activity, this rapid depletion of P-700 activity correlates well with the observed losses of activity of photosynthesis, photoreduction, and of the PSI-dependent reduction of methyl viologen with ascorbate-DCPIP as the electron donor system. In Figure 8 it can be seen that the reduction of methyl viologen, dependent upon both photosystems, is also inhibited by irradiation in LS-4. The recovery of the rate of this reaction to approximately the control rate during a dark period following irradiation is accompanied by a restoration of the light-induced A change attributable to P-700; the extent and rate of this recovery of activity parallels that seen for the recovery of photosynthesis in LS-4 (Fig. 1). The restoration of photosynthetic activity and P-700 activity are presented by either low concentrations of chloramphenicol, spectinomycin, and lincomycin or by anaerobic conditions (data not shown). Cycloheximide addition did not affect the recovery of photosynthesis. Specific details of the action of the antibiotics against the recovery phenomenon appear elsewhere (15). Photosynthetic competence and P-700 activity of irradiated cells of LS-41 are restored only when irradiated cells are returned to normal growing conditions for a period of time sufficient for a division cycle to occur (minimally 8 hr).
Plastoquinone and α-Tocopherol. The data of Figure 4 (lower) reveal that LS-41 cells lose both PSI and PSII activities during the course of photoactivation. Analyses of the total plastoquinone pool of irradiated and nonirradiated cells show that an appreciable loss (90%) occurs after a 5-hr exposure in LS-41 but not in the WT of LS-4 (Table II). Also accompanying this loss is the destruction of the α-tocopherol pool. Since the rates of destruction of these two components occur roughly in parallel with the loss of PSII activity, but slower than the course of inactivation of photosynthesis, it is viewed that this degradation is not primary in explaining the light lability of photosynthesis. It is apparent that the inactivation of photosynthesis in LS-41 results in secondary changes in several chloroplast components including plastoquinone and α-tocopherol. Abundant evidence exists showing that plastoquinone is the primary electron acceptor of PSII; the role of α-tocopherol, if any, in the photosynthetic electron transport system has been recently discussed (2, 9) but never conclusively demonstrated. Its more accepted role, as an antioxidant serving to protect polyunsaturated fatty acids of membrane lipids against peroxidation, might be more pertinent for the case in study. Clearly, LS-41 is extremely liable to attack by free radicals generated during irradiation and both the carotenoid and α-tocopherol pools are largely depleted in an attempt to protect the photosynthetic system. Whether the level of the additional cellular antioxidant, ascorbic acid, is affected during photoactivation was not studied.

Cytochromes. Difference spectra analyses were made on the Cyt components remaining bound to isolated chloroplast fragments and the data obtained are summarized in Table III. No major loss of any Cyt was noted. The apparent changes in the plastoquinone/Cyt ratio to the chlorophyll content (Table I) that occurs during the irradiation period. Although other chloroplast components of LS-41 normally associated with PSI activity, i.e. plastoquinone, are affected by irradiation, this loss and associated damage to PSI does not extend to Cyt b-559 (H.P. or L.P.). Similarly, the Cyt normally associated with PSI, i.e. Cyt f and Cyt b-562, are unaffected by the irradiation. Since neither plastocyanin nor Cyt f is damaged during the inactivation of PSI and the apparent photodestruction of P-700 activity, it is clear that the electron donor system to P-700 is not altered and explains further why the kinetics of oxidation and reduction of P-700 are not altered during the inactivation process.

The behavior of the two LS mutants reported here appear somewhat different. The results of experiments with a representative of Plant Physiol. All rights reserved.

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Table III. Cytochrome levels of washed chloroplast membrane preparation of Scenedesmus obliquus wild-type and of mutants LS-41 and LS-4.

<table>
<thead>
<tr>
<th>Cytochrome</th>
<th>ScD34-W.T.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>untreated</td>
</tr>
<tr>
<td>Cyt b-559</td>
<td>428</td>
</tr>
<tr>
<td>Cyt b-559</td>
<td>1340</td>
</tr>
<tr>
<td>Cyt f</td>
<td>581</td>
</tr>
<tr>
<td>Cyt f</td>
<td>742</td>
</tr>
</tbody>
</table>

represents an amplified example of the normal sensitivity of photosynthesis to the combination of high light intensity and the presence of O2. The pigmentation of LS-4 is not affected during the inactivation (Table I) unlike other LS mutants of algae previously described (10). Because of the loss of Chl in LS-41 and the extensive damage to PSI after prolonged irradiation, this mutant is more typical of the pigment mutants of Chlorella as originally described by Claes (10) although the pattern for carotenoid biosynthesis is not altered in this mutant.

The reversible inhibition of photosynthesis and photoreduction and the rapid resynthesis of P-700 following inactivation is part of the uniqueness of LS-4; this type of mutant offers unique features for studying the association between PSI and PSII without the addition of anything but light. We deduce from our experimental data that the lability of P-700 activity to higher intensities of white light may be the underlying cause of the general sensitivity of photosynthesis to the photodynamic conditions prevalent under natural circumstances.

Acknowledgments. The expert technical assistance of M. Frick, J. Williams, and J. Wong throughout the course of this study is gratefully acknowledged.

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