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Photosynthetic Electron Transport Chain of Chlamydomonas reinhardi. III. Light-Induced Absorbance Changes in Chloroplast Fragments of the Wild Type and Mutant Strains[1]

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Summary. Light-induced absorbance changes were investigated in chloroplast fragments of wild type *Chlamydomonas reinhardi* and 5 different mutant strains having impaired photosynthesis. Two absorbance changes were detected, 1 having a maximum at 553 nm and the other at 559 nm. The component exhibiting the 553 nm change is a cytochrome similar to cytochrome *f* from higher plant chloroplasts. The component exhibiting the 559 nm change has the properties of a cytochrome similar to cytochrome b_3. Two of the mutant strains (*ac-115* and *ac-141*) were found to lack the 559 cytochrome and light induced only the oxidation of the 553 cytochrome. A third mutant strain (*ac-206*), previously shown to lack the 553 cytochrome, exhibited only the light-induced reduction of the 559 cytochrome. A fourth mutant strain (*ac-208*), shown to lack plastocyanin, exhibited absorbance changes attributable to both cytochromes. However, light was capable of inducing the reduction of the 559 cytochrome but not its oxidation. On the other hand, light induced the oxidation of the 553 cytochrome but not its reduction.

These observations are discussed in terms of the series formulation for photosynthetic electron transport in which the 559 cytochrome is reduced by system II and transfers electrons via the component affected in *ac-21* to the 553 cytochrome. Accordingly, system I sensitizes the oxidation of the 3 components of the electron transport chain.

Reactions of the photosynthetic electron transport chain of the unicellular green alga *Chlamydomonas reinhardi* have been investigated using the wild type strain and mutant strains that are incapable of carrying out normal photosynthesis (4, 8, 9, 12). The purpose of the investigations with the mutant strains is to determine the nature of the components of the photosynthetic electron transport chain and the sequence in which they act (8). For example, recent investigations of NADP photoreduction and photosynthetic phosphorylation with 2 different mutant strains (4), one lacking a *c*-type cytochrome, the other plastocyanin, suggested that these 2 chloroplast components function in series in the photosynthetic electron transport chain and that their sequence is system II → cytochrome 553 → plastocyanin → system I, where system I and system II refer to 2 different photochemical reaction centers (3).

A series of experiments concerned with both in vivo and in vitro light-induced absorbance changes in the wild type and certain mutant strains has been initiated. Chloroplast fragments were used in the experiments described here, for in contrast to intact cells, the fragments lack the relatively large light-induced absorbance change at 515 nm that tends to obscure absorbance changes in the region from 550 to 560 nm. The results obtained with chloroplast fragments provide evidence for the participation of 2 different cytochromes in the photosynthetic electron transport chain of *C. reinhardi* as well as for plastocyanin and a component whose chemical nature is unknown. The evidence will be discussed in terms of the series formulation for photosynthetic electron transport postulated originally by Hill and Bendall (5).

Materials and Methods

Organisms and Conditions of Growth. The wild type strain (137c) of *C. reinhardi* and 5 different mutant strains derived from it (*ac-21, ac-115, ac-141, ac-206, and ac-208*) were used. Some of the photosynthetic properties of these strains have already

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been described (4, 8, 9, 12). Whole cells of each strain are unable to fix carbon dioxide by photosynthesis because photosynthetic electron transport is blocked. This block is detected by the observation that chloroplast fragments of each strain fail to photoreduce NADP when the source of electrons is from water. With the exception of *ac-208*, the block in each of the other mutant strains can be by-passed when reduced DPIP is provided as the source of electrons for the photoreduction of NADP (4, 8, 9).

Chloroplast fragments were prepared from cells of logarithmic phase cultures grown in Tris-acetate-phosphate medium (4) at 25°. Light was provided by daylight fluorescent lamps at an intensity of 4000 lux for cultures of wild type, *ac-115*, *ac-141*, and *ac-206* and at 2000 lux for cultures of *ac-21* and *ac-208*.

Preparation of Chloroplast Fragments for Spectroscopic Measurements. Cells were harvested by centrifugation at 4000 × *g* for 2 minutes at 0°. They were washed once and resuspended in 5 ml of a 0.01 M phosphate buffer, pH 7.0, containing 0.02 M KCl and 0.0025 M $MgCl_2$. The cells were disrupted by sonic disintegration for 30 seconds at 0° using a Mullard ultrasonic disintegrator. The preparation was then centrifuged at 480 × *g* for 6 minutes at −5° to sediment whole cells. The supernate, containing the chloroplast fragments, was centrifuged at 20,000 × *g* for 20 minutes at −5°. The sediment from this centrifugation contained the chloroplast fragments, and they were resuspended in 2 to 5 ml of the phosphate-KCl-$MgCl_2$ buffer. This suspension, containing large aggregates of chloroplast fragments, was dispersed in a Ten-Broeck homogenizer.

The chlorophyll content of the chloroplast fragment preparations was determined by a modification (1) of the procedure of Mackinney (10)

Absorbance Changes. The measurements of absorbance changes were made with an Aminco-Chance double beam spectrophotometer fitted with a side illumination attachment for the actinic light. The actinic light, provided by an incandescent lamp (GE-CPR, 6v, 18 amp), was passed through either a 650 nm or a 720 nm interference filter, each having a 10 nm band width. The energy from the actinic light at the position of the sample was measured with a Yellow Springs Instrument Company Radiometer Model 65. It was 2.2 × 10^4 ergs/cm²•sec for the light transmitted through the 720 nm filter and 2.3 × 10^5 ergs/cm² sec for the light transmitted through the 650 nm filter. The photomultiplier tube of the spectrophotometer (RCA 6903) was protected from both the red and far-red actinic light by a Corning glass filter (4076) in combination with a Wrattan filter (no. 57). The temperature during the measurements was maintained at 25°.

Measurements were made on 1.6 ml samples of chloroplast fragments (equivalent to 0.15 to 0.16 mg chlorophyll) suspended in the phosphate-KCl-$MgCl_2$ buffer and contained in a square cuvette having a 1 cm light path.

The determination of absorbance changes was carried out at a sensitivity of either 0.0086 or 0.0043 absorbance units for full scale of the recorder chart (Varian G-14). The entrance and exit slit widths of the monochrometer were 0.3 mm.

For all of the measurements reported here λ_1 refers to the wavelength of the measuring beam. The wavelength of the reference beam (λ_2) was in all instances 542 nm.

Chemical oxidation of the chloroplast fragments was obtained by adding 1 μmole of potassium ferricyanide to the sample and chemical reduction was obtained by adding 1 μmole of ascorbate. Difference spectra comparing chemically oxidized versus chemically reduced preparations were obtained from the difference in absorbance between a reduced sample and an oxidized sample, both of which had identical absorbances over the spectral range under consideration prior to the addition of either ferricyanide or ascorbate.

The effects of DCMU[3] were studied at a final concentration of 10 μmolar.

Results

When chloroplast fragments of the wild type strain were illuminated with 720 nm light a decrease in absorbance was detected at λ_1 = 553 nm and λ_1 = 559 nm whereas illumination with 650 nm light resulted in an increase in absorbance at these wavelengths (fig 1). When DCMU was added,

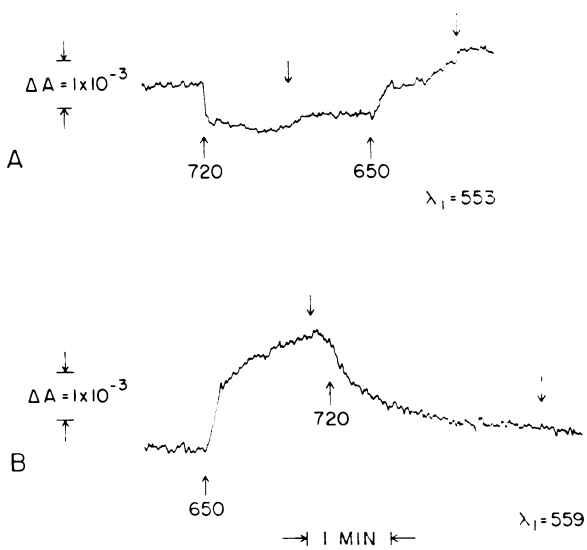

FIG. 1. Light-induced absorbance changes at 553 nm (part A) and 559 nm (part B), in chloroplast fragments of wild type *C. reinhardi*. In this figure and in figures 2, 3, 4, 6, 7, and 9 through 12, λ_1 refers to the wavelength of the measuring light and 720 and 650 refer to the far-red and red actinic illumination respectively. Arrows pointing upward indicate actinic illumination on, and arrows pointing downward indicate actinic illumination off.

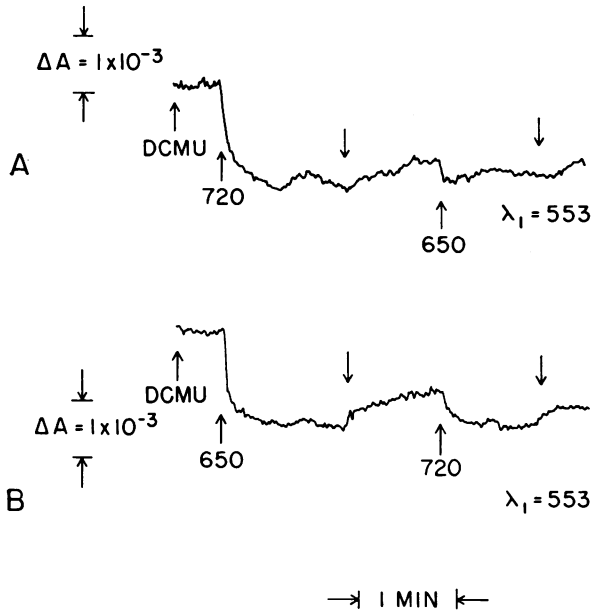

FIG. 2. The effect of DCMU on light-induced absorbance changes at 553 nm in chloroplast fragments of wild type *C. reinhardi*. Before the addition of the DCMU the preparations were illuminated with 650 actinic light. The light was turned off and DCMU was added before the actinic illumination was given again.

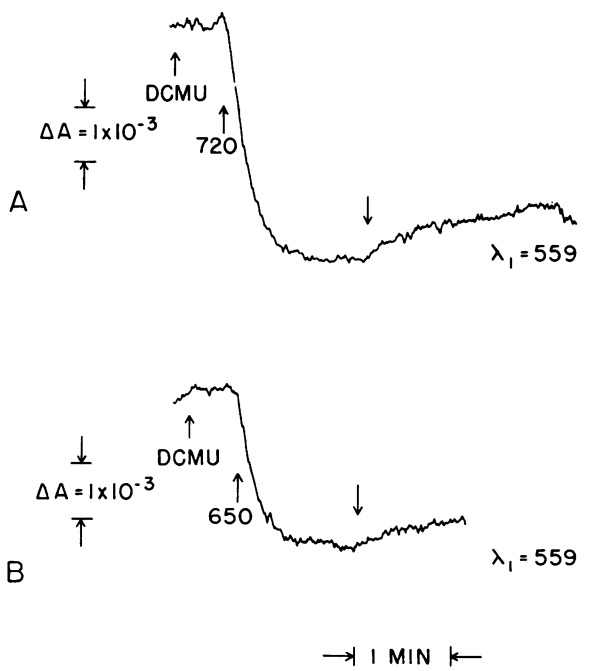

FIG. 3. The effect of DCMU on light-induced absorbance changes at 559 nm in chloroplast fragments of wild type *C. reinhardi*. The conditions for assay were the same as those given under figure 2.

illumination with either 650 nm or 720 nm light resulted in a decrease in absorbance at 553 and 559 nm (fig 2, 3). The absorbance changes detected in the wild type strain can be ascribed to 2 components; one showing a maximum absorbance change at 559 nm and the other showing a maximum absorbance change at 553 nm. This resolution into 2 components was achieved by studying the absorbance changes in chloroplast fragments of 3 different mutant strains; *ac-115*, *ac-141*, and *ac-206*.

The mutant strain *ac-206* lacks cytochrome 553 (4) present in the wild type strain and has a re-

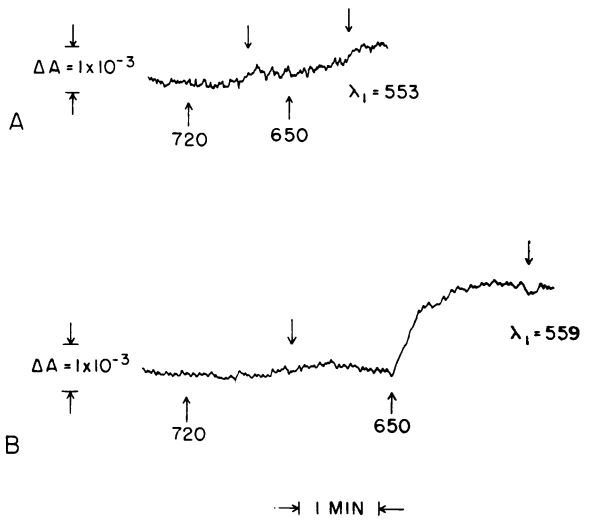

FIG. 4. Light-induced absorbance changes at 553 and 559 nm in chloroplast fragments of *ac-206*.

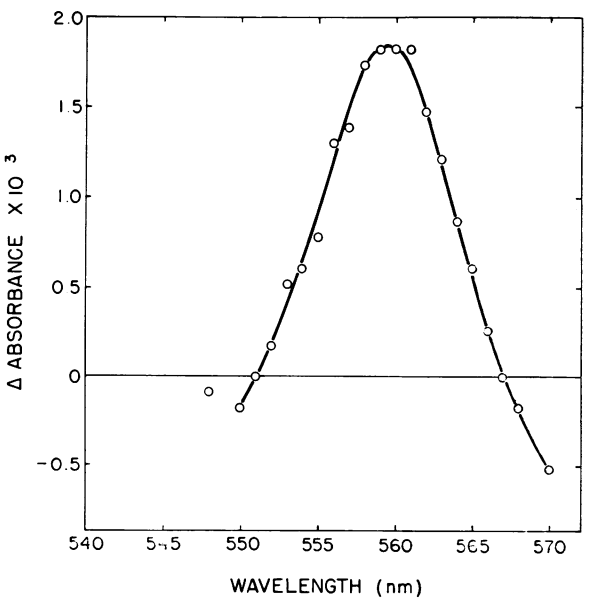

FIG. 5. Light-minus-dark difference spectrum for chloroplast fragments of *ac-206*. Red actinic light was used and sufficient dark time was allowed between each measurement to permit complete autoxidation.

duced α band at 553 nm. This cytochrome resembles cytochrome *f* of higher plants (*2*, 12). Chloroplast fragments of *ac-206* failed to show any marked light-induced absorbance changes at 553 nm (fig 4A). As expected, no marked changes were detected at this wavelength after the chloroplast fragments were either chemically oxidized with ferricyanide or reduced with ascorbate. On the other hand, a light-induced absorbance change was detected at 559 nm. This change was induced by 650 nm light but not by 720 nm light (fig 4B). The light-minus-dark difference spectrum for chloroplast fragments of *ac-206* is shown in figure 5. Whereas a peak at 559 nm is evident, the peak at 553 nm for the reduced α band of cytochrome 553 is absent. The peak at 559 nm may be attributed to the α band of a cytochrome similar to cytochrome b_3 from higher plants (6).

In contrast to chloroplast fragments of both wild type and *ac-206*, the mutant strains *ac-115* and *ac-141* failed to exhibit any light-induced absorbance changes at 559 nm. Figure 6 shows the results obtained with *ac-141*. Indentical results were obtained throughout with *ac-115*. Neither mutant strain showed any marked light-induced absorbance change at 553 nm until the preparations of chloroplast fragments had been reduced with ascorbate (fig 7). It will be noted from figure 7B that in the presence of ascorbate either 650 nm or 720 nm light was capable of bringing about a decrease in absorbance at 553 nm. In this respect, both *ac-115* and *ac-141* responded in the same fashion as DCMU-treated wild type (fig 2). Thus, in both mutant

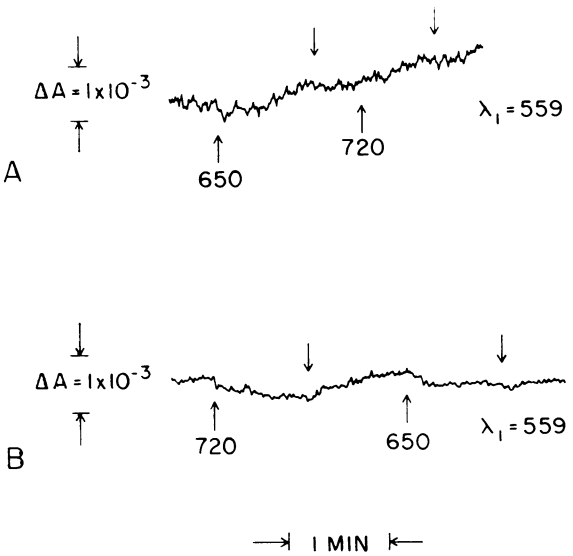

FIG. 6. Light-induced absorbance changes at 559 nm in chloroplast fragments of *ac-141*. In part B the preparation contained 1 μmole of ascorbate.

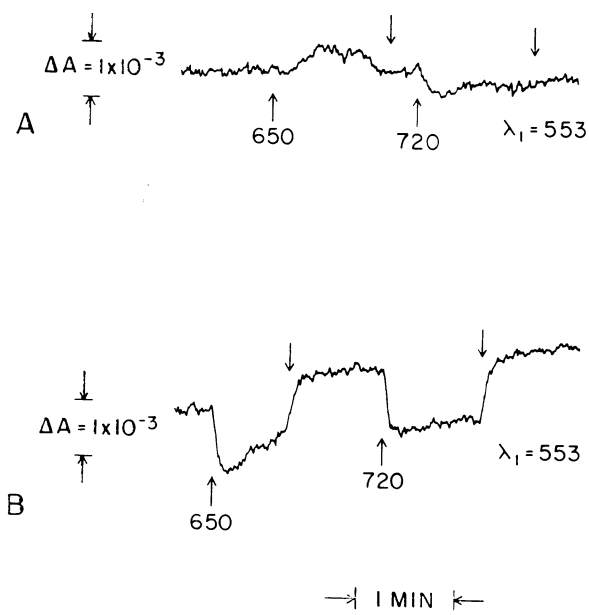

FIG. 7. Light-induced absorbance changes at 553 nm in chloroplast fragments of *ac-141*. In part B the preparation contained 1 μmole of ascorbate.

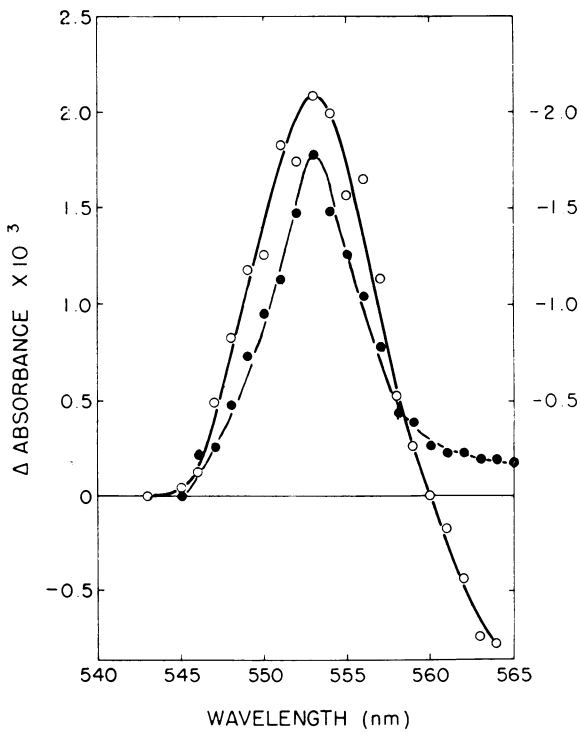

FIG. 8. Light-minus-dark difference spectrum (solid circles) and chemically reduced-minus-oxidized difference spectrum (open circles) for chloroplast fragments of *ac-141*. For the former, far-red actinic light was used and the preparations contained 1 μmole of ascorbate. The ordinate for this curve is on the right. For the latter, 2 samples were compared. One sample contained 1 μmole of ascorbate and the other sample contained 1 μmole of ferricyanide. The ordinate for this curve is on the left.

strains light is unable to sensitize any significant reduction of cytochrome 553, but it can sensitize its oxidation. The light-minus-dark difference spectrum sensitized by 720 nm light in preparations chemically reduced with ascorbate (fig 8) failed to reveal any absorbance change having a maximum at 559 nm in either *ac-115* or *ac-141* whereas a change having a maximum at 553 nm was revealed for the oxidation of cytochrome 553. This spectrum is to be contrasted with that of *ac-206* which lacks cytochrome 553 (fig 5).

The absence of any marked absorbance changes at 559 nm can be attributed to the absence of a cytochrome as revealed by the difference spectrum obtained with chemically oxidized and reduced chloroplast fragments of either *ac-115* or *ac-141*. The reduced-minus-oxidized difference spectrum for *ac-141* and for *ac-115* revealed a single peak at 553 nm representing the reduced α band of cytochrome 553 (fig 8).

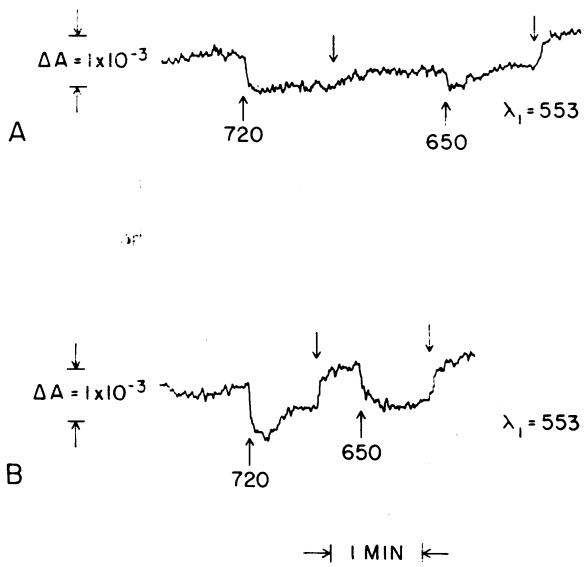

FIG. 9. Light-induced absorbance changes at 553 nm in chloroplast fragments of *ac-21*. In Part B, the preparation contained 1 μmole of ascorbate.

Investigations of a fourth mutant strain, *ac-21*, have shown that the oxidation and reduction of the 2 cytochromes exhibiting absorbance changes at 553 and 559 nm respectively are coupled via a component missing from this mutant strain. When chloroplast fragments of *ac-21* were illuminated with either 720 nm or 650 nm light, the oxidation of cytochrome 553 was detected (fig 9). In this regard *ac-21* resembled both *ac-115* and *ac-141*. However, unlike these mutant strains, *ac-21* was found to exhibit the light-induced absorbance change at 559 nm (fig 10). The 650 nm light induced an increase in absorbance indicative of the reduction of cytochrome 559, but unlike the wild type strain the cytochrome 559 was not oxidized by 720 nm light.

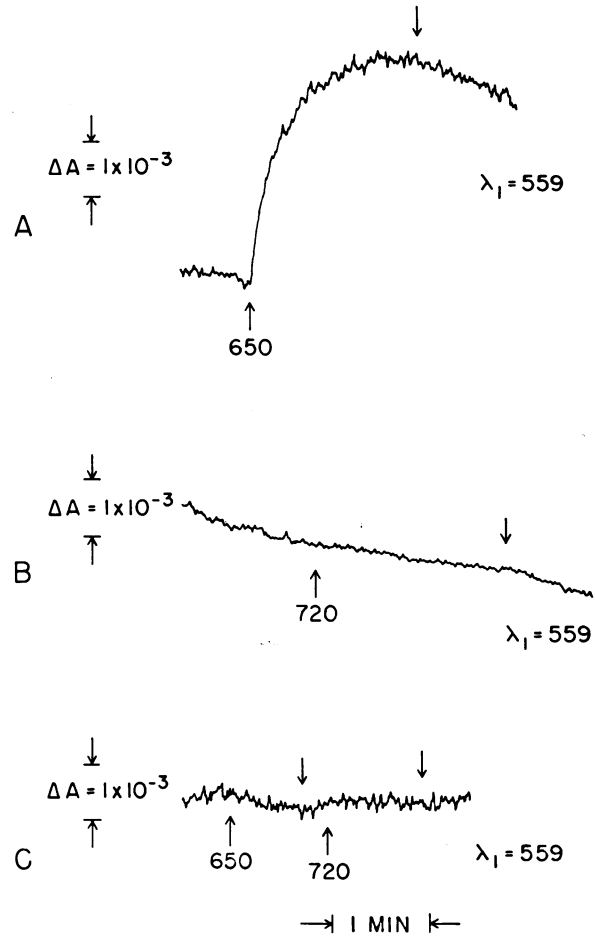

FIG. 10. Light-induced absorbance changes at 559 nm in chloroplast fragments of *ac-21*. In Part B, the preparation was reduced with 650 nm light, then illuminated with 720 nm light. Note that 720 nm light did not stimulate the rate of oxidation. In part C, the preparation contained 1 μmole of ascorbate.

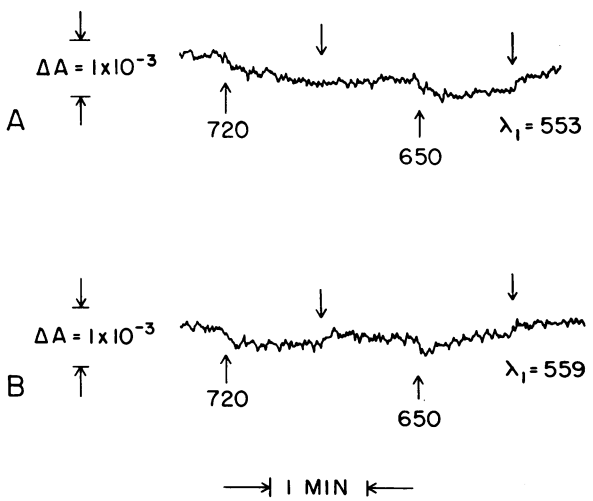

FIG. 11. Light-induced absorbance changes at 553 and 559 nm in chloroplast fragments of *ac-208*.

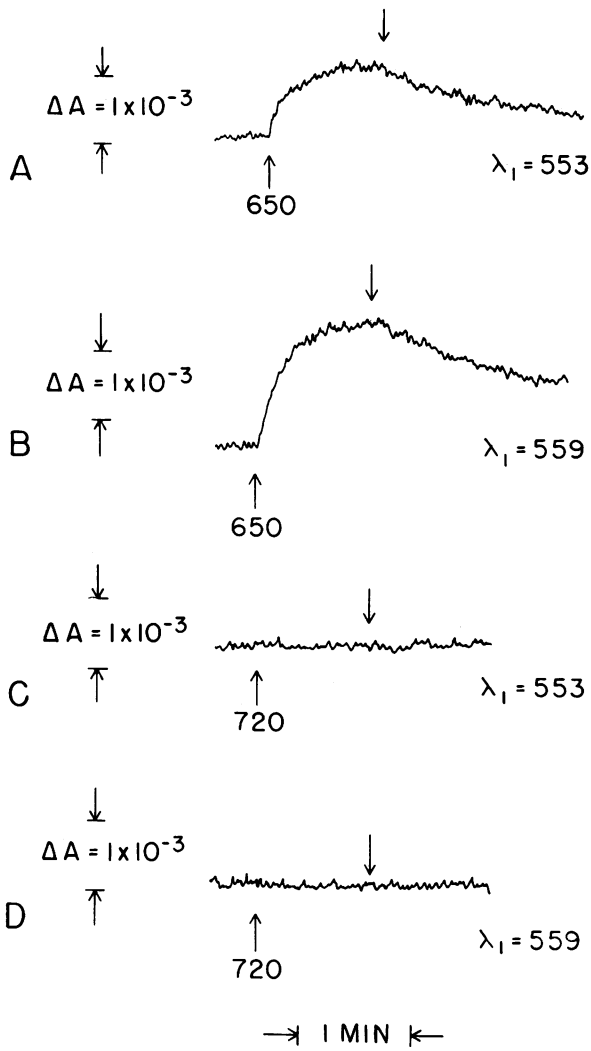

FIG. 12. Light-induced absorbance changes at 553 and 559 nm in chloroplast fragments of *ac-208*. Parts A and B contained 1 μmole of ferricyanide and parts C and D contained 1 μmole of ascorbate.

In fact, light-induced oxidation was not detected even after the preparations had been reduced with ascorbate in the dark. In contrast, preparations that had been oxidized in the dark with ferricyanide exhibited the light-induced reduction of cytochrome 559 but not that of cytochrome 553.

The fifth mutant strain considered here, *ac-208*, lacks the copper protein, plastocyanin (4). No marked light-induced absorbance changes were detected with chloroplast fragments of *ac-208* unless ferricyanide was added (fig 11, 12). In the presence of ferricyanide (fig 12A, 12B) an increase of absorbance was detected at both 553 and 559 nm. In other words, when the preparations were oxidized chemically, light was capable of inducing a reduction of both cytochrome 559 and cytochrome 553. However, if the preparations were reduced chemically with ascorbate (fig 12C, 12D), there was no light-induced oxidation of either cytochrome.

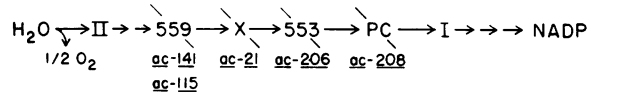

FIG. 13. A series of formulation for the photosynthetic electron transport chain of *C. reinhardi*. The dashed lines refer to the components missing in the different mutant strains. Further explanation in the text.

Discussion

The results obtained with the wild type and mutant strains are summarized in table 1. They can be accommodated most simply by the hypothesis of the series formulation for photosynthetic electron transport proposed originally by Hill and Bendall (5) and extended by other investigators (13). According to the series formulation and the results presented here, a partial sequence of the photosynthetic electron transport chain of *C. reinhardi* can be diagrammed as shown in figure 13. The formulation is not meant to convey that only 4 components lie between systems I and II, but rather that there are at least 4 components as deduced from the behavior of the mutant strains.

The 553 nm component, either missing from or inactive in *ac-206*, appears to be a *c*-type cytochrome having a reduced-minus-oxidized difference spectrum (4) similar to cytochrome *f*, the *c*-type cytochrome obtained from chloroplasts of higher plants (2). The oxidation-reduction potential for the *c*-type cytochrome from *C. reinhardi* is +0.37 volt (Gorman and Levine, unpublished results), a value similar to that reported for the *c*-type cytochrome

Table I. *Light-induced Absorbance Changes at 553 and 559 nm Exhibited by Wild Type and Mutant Strains of* C. reinhardi

Wave length of measuring light	553 nm		559 nm	
Wave length of actinic light	650 nm	720 nm	650 nm	720 nm
wild type	reduction	oxidation	reduction	oxidation
ac-206	no change	no change	reduction	no change
ac-141 (or ac-115)	oxidation	oxidation	no change	no change
ac-21	oxidation	oxidation	reduction	no change
ac-208	reduction	no change	reduction	no change

from the chloroplasts of other plants (2, 7, 11, 14).

The component exhibiting an absorbance change at 559 nm may be a *b*-type cytochrome. A *b*-type cytochrome from plants, having a reduced α band at 559.7 nm, and called cytochrome b_3 has been described by Hill and Scarisbrick (6). It was found in both the green and non-green tissue of higher plants, and it was autoxidizable and could be reduced with ascorbate. In addition, it was reported that cytochrome b_3 was denatured by acetone extraction of plant tissue whereas cytochrome *f* and cytochrome b_6 were found to be more resistant to extraction.

The component in *C. reinhardi* exhibiting an absorbance change at 559 nm and either missing from or inactive in *ac-141* and *ac-115* shares some of the properties of cytochrome b_3, for its reduced absorbance maximum is the same as that given for cytochrome b_3 (fig 5), it is autoxidizable (fig 10B, 12B), and it is reduced with ascorbate. In addition, it has not been detected in acetone extracted cells of *C. reinhardi* whereas a *b*-type cytochrome having the properties of cytochrome b_6 has (12). Accordingly, this component of the photosynthetic electron transport chain of *C. reinhardi* is tentatively identified as a *b*-type cytochrome.

It has been demonstrated (4) that the mutant strain *ac-208* lacks the copper protein plastocyanin. No marked light-induced absorbance changes were detected with chloroplast fragments of this strain until the preparations were oxidized with ferricyanide (fig 12). Following the addition of ferricyanide, 650 nm light was capable of inducing the reduction of the 553 and 559 cytochromes. However, when ascorbate was added to the preparations, light was incapable of inducing the oxidation of either cytochrome.

The final mutant strain under consideration here is *ac-21*. Neither cytochrome 559 nor cytochrome 553 are missing from this mutant strain. However, light will induce the reduction of cytochrome 559 but not its oxidation, and light will induce the oxidation of cytochrome 553 but not its reduction. A component may be missing that is required to couple the oxidation and reduction of the 2 cytochromes. Alternatively, the component may be present but in altered form that is less effective than the wild type form. Some dark reduction of cytochrome 553 was detected in the mutant strain following illumination with 650 nm light (fig 9A). This observation suggests that some reducing equivalents, produced by 650 nm light, may be transferred more slowly to cytochrome 553 in the mutant strain than they are in the wild type strain. The relatively rapid oxidation of cytochrome 553 with 650 nm light in the mutant strain would then prevail over the slower reduction.

In the photosynthetic electron transport chain of *C. reinhardi* 650 nm light induces the reduction of 2 cytochromes and 720 nm light induces their oxidation. When cytochrome 559 is absent, as in *ac-115* and *ac-141*, light can induce only the oxidation of cytochrome 553. When cytochrome 553 is absent, as in *ac-206*, light can induce only the reduction of cytochrome 559. That both cytochromes are in series is deduced from the observations with *ac-21* in which light induces only the reduction of cytochrome 559 and the oxidation of cytochrome 553. The mutant strain *ac-21* must, therefore, lack (or possess in inactive form) a third component that lies between the 2 cytochromes in the chain. Finally, when plastocyanin is absent, as in *ac-208*, light can induce only the reduction of the 2 cytochromes. Therefore, plastocyanin is required for their light-induced oxidation. If plastocyanin, isolated and purified from the wild type strain, is added to chloroplast fragments of *ac-208* the light-induced oxidation of both cytochromes is observed (Gorman and Levine, unpublished observation). It has been shown elsewhere (4) that plastocyanin is required in *C. reinhardi* for NADP photoreduction when the source of electrons is reduced 2, 6-dichlorophenol-indophenol. However, neither cytochrome 559 nor cytochrome 553 are required, for this reaction occurs in *ac-115*, *ac-141*, and *ac-206* (4, 8, 9).

It will be noted from the light-minus-dark difference spectra (fig 5, 8) that there is no evidence for a light-induced absorbance change having a peak at 563 nm, the reported peak (12) for the reduced α band of a *b*-type cytochrome of *C. reinhardi* resembling cytochrome b_6. Though the experiments reveal no evidence for this cytochrome in preparations of chloroplast fragments, a light-induced absorbance change having a peak at 564 nm has been detected in whole cells (Levine, unpublished observation). It would appear, therefore, that there are 2 *b*-type cytochromes in *C. reinhardi* and that they have different sensitivities to extraction and to cell breakage, for one, cytochrome 559, is denatured by acetone extraction and has not been detected chemically and the other, cytochrome 563 is denatured by cell breakage and has not been detected in chloroplast fragments.

Acknowledgments

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