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The Photosynthetic Electron Transport Chain of Chlamydomonas reinhardi. VIII. The 520 nm Light-induced Absorbance Change in the Wild-type and Mutant Strains[1]

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Abstract. **The 520 nm light-induced absorbance change in wild-type and 4 mutant strains of *Chlamydomonas reinhardi* was investigated. In the wild-type strain the absorbance change is composed of at least 2 components, P520 I and P520 II, sensitized by Systems I and II respectively. Some of the properties of these components can be studied by using the appropriate photosynthetic mutant strain. A group of mutant strains modified in the photochemical complex of System II shows only the P520 I absorbance change, whereas a mutant strain deficient in active P700 exhibits only the P520 II absorbance change. The possible relationship between these absorbance changes and the photosynthetic electron transport pathway is discussed.**

The light-induced absorbance change with a maximum in the 515 nm to 520 nm region in green plants was first discovered by Duysens (8) who observed that upon illumination with red light, whole cells of *Chlorella* gave an increase in absorbance at 515 nm. Chance and Strehler (6) showed that this absorbance increase could be obtained by the oxygenation of anaerobic cells in the dark. They attributed the change to a carotenoid since it is absent in a mutant strain of *Chlamydomonas reinhardi* which lacks carotenoid (5, 25). Witt and his co-workers (22, 27, 28) observed that, under certain conditions, the absorbance change is sensitive to 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU) and that it has a System II action spectrum. They concluded that the absorbance increase at 515 nm is due to a change in chlorophyll *b* absorption and that this change could be used as an indicator of the operation of System II (28). On the other hand, Rubinstein and Rabinowitch (24) and Kok, Cooper, and Yang (16) have found that the change is sensitized by System I.

These apparent contradictions were resolved by Rubinstein (23) who demonstrated that different responses at 520 nm could be obtained under different experimental conditions. Under aerobic conditions, the absorbance change is partially sensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), whereas under anaerobic conditions, it is insensitive to the inhibitor. He therefore concluded that the absorbance change at 520 nm is due to both Systems I and II. These results were confirmed by Govindjee and Govindjee (14), and Fork and de Kouchkovsky (10). The latter workers also reported that there were no antagonistic effects of red and far-red light on this change.

In this paper we will present the results of an investigation of the 520 nm absorbance change in the wild-type and for mutant strains of *C. reinhardi*. The results will be discussed in terms of the series formulation in photosynthetic electron transport.

Materials and Methods

The wild-type and 4 mutant strains of *C. reinhardi* were used in these experiments. The strains *ac-115* and *ac-141* were derived from the parental strain by UV irradiation, and their photosynthetic properties have been described (18-20). F-1 and F-34 were obtained by treating the wild-type strain with a chemical mutagen, methylmethane sulfonate, and they were isolated by Mr. Pierre Bennoun as

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high fluorescence mutant strains using a recently-reported technique (4).

Cells of the wild-type and mutant strains were grown in 300 ml portions of tris-acetate-phosphate (TAP) medium under conditions previously described (19). Cells were harvested during the logarithmic phase of growth and resuspended in fresh TAP medium. Chloroplast fragments were prepared according to the methods of Gorman and Levine (12).

Light-induced absorbance changes at 520 nm were measured with an Aminco-Chance dual-wavelength spectrophotometer. The reference wavelength selected was 575 nm. All measurements were made with samples of intact cells (1.60 ml) contained in a square-sided cuvette having a 1 cm light path. Samples were stirred with a glass paddle attached to an Amino Rapid stirrer that was operated at the maximum amplitude. The temperature during the experiments was 25°.

Illumination of the sample was provided by an incandescent lamp (GE-CPR, 6v, 18 amp) fitted with either a 650 nm or 720 nm interference filter, each having a 10 nm half band width. The energy of the illumination at the position of the sample was measured with a Radiometer Model 65 (Yellow Springs Instrument Company). For the 650 nm actinic illumination the energy was 2.3×10^5 ergs sec^{-1} cm^{-2} and for the 720 nm actinic illumination it was 2.2×10^4 ergs sec^{-1} cm^{-2}. The photomultiplier tube of the spectrophotometer (RCA 6903) was protected from the actinic illumination by a combination of 2 filters: a Corning glass filter (9780) and a Wratten filter No. 57.

The absorbance changes were measured at a sensitivity of either 0.0086 or 0.0215 absorbance units for full scale deflection of the recorder chart (Varian G-14). The entrance and exit slit widths of the monochromator were 0.3 mm.

The overall response time of the instrument is limited by the response time of the recorder which is about 0.15 sec.

The photoreduction of NADP and of 2,6-dichlorophenol indophenol (DPIP) were measured in a Cary model 14 recording spectrophotometer according to the methods described by Gorman and Levine (12).

It was found in the course of the experiments that a partially purified preparation of ferredoxin-NADP reductase (Fd-NADP reductase) from the wild-type strain of *C. reinhardi* improved the rate of NADP photoreduction when included in the reaction mixture. Therefore, an excess of the enzyme was used when the photoreduction of NADP was assayed.

The method of Gorman and Levine (13) for the purification of plastocyanin from *C. reinhardi* was used for the preparation of the Fd-NADP reductase. This enzyme was eluted from the DEAE cellulose column by 0.05 M phosphate buffer, pH 7, during the first step of the purification procedure.

The activity of the Fd-NADP reductase was assayed by a procedure similar to that described by Avron and Jagendorf (3). The total volume of the reaction mixture was 1.0 ml, and it contained the following components in μmoles: potassium phosphate buffer, pH 7.0, 50; DPIP, 0.05; and NADPH, 0.1. The NADPH was omitted from the control sample. The reaction was started by the addition of the enzyme, and the reduction of DPIP was followed at 600 nm using a Cary model 14 recording spectrophotometer. The temperature during the assays was 25°.

It was found that 50 milliunits (mU) of the enzyme preparation was an excess for the photoreduction of NADP by chloroplast fragments. One unit (U) of Fd-NADP reductase is defined as that amount which will catalyze the reduction of 1 μmole of DPIP per min at 25°.

Chlorophyll was determined by a modification (2) of the procedure of Mackinney (21).

Results

The 520 nm Light-induced Absorbance Changes in Whole Cells of the Wild-type Strain. When cells of the wild-type strain of *C. reinhardi* are illuminated under aerobic conditions with 650 nm light which activates both Systems I and II, there is an increase and then a decrease in absorbance at 520 nm (fig 1,a′). Following the illumination, there is always an overshoot of the dark base line. With 720 nm light which primarily excites System I, there is also an increase in absorbance (fig 1a), but the kinetics of the absorbance change are different. The time course of this change shows an initial fast increase followed by a slower increase in absorbance of small magnitude. Also, there is no overshoot of the base line after the illumination.

The effects of DCMU upon these changes are shown in figure 1, b′ and b. In the presence of this inhibitor the change induced by 650 nm light becomes

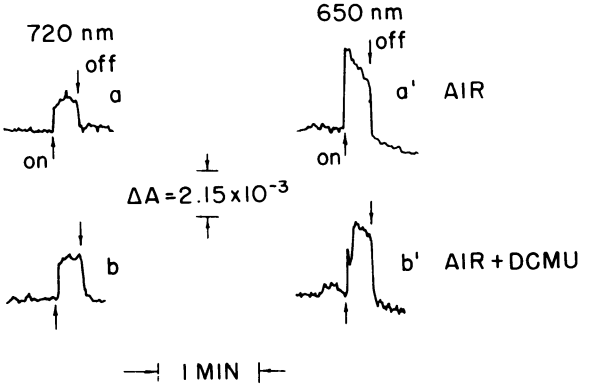

FIG. 1. The 520 nm light-induced absorbance change in whole cells of wild type: (a) and (a′) control sample; (b) and (b′) after the addition of DCMU (1.2×10^{-5} M). Chlorophyll concentration 75 μg/ml. Preceding dark time 10 min. Gas phase air.

similar to that obtained with 720 nm light in the absence of DCMU; there is an initial fast transitory increase in absorbance followed by a slower increase. The initial increase in absorbance is sometimes seen as a spike. On the other hand, DCMU has little effect on the 720 nm-induced change.

Figure 2 shows the 500 nm to 545 nm region of the light-minus-dark difference spectrum of an aerobic suspension of the wild-type cells. The difference spectrum obtained with 650 nm actinic light has a peak at 518 nm. This is in agreement with the results obtained by Chance and Strehler (6) for the wild-type strain of *C. reinhardi.*

The effects of DCMU upon the 650 nm-induced absorbance change suggest that in the absence of this inhibitor the absorbance change may be sensitized by both Systems I and II. If this is true, then mutant strains that lack System II activity should exhibit the 520 nm absorbance change sensitized by System I. Conversely, mutant strains deficient in System I activity should show only the absorbance change sensitized by System II.

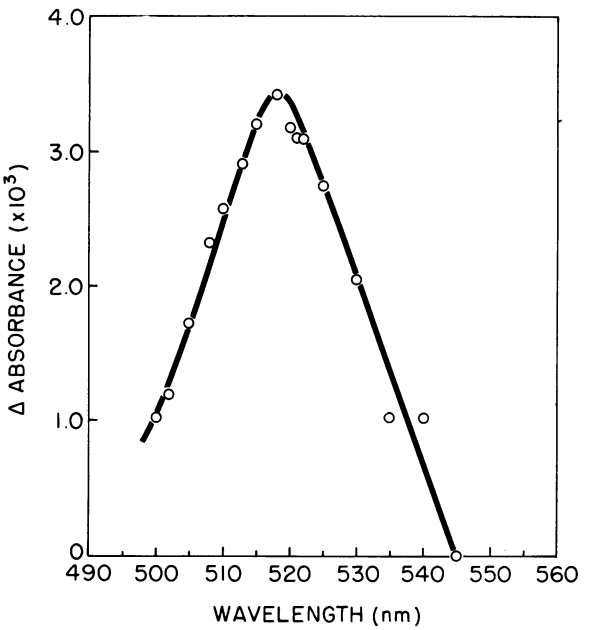

FIG. 2. The light-minus-dark difference spectrum of whole cells of the wild-type strain. Chlorophyll concentration 75 μg/ml. Preceding dark time 10 min. Gas phase air. Actinic light 650 nm. The absorbance changes were measured at a sensitivity of 0.0086 absorbance units for full deflection of the recorder chart.

The 520 nm Absorbance Change in Whole Cells of ac-115, ac-141, *and F-34.* The mutant strains, *ac-115, ac-141,* and F-34 have lesions in the photosynthetic electron transport chain close to System II. Some of the photochemical capacities of chloroplast preparations from these strains are shown in table I.

Light-induced absorbance changes of the cytochromes in the chloroplast fragments of *ac-115* and

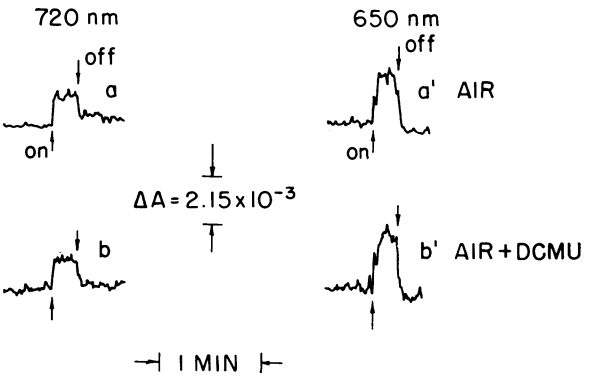

FIG. 3. The 520 nm light-induced absorbance change in whole cells of *ac-141:* (a) and (a′) control sample; (b) and (b′) after the addition of DCMU (1.2×10^{-5} M). Chlorophyll concentration 75 μg/ml. Preceding dark time 10 min. Gas phase air.

ac-141 have been described (18). These 2 strains lack an active cytochrome 559, and the light-minus-dark difference spectrum of their chloroplast fragments shows a peak only at 553 nm. Chloroplast fragments prepared from F-34 exhibit the same properties (Chua and Levine, unpublished results).

The fluorescence properties of *ac-115* and *ac-141* have been reported recently by Lavorel and Levine (17). Both strains show abnormally high fluorescence levels in the initial *O* state as compared to the maximum *P* state. Furthermore, *ac-115* exhibits very low luminescence. These results seem to indicate that in these mutant strains Q, the primary electron acceptor of System II, is either absent or inactive suggesting that the mutation has modified the photochemical complex of System II [for a discussion of the relationship between Q and cytochrome 559 see (17)]. The fluorescence properties of F-34 have not been studied.

The 520 nm absorbance changes were investigated in these 3 mutant strains, and similar results were obtained with each. Figure 3 illustrates the absorbance change in *ac-141.*

The characteristics of the absorbance changes induced by 650 nm and 720 nm light (fig 3, a and a′) are similar to those of the wild-type strain treated with DCMU. Furthermore, addition of DCMU has no obvious effect upon these changes (fig 3, b and b′). These results are consistent with the observation that electron flow in *ac-141,* as well as in *ac-115* and F-34, is blocked close to System II.

The 520 nm Absorbance Change in Whole Cells of F-1. The photochemical characteristics of F-1 are shown in table I.

The light-induced absorbance changes of cytochrome 553, cytochrome 559, and P700 were investigated with chloroplast fragments using the methods previously described (11, 19). Both cytochromes can be reduced by 650 nm light but cannot be oxidized by 720 nm light. Repeated attempts to show chemical or light-induced bleaching at 700 nm

Table I. *Photochemical Reactions of Chloroplast Fragments of Wild-type and Mutant Strains*

Columns 2 and 3 are the Hill reactions with DPIP and NADP as the respective electron acceptors. Column 4 is the photoreduction of NADP with the DPIP-ascorbate couple as the electron donor.

For the Hill reaction with DPIP the reaction mixture (1.0 ml) contained chloroplast fragments (10 μg chlorophyll) and the following in μmoles: potassium phosphate buffer, pH 7.0, 10; KCl, 20, $MgCl_2$, 2.5; and DPIP, 0.05. The DPIP was omitted from the control reaction mixture.

For the Hill reaction with NADP the reaction mixture (1.0 ml) contained chloroplast fragments (10 μg chlorophyll) and the following in μmoles: potassium phosphate buffer, pH 7.0, 10; KCl, 20; $MgCl_2$, 2.5; ferredoxin purified from wild-type *C. reinhardi*, 0.005; and NADP, 0.5. About 50 mU of Fd-NADP reductase was also included in the reaction mixture. For the photoreduction of NADP using the DPIP-ascorbate couple the reaction mixture contained, in addition to the above, the following in μmoles: DPIP, 0.05; sodium ascorbate, 8; and DCMU, 0.02. The reaction was run in a cuvette placed in the sample compartment of the spectrophotometer. Ferredoxin and NADP were omitted from the control reaction mixture in the reference cuvette.

The temperature during the reaction was 25°.

Strain	μmoles oxidant reduced/mg chlorophyll•hr		
	DPIP	NADP	NADP (DPIP-ascorbate)
wild-type	125	210	180
ac-115	<2	<2	150
ac-141	<2	<2	132
F-34	<2	<2	178
F-1	22	<2	<2

were unsuccessful (B. Moll, unpublished results). Thus the characteristics of F-1, are similar to those of *ac-80a* reported earlier (11), and they indicate that F-1 is deficient in active P700, the reaction center chlorophyll of System I.

Figure 4 shows the 520 nm absorbance changes of an aerobic cell suspension of F-1. Illumination with 650 nm light results in an absorbance increase

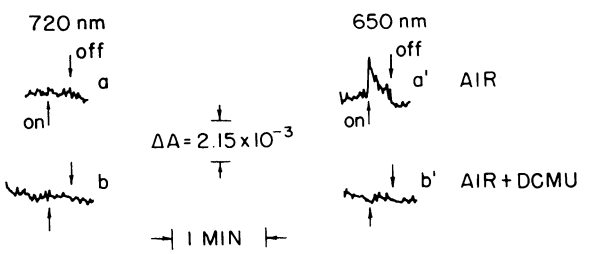

FIG. 4. The 520 nm light-induced absorbance change in whole cells of F-1: (a) and (a′) control sample; (b) and (b′) after the addition of DCMU (1.2×10^{-5} M). Chlorophyll concentration 75 μg/ml. Preceding dark time 10 min. Gas phase air.

followed by a fast decay in the light and an overshoot of the base line after the illumination (fig 4, a′). This increase in absorbance can be abolished by the addition of DCMU (fig 4, b′) indicating that the change is sensitized by System II. No absorbance change is observed with 720 nm illumination.

Discussion

The results reported in this paper suggest that the 520 nm absorbance change in the green alga *C. reinhardi* is sensitized by both Systems I and II. In the wild-type cells, the absorbance change is composed of at least 2 components: P520 I and P520 II associated with Systems I and II respectively. The P520 I absorbance change is observed in the DCMU-treated wild-type cells as well as in mutant strains modified in the photochemical complex of System II (fig 1 b′ and fig 3 a′). The time course of the absorbance change shows an initial fast transient followed by a slower increase in absorbance. The P520 II absorbance change, on the other hand, shows different kinetics in red light. The absorbance increase exhibits a decay in the light followed by an overshoot of the base line after the illumination (fig 4 a′). This is observed in a mutant strain lacking active P700, the reaction center chlorophyll of System I.

Although several hypothese have been proposed (10, 27, 28) the exact mechanism by which the 520 nm absorbance change is coupled to the photosynthetic electron transport pathway still remains unclear. Illumination of cells with either red or far-red light results in an increase in absorbance, and no antagonistic effects similar to those found with cytochromes 553 and 559 have been observed (19). Therefore, the pigment(s) that are responsible for the absorbance change do not seem to be situated directly in the path of electron transport from water to NADP. This contention is further supported by the observation that mutant strains in which electron transport is blocked still show this change on illumination. Furthermore, chloroplast fragments prepared after the sonic disintegration of cells can photoreduce NADP but they do not show the 520 nm absorbance change.

Fork and de Kouchkovsky (10) suggested that part of the absorbance change may be associated with the primary reaction of System II. However, 2 lines of evidence drawn from the results reported in this paper are incompatible with this hypothesis:

(1) The primary reaction of System II can occur even in the presence of DCMU since Q, the primary electron acceptor of System II, can be reduced in the light, and can become reoxidized in the dark either by oxygen or by a back reaction (9, 10). The hypothesis predicts that part of the 520 nm change should still persist in a System I mutant treated with DCMU. However, our results

show that the 520 nm change of F-1, a mutant strain deficient in active P700, is completely abolished by DCMU (see fig 4 b′).

(2) The photochemical complex of System II in the mutant strains *ac-115* and *ac-141* has been shown to be modified (17). The primary reaction of System II which still functions in DCMU-treated wild-type cells becomes inoperative in these mutant strains. According to the hypothesis these mutant strains should show a 520 nm change different from that of the DCMU-treated wild-type cells under red light illumination which activates both photosystems. However, the results reported in this paper point to the contrary. With 650 nm actinic light, the time course of the 520 nm absorbance change of these mutant strains is similar to that of the DCMU-treated wild-type cells (compare fig 1 b′ and fig 3 b′).

Experiments reported elsewhere (7) concerning the effects of the redox level on the absorbance change have led to the suggestion that the P520 I change may occur in association with a photo-oxidation and the P520 II change with a photoreduction. The identity of the pigment(s) responsible for these changes is unknown as is indeed the case for the 520 nm absorbance change in green plants in general. Several pigments including chlorophyll *b* (9, 27, 28) and carotenoid (5, 6, 15) have been suggested as possible candidates. In our experiments no attempts were made to establish the identities of the pigment(s) responsible for the P520 I and P520 II absorbance changes. Both changes might be mediated by the same pigment or by 2 different pigments. The differences in kinetics of the 2 changes under red light illumination suggest that 2 different pigments are involved. However, the alternative of a single pigment cannot be ruled out since the different characteristics of the 2 absorbance changes can be explained if it is assumed that the same pigment is located in 2 different physico-chemical environments, 1 associated with each photosystem.

The precise mechanisms by which the 520 nm absorbance change is brought about in green plants remain largely speculative. In the purple photosynthetic bacterium, *Rhodopseudomonas spheroides*, an absorbance change at 520 nm has been attributed to carotenoid, and it was found that 1 quantum of light brings about the shift in absorbance of 3 carotenoid molecules (1). If a similar mechanism were operating in the 520 nm absorbance change in green plants, then the change itself cannot be due to a redox reaction. Perhaps it is brought about in response to a change in the molecular environment of the pigment(s) (26) as a result of electron transport through Systems I and II.

Acknowledgments

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