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Mechanism of Photoactivation of Electron Transport in Intact *Bryopsis* Chloroplasts[1]

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ABSTRACT

The mechanism of photoactivation of photosystem I electron transport was studied in intact *Bryopsis corticulans* chloroplasts. The evidence from chemical and photochemical studies suggests that photoactivation is a consequence of a reduction of an electron transport component, presumably ferredoxin-NADP$^+$ reductase. O_2 does not act as a mediator of the process but rather acts as an electron acceptor after photoactivation has occurred. We suggest that the initial function of the chloroplasts in a transition from dark to light is to initiate pseudocyclic electron flow.

When dark-adapted algal cells, leaves, or intact chloroplasts are illuminated with strong light, the yield of Chl fluorescence shows a characteristic induction phenomenon (3, 6–8, 10–12, 14, 15, 18). The most pronounced transient of Chl fluorescence (termed DPS_1;[3] see Refs. 6 and 23) has been proposed to be related to photoactivation of the electron transport on the reducing side of photosystem I (6, 17, 18, 20). The rise to the 'P' level was suggested to be due to a 'traffic jam' of electrons up to the electron acceptor 'X' of photosystem I (12). Recently, Satoh and Katoh (18) and Satoh (17) showed that the photoactivation site is located after ferredoxin and before the site where NADP$^+$ accepts electrons. An activity change of the ferredoxin-NADP$^+$ reductase upon a dark-light transition of the chloroplasts was observed (17). Therefore, it was suggested that the first light-induced enzymic activation in chloroplasts is the photoactivation of ferredoxin-NADP$^+$ reductase.

The mechanism of the photoactivation process is not clear. Some information is, nevertheless, available. First, the photoactivation is greatly retarded in the absence of O_2 (3, 13, 20–22), indicating that O_2 plays some role in the process. Second, the rate of the photoactivation was high when the stromal pH was high and low at acidic pH (24). Third, the photoactivation is sensitive to any structural alteration of the enzyme ferredoxin-NADP$^+$ reductase or to the thylakoid membrane containing it (17). There have been many reports on the photoactivation of the enzymes of CO_2 fixation and this process is rather well known (1, 4, 9). However, these enzymes are soluble in the stroma. Ferredoxin-NADP$^+$ reductase is a membrane-bound electron carrier protein, and apparently the first transition occurring upon activation of the chloroplast enzymic system is the photoactivation of this component. Therefore, it was interesting and physiologically important to clarify the mechanism of this photoactivation process.

In this work, we present a tentative mechanism for the photoactivation as it occurs in intact *Bryopsis* chloroplasts. The specific role of O_2 in this process is elucidated.

MATERIALS AND METHODS

The marine green alga, *Bryopsis corticulans* was collected at Monterey Bay, CA. The algae were kept in sea water in the laboratory at 13°C under fluorescent illumination (12-h photoperiod, 8 μmol quanta m^{-2} s^{-1}).

Preparation of intact chloroplasts was as described previously (7, 24). Chloroplasts were kept in the dark for 2 h or more before use. Chl fluorescence was measured as described previously (17). The intensity of the actinic light was 20 w/m^2. Measurements of the rapid kinetics of Chl fluorescence were done as reported by Fork *et al.* (5). A Nicolet 1010 signal averager was used as a transient time converter. The intensity of the actinic light was 11 w/m^2.

Light-induced *A* changes of Cyt *f* were observed at 420 nm as described previously (20). The filter time constant of the system was 50 ms and the intensity of the red actinic light was 240 w/m^2.

In some experiments, added dithionite, DTT, or ascorbate was removed by centrifugation. Centrifugation was done twice at 1,000*g* for 15 s. The reaction mixture was the same as the chloroplast washing and resuspending medium and contained, in a final volume of 2 ml, 1.0 M sorbitol, 2 mM EDTA, 11 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM $NaNO_3$, and 50 mM Hepes (pH 7.5).

The concentration of Chl was determined according to the method of Arnon (2).

RESULTS AND DISCUSSION

As mentioned previously, O_2 is required for the photoactivation process (3, 13, 20–22). Figure 1 shows time courses of Chl fluorescence in the presence and absence of O_2 in intact *Bryopsis* chloroplasts. In order to consume oxygen in the reaction mixture, dithionite or glucose + glucose oxidase was added in Figure 1, b and c, respectively. In the presence of 1.0 M sorbitol, the chloroplasts were not leaky to dithionite in the time range used in these experiments (data not shown). In the case where reaction mixture was equilibrated with air (Fig. 1a), the typical DPS_1M_1 transient was observed. On the other hand, in the absence of O_2 the PS_1M_1 transient was largely inhibited (Fig. 1, b and c).

In the cases of Figure 1, b and c, enough dithionite or β-D-glucose was added to consume almost all O_2 in the reaction mixture. Figure 2 shows the effects of various concentrations of β-D-glucose on the extent of the PS_1 decline in the presence of glucose oxidase. Addition of glucose even as low as 0.1 mM decreased the extent of the PS_1 decline and at 0.5 mM glucose the

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[3] DPS_1 transition, the increase and decrease in Chl fluorescence which is inhibited by methyl viologen and which occurs from 50 ms to 5 s after the onset of illumination of chloroplasts.

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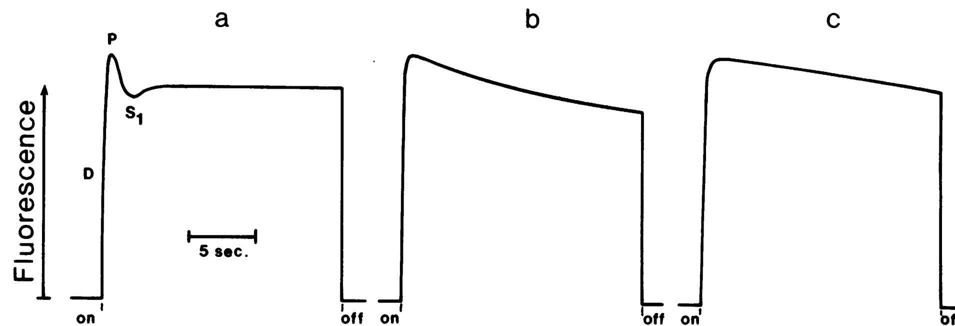

FIG. 1. Time courses of Chl fluorescence in the presence and absence of oxygen. a, With no addition. b, + 5 mM dithionite. c, + 10 mM β-D-glucose, 20 units/ml glucose oxidase, and 60 units/ml catalase. Fluorescence was measured 3 min after the addition of the reagents. The reaction mixture contained, in 2 ml, 1.0 M sorbitol, 2 mM EDTA, 11 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM $NaNO_3$, 50 mM Hepes (pH 7.5), and chloroplasts equivalent to 2.11 μg Chl/ml. The intensity of the actinic light was 20 w/m^2.

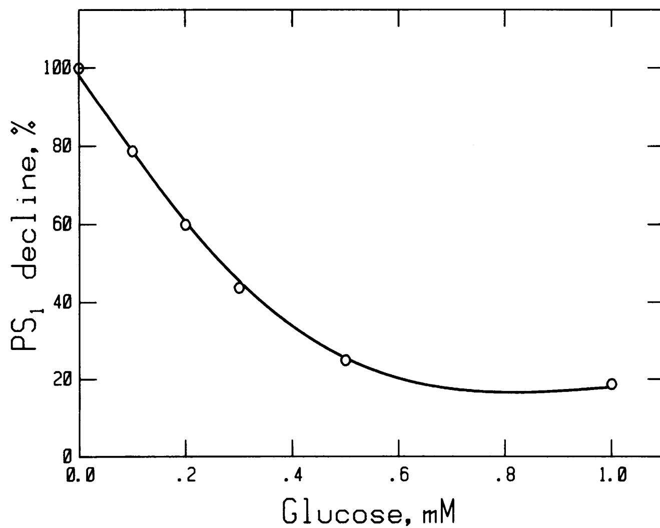

FIG. 2. Effects of glucose concentration on the extent of the PS_1 decline of Chl fluorescence. Twenty units/ml glucose oxidase and 60 units/ml catalase were added 3 min before the measurements. Concentrations of β-D-glucose are indicated. Chl concentration was 4.89 μg/ml. Other conditions were the same as in Figure 1.

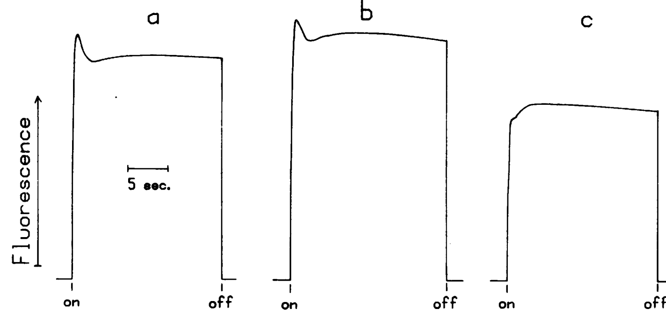

FIG. 3. Effects of pre-illumination of the chloroplasts in the presence of dithionite on the time course of Chl fluorescence. c, The chloroplasts were pre-illuminated for 10 s with the actinic light 3 min after the incubation of the chloroplasts with 5 mM dithionite. b, Without the pre-illumination of the chloroplasts. Dithionite was removed by two centrifugations. a, Without dithionite and any pretreatment. Chl concentrations were 2.75, 3.41, and 2.40 μg/ml for a, b, and c, respectively. Other conditions were the same as in Figure 1.

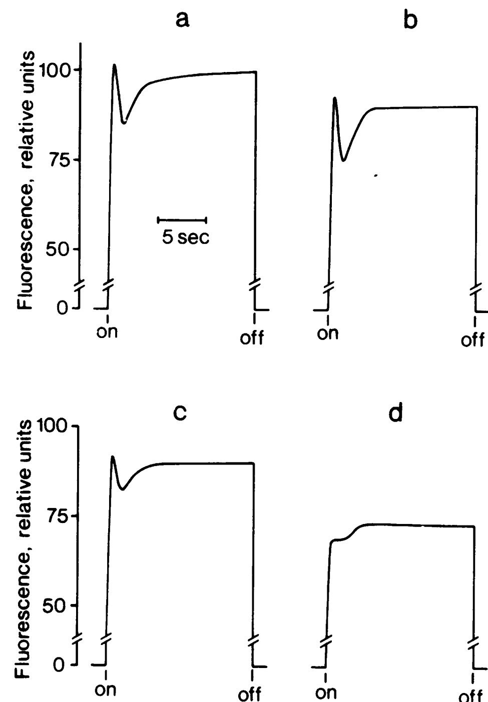

FIG. 4. Time courses of Chl fluorescence after incubation of the chloroplasts with ascorbate, DTT, or dithionite at 0.25 M sorbitol. Chloroplasts were incubated for 5 min with no reductant (a), with 10 mM ascorbate (b), with 10 mM dithiothreitol (c), or with 5 mM dithionite (d). The incubation medium contained 0.25 M sorbitol, 50 mM Hepes (pH 7.5), 2 mM EDTA, 11 mM $MgCl_2$, 1 mM $MnCl_2$, and 2 mM $NaNO_3$. The reductants were removed by washing the chloroplasts with two centrifugations (see text). Chl concentrations were 2.86, 2.44, 2.59, and 2.31 μg/ml for a, b, c, and d, respectively. Other conditions were the same as in Figure 1.

PS_1 decline became difficult to distinguish from a slow decay which had no relation to photoactivation (Fig. 2). These results indicate that the photoactivation process is highly dependent on the O_2 concentration in the reaction mixture. Such a dependency could reflect either a requirement for O_2 in the photoactivation process *per se* and/or a requirement for O_2 in the Mehler reaction after the photoactivation process has taken place. Therefore, we tested whether the photoactivation process would occur in the absence of O_2 (Fig. 3). Chloroplasts were pre-illuminated for 10 s in the absence of O_2 (in the presence of 5 mM dithionite) and then washed twice by centrifugation to remove the dithionite (Fig. 3c). The fluorescence time course of the chloroplasts showed no DPS_1 transient, indicating that the photoactivation process had already taken place. Addition of dithionite without pre-illumination had little effect on the DPS_1 transient (Fig. 3b). These data show clearly that O_2 is not necessary for the photoactivation process itself. It is well known that O_2 acts as an electron acceptor in photosynthesis (16). Therefore, it is reasonable to assume that O_2

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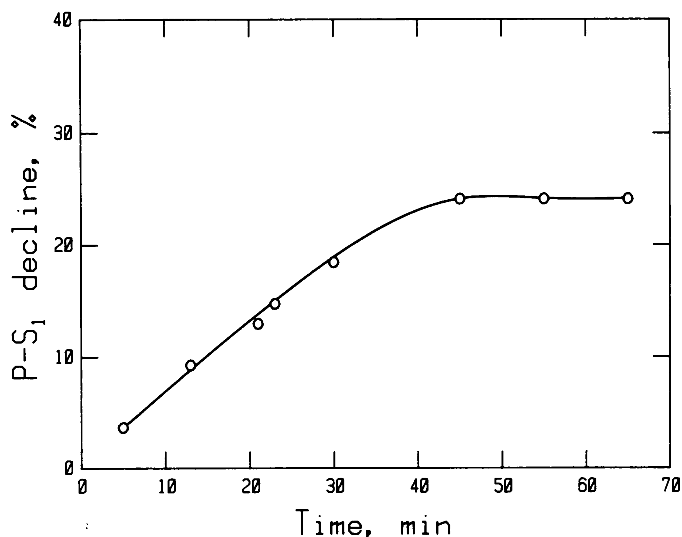

FIG. 5. Recovery of the extent of the PS_1 decline after the removal of the dithionite. The chloroplasts were treated with dithionite as in Figure 4d and the extents of the PS_1 decline were measured at various times after the removal of the dithionite, and plotted as percentage of the control. The chloroplasts were kept in the dark. Chl concentration was 4.3 μg/ml. Other conditions were the same as in Figure 1.

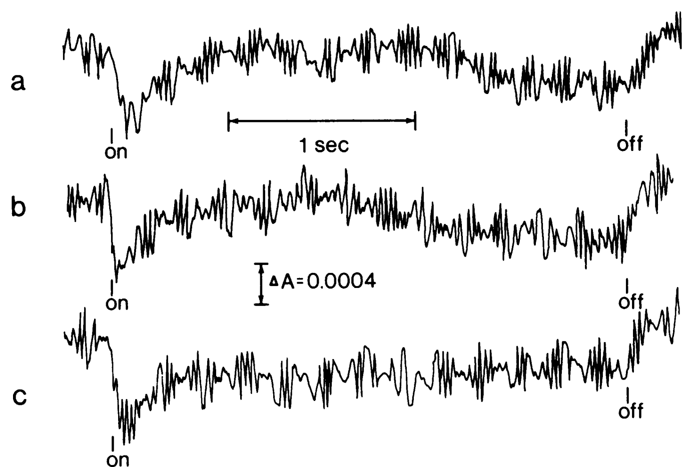

FIG. 6. Time courses of photooxidation of Cyt *f* after incubation of the chloroplasts with ascorbate or DTT at 0.25 M sorbitol. The photooxidation of Cyt *f* was measured by observing the light-induced *A* change at 420 nm. The chloroplasts were treated with no reductant (a), with ascorbate (b), or DTT (c) as in Figure 6. Chl concentrations were 44.3, 42.6, and 42.9 μg/ml for a, b, and c, respectively. Other conditions were the same as in Figure 1.

acts as an electron acceptor after chloroplasts are photoactivated. In fact, Satoh *et al.* (20) and Katoh (19) observed that, even after the pre-illumination of the chloroplasts in the presence of dithionite, Cyt *f* remained reduced after transient oxidation during illumination of the chloroplasts. We also observed that, in the absence of O_2, the extent of Chl fluorescence stayed at a high level, showing that Q was largely reduced even in photoactivated chloroplasts (data not shown).

As was shown recently (17), the photoactivation process can be observed in *Bryopsis* chloroplasts which have envelopes that are leaky to small molecules. Using similar conditions, we observed the effects of reductants on the photoactivation process. Figure 4 shows time courses of Chl fluorescence after incubation of the chloroplasts in the dark with and without several reductants at 0.25 M sorbitol concentration. The reductants were removed by two centrifugations of the chloroplasts. As was shown previously (17), the dark inactivation process is very slow in *Bryopsis* chloroplasts. Therefore, we can see whether the activation of electron transport has taken place if we measure the induction of Chl fluorescence in less than 10 min after the removal of the reductants. The time course of Chl fluorescence in the chloroplasts which had been incubated with ascorbate (Fig. 4b) was similar to that in control chloroplasts which had been incubated with no reductant (Fig. 4a). Incubation of the chloroplasts with DTT slightly decreased the DPS_1 transient (Fig. 4c). A dramatic effect was obtained by the addition of dithionite (Fig. 4d). Incubation of the chloroplasts with dithionite largely eliminated the DPS_1 transient of Chl fluorescence, showing that the photoactivation process had already taken place. These results show that the reduction of a certain substance which can be reduced by dithionite ($E_{m,7}$ = −1.12 v) and partly by DTT ($E_{m,7}$ = −0.33 v) but not by ascorbate ($E_{m,7}$ = +0.058 v) (*i.e.* ferredoxin-$NADP^+$ reductase; see Ref. 17) is involved in the photoactivation process.

In order to check if this reductant effect is the same as the photoactivation of the chloroplasts, the dark recovery of the DPS_1 transient after the removal of the dithionite was observed (Fig. 5). The extent of the PS_1 decline recovered slowly and reached a maximum after about 40 min dark incubation. Although the maximum extent recovered was smaller, this dark recovery curve was similar to that after photoactivation of the chloroplasts (see Fig. 4 in Ref. 17). This shows that the reductant-induced decrease of the DPS_1 transient had similar characteristics to the light-induced decrease with respect to their dark recoveries. The smaller recovery might be due to further damages of the chloroplasts by centrifugation.

Figure 6 shows the induction of photooxidation of Cyt *f* after the reductant treatments. The reduction of the Cyt after the initial rapid oxidation indicates that there may be a block on the reducing side of PSI and the reoxidation during the illumination may correspond to the photoactivation of the blocked site (21). The ascorbate treatment had no effect on this induction of Cyt *f* photooxidation (Fig. 6b). However, the DTT treatment decreased the transient reduction of Cyt *f* (Fig. 6c), showing that some activation had already been induced by this treatment. After the dithionite treatment, the initial rate and also the extent of photooxidation of Cyt *f* became so small that it was impossible to determine how far the activation had taken place (data not shown). The reason for this is not clear at present. However, the observation that a moderate reductant (DTT) decreased the transient reduction of Cyt *f* is in agreement with the results of Figure 4.

All the results presented in this paper suggest that the photoreduction of a hypothetical compound with a very low redox potential (probably lower than −0.33 v) is involved in the photoactivation process and after this photoactivation has occurred, electrons begin to flow to O_2, inducing a pseudocyclic electron flow through PSI and PSII. The initiation of this pseudocyclic electron flow may be the first photoactivation event in chloroplasts. Inasmuch as O_2 is abundant under normal conditions, illumination of chloroplasts would cause a large Mg^{2+} efflux from inside of the thylakoid membranes and an alkalinization of stromal pH, conditions that are favorable for a high rate of photosynthesis (4, 9). It seems probable that $NADP^+$ initially may not exist in sufficient amounts to act as an electron acceptor in the chloroplasts when CO_2 fixation enzymes are still inactive in the dark and before the completion of the photoactivation of these enzymes. Therefore, this pseudocyclic electron transport reaction may be needed to make favorable conditions for photosynthesis to proceed faster, suggesting that this may be the physiological function of this process. The results obtained by Radmer and Ollinger (16) are in agreement with this idea. They found no significant lag in O_2 uptake in *Scenedesmus* which showed a marked lag in CO_2 fixation. They also found no appreciable light-driven O_2 uptake in a mutant lacking PSI.

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Some enzymes related to the carbon cycle in chloroplasts are also known to be activated or inactivated through photoreduction by PSI (1, 4, 9). Reduction of S-S bonds is thought to be involved in these processes and addition of DTT caused the activation or inactivation of several enzymes in the dark (1, 4, 9). In the case reported here, incubation of the chloroplasts with DTT in the dark only partly decreased the DPS_1 transient (Figs. 4c and 6c). Therefore, the mechanism of the photoactivation reported in this paper may be different from the mechanism for activation of the enzymes of CO_2 fixation.

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