Correlation between Changes in Light Energy Distribution and Changes in Thylakoid Membrane Polypeptide Phosphorylation in *Chlamydomonas Reinhardtii*

FRANCIS-ANDRÉ WOLLMAN and PHILIPPE DELEPELAIRE
Institut de Biologie Physico-Chimique, 75005 Paris, France

**ABSTRACT**  We have used a new method to extensively modify the redox state of the plastoquinone pool in *Chlamydomonas reinhardtii* intact cells. This was achieved by an anaerobic treatment that inhibits the chlororespiratory pathway recently described by P. Bennoun (*Proc. Natl. Acad. Sci. USA*, 1982, 79:4352–4356). A state I (plus 3,4-dichlorophenyl-1,1-dimethylurea) → anaerobic state transition induced a decrease in the maximal fluorescence yield at room temperature and in the $F_{\text{m}}/F_{\text{ps}}$ ratio at 77°K, which was three times larger than in a classical state I → state II transition. The fluorescence changes observed in vivo were similar in amplitude to those observed in vitro upon transfer to the light of dark-adapted, broken chloroplasts incubated in the presence of ATP. We then compared the phosphorylation pattern of thylakoid polypeptides in *C. reinhardtii* in vitro and in vivo using $\gamma$-[32P]ATP and [32P]orthophosphate labeling, respectively. The same set of polypeptides, mainly light-harvesting complex polypeptides, was phosphorylated in both cases. We observed that this phosphorylation process is reversible and is mediated by the redox state of the plastoquinone pool in vivo as well as in vitro. Similar changes of even larger amplitude were observed with the F34 mutant intact cells lacking in photosystem II centers. The presence of the photosystem II centers is then not required for the occurrence of the plastoquinone-mediated phosphorylation of light-harvesting complex polypeptides.

It is now well established that several thylakoid membrane polypeptides (mainly the light-harvesting complex [LHC] polypeptides) can be phosphorylated in vitro (4, 5) and that their phosphorylation level is controlled by the redox state of a component of the electron transfer chain located between the two photosystems, most likely the plastoquinone (PQ) pool (2, 17). It has been shown in vitro that, upon reduction of the PQ pool, the LHC becomes phosphorylated and that its ability to transfer excitation energy to the photosystem I (PSI) reaction centers is increased. Conversely, the oxidation of the PQ pool is accompanied by a dephosphorylation of the LHC and a better sensitization of the photosystem II (PSII) reaction centers by this chlorophyll-protein complex. This phosphorylation-dephosphorylation process is mediated by two membrane bound enzymes, a kinase and a phosphatase (6, 7). Whereas the phosphatase is always active, the activity of the kinase would be regulated by the redox state of the PQ pool.

Several authors have emphasized that such a mechanism might serve in vivo as a regulation process in the cases where the photochemical rates of the two photosystems are unbalanced (13, 21). However, although likely, no convincing in vivo experiment has been reported in higher plants to support this hypothesis. Nevertheless, Allen and Bennett (1) have shown that this PQ-mediated phosphorylation process of several polypeptides occurs in isolated intact chloroplasts as well as in purified thylakoid membranes.

Owens and Ohad (20) used the unicellular green alga, *Chlamydomonas reinhardtii*, to compare the in vitro and in vivo phosphorylation patterns of thylakoid membranes polypeptides. Their in vitro labeling experiments confirmed the main results previously reported for higher plant chloroplasts. They could not find, however, any significant change in the phosphorylation level of the thylakoid membrane polypep-
tides upon illumination of intact cells in red or far red light as compared with dark-adapted cells. They then concluded that the state I-state II transition first reported by Bonaventura and Myers (12) in intact cells was not controlled by such a phosphorylation-dephosphorylation process in vivo.

Such a discrepancy between in vivo and in vitro results may arise for two quite different reasons: (a) the relative activity of the membrane bound kinase and phosphatase are strongly modified in the course of chloroplast isolation for in vitro studies; (b) the methods previously used to modify the redox state of the PQ pool in vivo are not as efficient as those used in vitro. Consequently, no significant changes in the phosphorylation level of the thylakoid membrane polypeptides would have been observed in vivo.

The redox state of the plastoquinone pool in dark-adapted algae is controlled by the relative concentrations of an endogenous reductant and of molecular O$_2$ (16). Bennoun (11) has recently shown that the PQ pool in the thylakoid membrane is a redox carrier common to two distinct electron transfer chains: the photosynthetic one, from H$_2$O to NAD(P)H, and a respiratory-like one, from NAD(P)H to O$_2$. This second electron transfer chain in the chloroplast, which was referred to as chlororespiration, is inhibited in anaerobic conditions and, in most of the cases studied so far, by heme-proteins inhibitors, such as carbon or nitrogen-monoxide or cyanide leading, in the dark, to a nearly complete reduction of the PQ pool. Such inhibitors can then be used to drive the reduction of the PQ pool much more efficiently than the use of light II (~650 nm) and may improve the conditions under which phosphorylated polypeptides are observed in thylakoids of intact cells.

Alternatively oxidation of the PQ pool can be improved by the use of mutants lacking in PSI II centers. In such a case, the mere illumination of the cells induces an oxidation of the PQ pool by PSI with no concomitant input of reducing equivalents coming from PSI II.

We have compared, in this paper, the phosphorylation pattern of thylakoid polypeptides and the light energy distribution in C. reinhardtii under experimental conditions where the PQ pool is maximally oxidized or reduced both in vivo and in vitro. We show that changes in the light energy distribution between the two photosystems are associated in vivo as well as in vitro to changes in the amount of phosphate incorporated in several thylakoid polypeptides. This phosphorylation process is mediated by the redox state of intersystem electron carriers in both cases and does not depend on the presence of PSI II centers in the thylakoid membrane.

MATERIALS AND METHODS

Two different strains of C. reinhardtii were used in this work: a wild-type strain (WT) and a mutant strain devoid of photosystem II reaction centers (F34). Both were grown in Tris-acetate-phosphate (TAP) medium under a continuous illumination of 200 lux (cool fluorescent light). The cultures were used in mid-logarithmic growth phase (3 x 10$^6$ cells/ml).

In Vitro Experiments: Wild-type cells were broken and resuspended in 20 mM HEPES pH 7.5, 0.1 M sucrose, and 10 mM NaCl according to already published procedure (23): 5 mM MgCl$_2$ was present throughout the experiment. 1 x [32P]ATP (specific activity, 1 x 10$^6$ cpm/umol) at a concentration of 100 µM was then added to the preparation which was split into three aliquots: the first one was kept in the dark (under those conditions, the PQ pool is fully oxidized), the second one was illuminated with white light at 200 lux (PQ pool fully reduced), and the third one was illuminated with white light in the presence of 10$^{-5}$ M (plus 3,4-dichlorophenyl-1,1-dimethylethyl) (PQ pool oxidized). The incubation time was 150 min. The reaction was stopped by the addition of NaF (20 mM) and rapid cooling down to 4°C.

In Vivo Experiments: Cells were harvested by low speed centrifugation (600 g, 10 min) and resuspended at 3 x 10$^6$ cells/ml in a phosphate depleted TAP medium containing [32P]orthophosphate (specific activity, 1 µCi/ml). They were incubated at 200 lux in this medium for 120 min to reach a steady-state level of phosphorylation. They were then incubated for 15 min under various conditions to get an oxidation or a reduction of the PQ pool; reduction of the PQ pool was achieved by adding in the dark either NaN$_3$ which inhibits the output of the chlororespiratory pathway (cf. Introduction) or glucose/glucose-oxidase (20 mM-2 mg/ml, respectively) which takes up O$_2$, the terminal electron acceptor of the chlororespiratory pathway.

Fluorescence Experiments: Fluorescence measurements at room temperature were performed as in (23). 1 min before recording the fluorescence induction curve, 10$^{-5}$ M DCMU was added in the dark to all the samples, except when otherwise indicated. 77K fluorescence emission spectra were recorded as described previously (24).

RESULTS

In Vitro Experiments: WT Strain

The labeling patterns of phosphorylated thylakoid membrane polypeptides from broken cell preparation of the wild-type strain under different experimental conditions are shown in Fig. 1. When the broken cell preparation is illuminated to reduce the PQ pool (Fig. 1, lane 3), five distinct polypeptides in the range 25-35 kdaltons (9-11, 13, and 16) are heavily labeled and several others are also faintly labeled (two in the low molecular weight range, A and B and a few in the high molecular weight range). Among these polypeptides, 10, 11, 13, and 16 have been shown to be LHC apoproteins (15).

Striking differences in this labeling pattern are observed when the broken cells are incubated in the dark to oxidize the PQ pool (Fig. 1, lane 2). Much less phosphate is incorporated in the thylakoid proteins. This holds true in particular for polypeptides 13 and 16 which are completely dephosphorylated. Polypeptides 9, 10, 11, and 4 are dephosphorylated by ~50%. No net dephosphorylation occurs however for band B and the high molecular weight polypeptides. On lane 4, Fig. 1, is shown the phosphorylation pattern obtained for a broken cell preparation illuminated in the presence of 10$^{-5}$ M DCMU. This pattern is nearly identical to that obtained with broken cells incubated in the dark (lane 2). This result confirms previous in vitro labeling experiments reported for C. reinhardtii (20) and is consistent with a phosphorylation of the LHC polypeptides mediated by the redox state of the PQ pool, as observed in higher plant chloroplasts.

In Fig. 2 are shown the fluorescence induction curves of these broken cell preparations observed in the presence of 10$^{-5}$ M DCMU. Illumination of the sample in the presence of ATP decreases the F$_{m}$ level by ~35% as compared with dark adapted preparations (Fig. 2, curves 1 and 2). However the addition of DCMU before the illumination period does not restore the high fluorescence state, as it would be expected.
Although, as far as phosphorylation is concerned, the two conditions, dark and light plus DCMU, are identical (Fig. 1, lanes 2 and 4). The $F_m$ level is even slightly lower when DCMU is present during the illumination period; a similar observation on higher plant chloroplasts has been reported by Bennett et al. (8). A deleterious effect of prolonged illumination of broken cells must then be considered. This emphasizes the existence of other quenching phenomena in vitro and draws the limits of experiments devoted to phosphorylation of LHC proteins through the exclusive use of fluorescence parameters.

In Vivo Experiments: WT strain

A typical state II to state I transition is shown in Fig. 3A. As previously observed (9), both $F_o$ and $F_m$ (observed in the presence of DCMU) are $\sim 15\%$ lower in state II than in state I. Similar variations occur in the half time of the fluorescence rise: it is $\sim 15\%$ shorter in state I than in state II. To get either a more complete reduction or oxidation of the PQ pool, cells were treated for 15 min either in the dark in the presence of glucose/glucose-oxidase (20 mM and 2 mg/ml, respectively) or preilluminated with far red light in the presence of $10^{-5}$ M DCMU. In the former case, molecular O$_2$, the terminal electron acceptor of the chlororespiratory pathway is no longer present and electrons accumulate in the dark in the PQ pool. The fluorescence induction curves observed in the presence of DCMU for these two conditions are shown in Fig. 3B. The $F_m$ level is reduced by a factor of 2 in the anaerobic state as compared with the sample preilluminated in the presence of DCMU. Similar changes were observed when NaN$_3$ was used instead of glucose/glucose-oxidase to inhibit the chlororespiratory pathway (not shown). No simple comparison can be made between the $F_o$ levels in Fig. 3B since the reduction of the PQ pool leads to a partial reduction of the primary and secondary acceptors of PSII and thus increases the $F_o$ level upon addition of DCMU. It is worth mentioning that the fluorescence changes observed in vivo are of even greater amplitude than those seen in vitro (compare Fig. 3B and Fig. 2).

The decrease observed in the $F_m$ level during a state I to state II transition is attributed to a more efficient energy transfer from the bulk antenna to the PSI centers at the expense of the PSII centers (22). An alternative description of the phenomenon arises from the analysis of $77^\circ K$ fluorescence emission spectra obtained with algae in state I or in state II; the $F_{685\text{nm}}/F_{715\text{nm}}$ ratio (respectively PSII and PSI fluorescence) decreases during a state I to state II transition (12). To rule out the possibility that inhibition of chlororespiration
would modify other quenching processes at room temperature than those controlled by the state of excitation energy transfer from LHC to PSI, we analyzed the 77°K fluorescence emission spectra of cells preincubated in the same conditions as in Fig. 3. A very large decrease in the F685 nm/F715 nm ratio is observed upon reduction of the PQ pool under anaerobic conditions (Fig. 4). Thus a similar reorganization of the antenna between the two photosystems occurs in a state I to state II transition and in a state I plus DCMU to an anaerobic state transition, though of much higher amplitude.

Fig. 5 shows the labeling patterns of thylakoid membrane polypeptides isolated from WT intact cells and treated under various conditions. Independently of a particular pretreatment, it is seen that the phosphorylated polypeptides are mainly the same as in the in vitro labeling experiment: polypeptides 9–11, 13, 16, A, and B are labeled. These polypeptides are phosphorylated in algae placed either in state I or in state II (Fig. 5, lanes 1 and 2). One observes however a slightly lower level of phosphorylation (by ~20%) of all these polypeptides, but band B, in state I than in state II. The changes observed in this latter transition are then of much lower amplitude than in the in vitro experiments presented in Fig. 1. The variations in the phosphorylation levels of the different thylakoid membrane polypeptides in vivo are much higher when we compare cells preilluminated in far red light in the presence of DCMU and lower curve, cells incubated in the dark in the presence of glucose/glucose-oxidase. The lines on the left of the figures indicate the I_m levels in each case.

In Vivo Experiments: F34 Mutant Strain

Fluorescence induction curves at room temperature and 77°K fluorescence emission spectra with the F34 mutant cells following various pretreatment conditions are shown in Fig. 6. Owing to the absence of PSI centers, there is no variable fluorescence at room temperature and the emission peaks at 685–695 nm are shifted towards 682 nm which corresponds to the emission band of “disconnected” LHC. The comparison between the cells incubated with glucose/glucose-oxidase in the dark and those preilluminated with far red light shows that a reorganization of antenna pigments around PSI centers occurs in the absence of PSII centers. The fluorescence changes are similar to those observed in the wild-type but of even higher amplitude. The low fluorescence state and low F682 nm/F715 nm ratio produced by anaerobic treatment correspond to an efficient excitation energy transfer from the LHC to PSI centers while preillumination with far red light induces a high fluorescence state and a high F682 nm/F715 nm ratio by decreasing the energy transfer from LHC to PSI centers.

The labeling patterns of the thylakoid membranes isolated from F34 cells incubated in the dark in the presence of glucose/glucose-oxidase (Fig. 7, lane 4) includes polypeptides 9–11, 13, 16, and thus shows great similarities with that of the WT incubated under the same conditions (Fig. 5, lane 4). The F34 mutant lacks ~10 polypeptides among which are polypeptides 6, 27, L5–L6 (10). The corresponding phosphorylated polypeptides, bands A and B, and that of high molecular weight, are no longer seen in the autoradiogram of the F34 thylakoid membranes, although this latter strain lacks the whole PSII reaction center. A nearly complete dephosphorylation of all the polypeptides occurs when the F34 cells...
are preilluminated with far red light (Fig. 7, lane 3). In this mutant, intermediary situations can easily be obtained where the PQ pool is only partially oxidized: to this end, F34 cells were incubated in the dark in the presence of glucose/glucose-oxidase and then illuminated with far red light. Depending on the intensity of this far-red light, a partial reoxidation of the PQ pool occurs and the characteristics of the labeling pattern of the thylakoid membrane polypeptides and of the fluorescence induction curve at room temperature are in between the two extreme cases previously studied (Fig. 6A, curve 3, and Fig. 7, lane 2). The associated changes in these three parameters, redox state of the PQ pool, fluorescence yield, and labeling pattern of the thylakoid membrane polypeptides, indicate that the absence of the PSII centers in the F34 mutant does not alter the main features of the regulation process observed with the WT.

In Table I are summarized the values of the main fluorescence parameters pertaining to the light energy distribution between the two photosystems in the different samples we have analyzed together with the corresponding relative amounts of phosphate incorporated in each of the phosphopoly peptides of the thylakoid membranes. The main LHC polypeptide, polypeptide 11, is the most heavily labeled in many cases. Using the WT intact cells, a fourfold increase in the average phosphorylation of the set of phosphopoly peptides occurs in the state I (+ DCMU) to anaerobic state transition while it is of only 1.3 times in a state I to state II transition. The largest increase in phosphorylation, about eight times, is obtained with the F34 mutant cells upon a state I to anaerobic state transition. This latter phosphorylation is accompanied by a ~70% decrease in the $F_o$ level at room temperature with F34 cells while it is ~50% in a similar transition with the WT intact cells, and of ~15% only in a state I to state II transition.

A noticeable feature of the phosphorylation changes shown in Table I is that the distribution of the phosphate groups among the set of phosphorylated polypeptides varies from one treatment condition to another. This is shown diagrammatically in Fig. 8 in the case of a state I to state II transition as compared with a state I (+ DCMU) to anaerobic state transition using the WT cells labeled in vivo. The relative increase in phosphorylation $(\Delta\text{ phospho})$ of polypeptide 13 is much higher than that of polypeptide 16 in the former transition while similar increases in phosphorylation of the two polypeptides are observed in the latter transition. These observations are consistent with the phosphorylation of the thylakoid polypeptides being a nonspecific process although it is a selective one (since only a few polypeptides can be labeled). The LHC as a whole would undergo change in its phosphorylation level irrespective of which constitutive polypeptide is the more affected. In addition, bands $A$ and $B$ undergo PQ-mediated changes in their phosphorylation level similar to those of the LHC polypeptides in the WT cells although they are not required for the reorganization of the antenna as shown by the analysis of the F34 mutant. We cannot however exclude that the reversible phosphorylation of these PSII polypeptides in the WT cells might be involved in the reversible association of the LHC with the PSII centers.

**DISCUSSION**

The PQ pool in the thylakoid membrane is common to a photosynthetic pathway and a chlororespiratory pathway (11). It can be reduced by either PSII or NADH, and oxidized by either PSI or O$_2$. When state I-state II transitions are monitored in vivo, the continuous activity of chlororespiration insures a partial reduction of the PQ pool and thus restricts the amplitude of redox changes at this level of the electron transport chain. Associated changes in phosphorylation of thylakoid polypeptides and in the energy distribution between the two photosystems are then expected to be smaller than those observed in vitro where the PQ pool is fully oxidized in the dark and fully reduced in the light provided that no
of the thylakoid polypeptides in *C. reinhardtii*. However, at variance between the in vivo and in vitro patterns of phosphorylation of Owens and Ohad (20), we observed a striking similarity anaerobically in the dark. In agreement with previous results with far-red light in the presence of DCMU or incubated observed in *C. reinhardtii* for WT cells either preilluminated times decrease in the illuminated in the presence of ATP. This compares well with these authors, we showed that this phosphorylation is a reversible process in vivo as well as in vitro. There was an extensive dephosphorylation of the phosphorylated polypeptides when the fully reduced PQ pool is oxidized either in vitro by incubation in the dark of preilluminated chloroplasts or in vivo during an anaerobic state to state I plus DCMU transition. There were only minor differences in the dephosphorylation patterns in vivo and in vitro; whereas polypeptide B got dephosphorylated in vivo upon oxidation of the PQ pool, it was still phosphorylated in vitro upon oxidation of the PQ pool.

Several authors have pointed out a specific involvement of the PSII centers in the regulation process due to the reversible phosphorylation of the LHC (3, 18). Owens and Ohad (20) concluded from their analysis of the T44 mutant of *C. reinhardtii* that no phosphorylation of LHC polypeptides occurs in the absence of PSII centers. The present study of the F34 mutant lacking in PSII centers, clearly shows that no such specific interaction between LHC and PSII centers is required to get either a reversible phosphorylation of LHC polypeptides or a reorganization of the antenna pigments. Besides the lack of PSII centers, the T44 mutant might then be defective in some other component of importance in the phosphorylation process.

The fluorescence and phosphorylation changes observed in the F34 mutant are of even greater amplitude than in the WT. Mutants lacking in PSII still show a cation controlled light energy distribution (19, 23). Wollman and Diner (23) have previously shown that the target for the reorganization of the antenna induced by cations was located within the LHC. The present work shows that the target for the regulation of the light energy distribution controlled by the redox

\[ \text{Relative amount of phosphate bound to the different polypeptides and fluorescence state parameters} \]

| Broken cells (WT) | Intact cells (WT) | Intact cells (F34) |
|-------------------|------------------|------------------|
| L                 | D                | DCMU + L         | State II  | State I  | State I + DCMU | State I  | State I  | State I - O2 |
| 9                 | 70               | 45               | 31        | 75       | 57             | 69       | 16       | 24          |
| 10                | 45               | 23               | 15        | 61       | 48             | 29       | 3        | 17          |
| 11                | 100              | 32               | 28        | 100      | 74             | 100      | 31       | 100         |
| 13                | 45               | —                | —         | 59       | 32             | 38       | 16       | 38          |
| 16                | 64               | —                | —         | 56       | 54             | 50       | 26       | 41          |
| A                 | 11               | 4                | 3         | 16       | 11             | 11       | —        | —           |
| B                 | 18               | 18               | 15        | 62       | 57             | 30       | 7        | —           |
| *f*<sub>tot</sub> | 65               | 100              | 57        | 86       | 100            | 52       | 100      | 32          |
| 4Ratio            | 52               | 100              | —         | —        | —              | —        | —        | —           |

\[ \text{Relative amount of phosphate bound to the different polypeptides (9-11, 13, 16, A, and B). L, broken cells WT incubated for 150 min in the light (cf. Lane 3, Fig. 1); D, broken cells incubated for 150 min in the dark (cf. Lane 2, Fig. 1); DCMU + L, broken cells incubated in the light in the presence of 10−3 M DCMU (cf. Lane 4, Fig. 1). State II, cells incubated in red light (cf. Lane 1, Fig. 5); State I, cells incubated in far-red light (cf. Lane 2, Fig. 5); O₂ cells incubated in the dark in the presence of glucose/glucose-oxidase (cf. Lane 4, Fig. 5); State I + DCMU, cells incubated in far-red light in the presence of 10−3 DCMU (cf. Lane 3, Fig. 5). O₂ cells incubated in the dark in the presence of glucose/glucose-oxidase (cf. Lane 4, Fig. 7); State I, cells incubated in far-red light (cf. Lane 3, Fig. 7); State I-O₂, cells incubated in far-red light in the presence of glucose/glucose-oxidase (cf. Lane 2, Fig. 7).} \]

\[ \text{A} \]

\[ \text{B} \]

\[ \text{9-11, 13, 16, A, and B) for two distinct transitions. (A) Light + DCMU to anaerobic state transition; (B) state I to state II transition. For each of these transitions, the absolute increase in phosphorylation for each polypeptide calculated from Table I, is normalized to the maximum one (polypeptide 11 and 13, respectively).} \]

\[ \text{efficient exogenous PSI acceptor has been added to the suspension.} \]

\[ \text{The inhibition of chlororespiration in vivo in anaerobic conditions, led to a complete reduction of the PQ pool in the dark. In a state I (+ DCMU) to anaerobic transition in vivo, the PQ pool underwent large redox changes, from a nearly fully oxidized state to a fully reduced state. This induced a decrease in maximum fluorescence yield that is more than three times larger than that in a conventional state I to state II transition. In these conditions, the extent of reorganization of the antenna between the two photosystems was comparable with that observed in vitro. For instance, Steinback et al. (21) have obtained a variation of the } \]

\[ \text{F}_{\text{max}}/F_{\text{ss}} \text{ ratio at } 77{\text{K}} \text{ from 1.67 to 0.91 using pea chloroplasts that were dark adapted or illuminated in the presence of LED. This compares well with the two times decrease in the } \]

\[ \text{F}_{\text{max}}/F_{\text{ss}} \text{ ratio that we observed in } \]

\[ \text{C. reinhardtii} \text{ for WT cells either preilluminated with far red light in the presence of DCMU or incubated anaerobically in the dark. In agreement with previous results of Owens and Ohad (20), we observed a striking similarity between the in vivo and in vitro patterns of phosphorylation of thylakoid polypeptides in } \]

\[ \text{C. reinhardtii}. \text{ However, at variance with these authors, we showed that this phosphorylation is a reversible process in vivo as well as in vitro. There was an extensive dephosphorylation of the phosphorylated polypeptides when the fully reduced PQ pool is oxidized either in vitro by incubation in the dark of preilluminated chloroplasts or in vivo during an anaerobic state to state I plus DCMU transition. There were only minor differences in the dephosphorylation patterns in vivo and in vitro; whereas polypeptide B got dephosphorylated in vivo upon oxidation of the PQ pool, it was still phosphorylated in vitro upon oxidation of the PQ pool. Several authors have pointed out a specific involvement of the PSII centers in the regulation process due to the reversible phosphorylation of the LHC (3, 18). Owens and Ohad (20) concluded from their analysis of the T44 mutant of } \]

\[ \text{C. reinhardtii} \text{ that no phosphorylation of LHC polypeptides occurs in the absence of PSII centers. The present study of the F34 mutant lacking in PSII centers, clearly shows that no such specific interaction between LHC and PSII centers is required to get either a reversible phosphorylation of LHC polypeptides or a reorganization of the antenna pigments. Besides the lack of PSII centers, the T44 mutant might then be defective in some other component of importance in the phosphorylation process. The fluorescence and phosphorylation changes observed in the F34 mutant are of even greater amplitude than in the WT. Mutants lacking in PSII still show a cation controlled light energy distribution (19, 23). Wollman and Diner (23) have previously shown that the target for the reorganization of the antenna induced by cations was located within the LHC. The present work shows that the target for the regulation of the light energy distribution controlled by the redox}\]
state of PQ pool in vivo is located between the LHC and PSI or within the LHC itself.

The authors gratefully acknowledge P. Bennoun, J. Lavergne, and P. Joliot for stimulating discussions in the course of this work and M. N. Mannevy for typing the manuscript. This work was supported by European Economic Community grant no. ESD-0177.

Received and accepted for publication 25 May 1983.

REFERENCES

1. Allen, J. F., and J. Bennett. 1981. Photosynthetic protein phosphorylation in intact chloroplasts. FEBS (Fed. Eur. Biochem. Soc.) Lett. 123:67-70.
2. Allen, J. F., J. Bennett, K. E. Steinback, and C. J. Arntzen. 1981. Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. Nature (Lond.). 291:25-29.
3. Barber, J. 1982. Influence of surface charges on thylakoid structure and function. Annu. Rev. Plant. Physiol. 33:261-295.
4. Bennett, J. 1977. Phosphorylation of chloroplast membrane proteins. Nature (Lond.). 269:344-346.
5. Bennett, J. 1979. Chloroplast phosphoproteins. Eur. J. Biochem. 99:133-137.
6. Bennett, J. 1979. Chloroplasts phosphoproteins. The protein kinase of thylakoid membranes is light independent. FEBS (Fed. Eur. Biochem. Soc.) Lett. 103:342-344.
7. Bennett, J. 1980. Chloroplasts phosphoproteins. Evidence for a thylakoid-bound, phosphoprotein phosphatase. Eur. J. Biochem. 104:85-89.
8. Bennett, J., K. E. Steinback, and C. J. Arntzen. 1980. Chloroplasts phosphoproteins: regulation of excitation energy transfer by phosphorylation of thylakoid membrane polypeptides. Proc. Natl. Acad. Sci. USA. 77:5253-5257.
9. Bennett, P. 1974. Correlation between states I and II in algae and effect of magnesium in chloroplasts. Biochim. Biophys. Acta. 368:141-147.
10. Bennett, P. B. A. Diner, F. A. Wollman, G. Schmidt, and N. H. Chua. 1981. Thylakoid polypeptides associated with Photosystem II in Chlamydomonas reinhardtii: comparison of PSI mutants and particles. In Photosynthesis III (G. Aoyama, ed.) Balaban Int. Sci. Serv. Philadelphia. 839-849.
11. Bennoun, P. 1982. Evidence for a respiratory chain in the chloroplast. Proc. Natl. Acad. Sci. USA. 79:4352-4356.
12. Bonaventura, C., and J. J. Myers. 1969. Fluorescence and oxygen evolution from Chlorella pyrenoidosa. Biochim. Biophys. Acta. 189:366-383.
13. Chow, W. S., A. Teller, D. J. Chapman, and J. Barber. 1981. State I state transition in leaves and its association with ATP induced chlorophyll-fluorescence quenching. Biochim. Biophys. Acta. 638:50-64.
14. Chua, N. H., and P. Bennoun. 1975. Thylakoid membrane polypeptide of Chlamydomonas reinhardtii wild-type and mutant strains deficient in Photosystem II reaction center. Proc. Natl. Acad. Sci. USA. 72:2175-2179.
15. Delepelaire, P., and N. H. Chua. 1981. Electrophoretic purifications of Chlorophyll a/b protein complexes from Chlamydomonas reinhardtii and spinach and analysis of their polypeptide compositions. J. Biol. Chem. 256:9300-9307.
16. Diner, B. A., and D. Maurerrell. 1973. Feedback controlling oxygen production in a cross-reaction between two photosystems in photosynthesis. Biochim. Biophys. Acta. 305:329-352.
17. Horton, P., J. F. Allen, M. T. Black, and J. Bennett. 1981. Regulation of phosphorylation of chloroplast membrane polypeptides by the redox state of the plastoquinone. FEBS (Fed. Eur. Biochem. Soc.) Lett. 125:193-196.
18. Horton, P., and M. T. Black. 1982. On the nature of the fluorescence decrease due to phosphorylation of chloroplast membrane proteins. Biochim. Biophys. Acta. 680:22-27.
19. Loew, K., and C. J. Arntzen. 1981. Cation mediated regulation of excitation energy distribution in chloroplasts lacking organized photosystem II complexes. Biochim. Biophys. Acta. 637:107-117.
20. Owens, G. C., and I. Ohad. 1982. Phosphorylation of Chlamydomonas reinhardtii chloroplast membrane proteins in vivo and in vitro. J. Cell. Biol. 93:712-718.
21. Steinback, K. E., S. Bose, and D. J. Kyle. 1982. Phosphorylation of the light harvesting chlorophyll-protein regulates excitation energy distribution between photosystem II and photosystem I. Arch. Biochem. Biophys. 216:356-361.
22. Williams, W. P. 1977. The two photosystems and their interactions. In Topic in Photosynthesis (Barber, J. ed.) Vol. 2, pp 99-147. Elsevier/North-Holland, Amsterdam.
23. Wollman, F. A., and B. Diner. 1980. Cation control of fluorescence emission, light scatter and membrane stacking in pigment mutants of Chlamydomonas reinhardtii. Arch. Biochem. Biophys. 204:646-659.
24. Wollman, F. A., and P. Bennoun. 1982. A new chlorophyll protein complex related to Photosystem I in Chlamydomonas reinhardtii. Biochim. Biophys. Acta. 680:325-360.