Role of the Cytochrome \( b_6-f \) Complex in the Redox-controlled Activity of \textit{Acetabularia} Thylakoid Protein Kinase*

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The regulation of the protein kinase activity responsible for the phosphorylation of the light-harvesting complex of photosystem II (LHCII) 27-kDa polypeptide involved in the State I-State II transitions in \textit{Acetabularia} thylakoids was investigated. The LHCII kinase of isolated thylakoids retains its activity in absence of light-driven electron flow or reductants added in the dark. However, the kinase is reversibly inactivated by addition of oxidants \textit{in vitro} or by far red (710 nm) light \textit{in vivo}. Inhibitors of the quinol oxidase site of the cytochrome \( b_6-f \) complex inactivate the LHCII kinase in the dark, and also in the light, or in presence of duroquinol when the plastoquinone pool is reduced. Inhibitors of the quinone reductase site of the \( b_6-f \) complex have practically no effect in the dark and stimulate the kinase activity in the light. Based on these data and on our previous report, showing specific loss of LHCII kinase activity in a \textit{Lemma} mutant lacking the cytochrome \( b_6-f \) complex (Gal, A., Shahak, Y., Schuster, G., and Ohad, I. (1987) \textit{FEBS Lett.} 221, 205-210), we propose that the activity of the LHCII kinase is regulated by the redox state of a cytochrome \( b_6-f \) complex component(s) which responds to the balance of electron flow from photosystem II via the plastoquinone pool to photosystem I.

The regulation of energy distribution between the two photosystems of oxygenic chloroplasts at limiting light intensity involves the phosphorylation of the chlorophyll \( a/b \) binding polypeptides forming the light-harvesting complex antenna of photosystem II (LHCII)\(^1\) (1-3). The LHCII kinase responsible for this phosphorylation is membrane bound, and it is activated when the plastoquinone pool is reduced either by light-dependent electron flow via PSII or in the dark, upon addition of duroquinol or dithionite (2, 4). Although the different aspects of the regulatory role of LHCII kinase have been extensively investigated (1-9), the exact mechanism of its activation by the reduced quinones is not yet completely understood.

It was previously suggested that a quinone-binding site might be involved in the process of activation of the LHCII kinase (10, 11). One of the basic tools for the elucidation of the mechanism of the LHCII kinase activation was the use of different quinone analogs as potential activators or inhibitors (1, 2, 10, 11). However, due to the fact that LHCII kinase activity cannot be elicited \textit{in vitro} without concomitant reduction of the PQ pool either by light-driven electron flow or by the addition of an appropriate reductant in the dark, the interpretation of the data obtained so far was rather difficult.

The observation that quinone analogs known to inhibit electron flow via the quinol-oxidizing site of the cytochrome \( b_6-f \) complex inhibited the LHCII kinase activity (10, 11), and our recent finding that the cytochrome \( b_6-f \) less mutant of \textit{Lemma perpusilla} does not phosphorylate LHCII but nevertheless exhibits redox-controlled kinase(s) activity of other PSII polypeptides (11), has pointed out the possibility that the cytochrome \( b_6-f \) complex might be involved in the activation of the LHCII kinase.

We have previously reported that the LHCII kinase of \textit{Prochloron}, a prokaryote symbiont with certain types of \textit{Acanthotaenia} of tropical seas, retains its activity in the dark incubated thylakoids in absence of electron flow or addition of reductants (12). However, the difficulty in obtaining sufficient biological material has prevented us from pursuing the study of this LHCII kinase system.

We have now found a similar LHCII kinase activity in the thylakoids of the green alga \textit{Acetabularia mediterranea}. The ability of this kinase system to phosphorylate the LHCII polypeptides in the dark offers a good experimental model for studying the activation mechanism of the LHCII kinase in general. In this work we describe the phenomenon of "dark" phosphorylation of LHCII polypeptides in \textit{A. mediterranea} and the effect of different herbicides and quinone analogs on this activity. The results of this work suggest that the LHCII kinase activity is regulated by the redox state of a cytochrome \( b_6-f \) complex component(s).

MATERIALS AND METHODS

Cell Growth and Preparation of Thylakoid Membranes—\textit{A. mediterranea} cells were cultivated at the Max-Planck-Institute for Cell Biology in Ladenburg (Federal Republic of Germany (F. R. G.)). The cells were grown in artificial sea water at 22 °C, using a 12:12 h light-dark regime (13). Batches of ~2000 cells in 2 liters of medium were sent to the Jerusalem laboratory by air carrier. The cells were gently handled throughout the transport (total delivery time ~7 h) and further kept under similar growth conditions for up to 2 weeks. Chloroplast and subsequent thylakoid membrane preparations were carried out according to Ref. 14. The membrane pellet was suspended in 50 mM sodium Tricine, pH 8.0, containing 5 mM MgCl\(_2\) (TM buffer), to 0.3-0.5 mg chlorophyll/ml, and stored on ice until use.
Chlamydomonas reinhardtii y-1 cells were grown and thylakoid membranes prepared as previously described (15).

When the isolated membranes of both types of cells were not used immediately, they were frozen in liquid nitrogen, and the frozen aliquots were kept at -80 °C for up to 2 months without loss of activity.

In Vitro Thylakoid Protein Phosphorylation—In vitro LHCII kinase activity was routinely assayed by incubating the thylakoid membrane (1-2 μg of chlorophyll/assay) in a reaction medium (final volume 100 μl) containing 50 mM Tris·Cl, pH 8.0, 10 mM MgCl2, 5 mM NaF, and 100 μM [γ-32P]ATP (5 Ci/mmol). Incubation was continued for 15 min at 25 °C, either in the light (100 W/m²) or in the dark. When indicated, 1 mM duroquinol was added to the dark incubations assay. The effect of the different inhibitors used was assayed by adding them to the phosphorylation incubation mixture, such that the final solvent concentration (either methanol or Me₂SO) did not exceed 2% (v/v).

Phosphorylation was stopped by rapid centrifugation of the assay mixture in a microufuge (Beckman Instruments, Inc.), and the membrane pellet was resuspended in electrophoresis sample buffer, and heated for 5 min at 80 °C prior to resolution of the thylakoid polypeptides by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, as described by Laemmli (16). Unless otherwise specified 14% polyacrylamide gels were run, using the midget electrophoresis unit LKB2050. The gels were stained with Coomassie Brilliant Blue R, dried, and exposed to x-ray film. For estimation of the LHCl phosphorylation, the stained LHCl polypeptide bands were excised from the dried gels, and Cerenkov radioactivity was counted. Estimation of total thylakoid polypeptide phosphorylation was carried out by drawing 50-pl samples from the phosphorylation incubation mixture onto Whatman 3MM paper discs, and the radioactivity of the 5% trichloroacetic acid-insoluble material was measured according to Bollum (17).

In Vivo Phosphorylation of Acetabularia Cells and Measurements of State Transitions—The cells were incubated for 24 h with [32P]orthophosphate (1.2 μCi/nmol) in the growth medium under normal light regime, prior to 30 min exposure to various illumination treatments as described below. White light was provided by cool fluorescent lamps (20 W/m²). Red or far red light was provided by a quartz halogen fiber light (Dolan Jenner Industries, Inc., Woburn, MA) passing through a 650-nm or 715-nm interference filter (Baird Atomic half-band width, 40 nm) giving incident light intensities of 30 and 20 W/m², respectively.

State 1-State II transitions monitored by the change in modulated chlorophyll fluorescence were measured according to Ref. 18. The algae were kept in growth medium throughout the measurement and retained their photosynthetic activity for several hours.

Fluorescence kinetics measurements were carried out as described before (12). For the estimation of the plastoquinone pool size, the area above the fluorescence induction curve of isolated membranes recorded in the absence of DCMU was compared to that of the same membranes recorded in the presence of DCMU.

Other Measurements—Chlorophyll was measured according to Arnon. (19), and protein concentration was determined by the method of Peterson (20).

The halogenated derivatives of 1,4-benzoquinones, tetrabromo-1,4-benzoquinone, 2,3-diodo-5-t-butyl-1,4-benzoquinone, and 2,3-dibromo-5-t-butyl-1,4-benzoquinone, were the generous gift of Dr. W. Oettmeier from the Ruhr University, Bochum (F. R. G.). Stigmatellin was kindly supplied by Dr. G. Hofle from Braunschweig University (F. R. G.). [γ-32P]ATP was purchased from Amersham Corp. (England).

RESULTS

In Vitro Phosphorylation of Isolated Thylakoids—Fig. 1 shows the phosphorylation pattern of the thylakoid membranes from A. mediterranea as compared to that of the green algae Chlamydomonas reinhardtii y-1 under different incubation conditions such as light, dark, or dark in the presence of duroquinol. As opposed to the phosphorylation pattern of Chlamydomonas thylakoids, the in vitro phosphorylation of Acetabularia LHCl polypeptides is light independent. Only the upper LHCl band (27 kDa) is phosphorylated when isolated Acetabularia thylakoids are incubated with [γ-32P]ATP in vitro.

Similarly to higher plants and green algae (21–23) some additional Acetabularia polypeptides besides the LHCl are phosphorylated as well. These include small molecular mass (~12–14 kDa) polypeptides, heavily labeled polypeptides in the 32–35-kDa region, and a 43-kDa polypeptide (Fig. 1). Like LHCl these polypeptides are also phosphorylated in the dark.

The kinetics of [32P] incorporation into the thylakoid polypeptides are presented in Fig. 2. The inset (Fig. 2) shows the time course of phosphorylation of the specific LHCl poly-
As can be observed, while in the light-incubated system the LHCII kinase is active from the onset of illumination, there is an apparent lag time of circa 3 min in the dark system until LHCII polypeptide phosphorylation can be detected. The kinetics of in vitro LHCII phosphorylation were also assayed in the dark in the presence of duroquinol. The phosphorylation kinetics in this case were similar to that of the light-incubated thylakoids and did not exhibit a lag period (data not shown).

In Vivo Phosphorylation of Acetabularia Thylakoids—To find out whether LHCII phosphorylation in the dark in absence of electron flow occurs also in vivo, the cells were incubated with [33P]orthophosphate as described under "Materials and Methods." As can be seen in Fig. 3, unlike the results obtained in vitro (Fig. 1), both LHCII polypeptides (27 and 26 kDa) were phosphorylated in vivo in the light and in the dark. The activation of the LHCII kinase in vivo in the dark could also be due to chlororespiration (24), and thus one cannot conclude that the same mechanisms operate in the dark in vivo and in vitro.

It has been demonstrated before that the oxidation of the plastoquinone pool following exposure of intact cells or plants to far red light (FR, 710 nm) inactivates the LHCII kinase, while illumination with red light (R, 650 nm) which causes the reduction of the plastoquinone pool, activates the LHCII kinase in vitro (25, 26). This process is associated with the phenomenon of state transition. When Acetabularia cells preincubated in the presence of [33P]orthophosphate in white light were exposed to red light (R, 650 nm) prior to exposure to far red light (FR, 710 nm), the phosphorylated 27-kDa LHCII band was dephosphorylated while the lower 26-kDa polypeptide band was not (Fig. 3). To ascertain that the red/far red light treatment induced state transitions, measurements of modulated fluorescence under similar light regime were carried out on whole cells. The results are presented in Fig. 4.

The fluorescence emitted by dark-adapted cells with onset of modulated red light (650 nm) decreases following continuous illumination for 10 min with added excess nonmodulated far red light I (710 nm, Fig. 4, 1). This decrease in fluorescence is ascribed to the increase in the linear electron flow due to the excitation of PSI by far red light (Emerson effect (27, 28)). The excess excitation of PSI causes a partial oxidation of the PQH2 pool and hence the inactivation of the kinase followed by the dephosphorylation of LHCII which can associate with PSII and increase its cross-section antenna (State I, (8, 18)). This is demonstrated by the increase in the fluorescence emission elicited by modulated red light upon removal of the far red light (Fig. 4, 2). The extent of the rise in fluorescence (∆F) normalized to the total fluorescence (∆F/F, Fig. 4) was 0.28. Continuation of illumination with red light (650 nm) for an additional 10 min (Fig. 4, 3) causes a decrease in the fluorescence due to the reduction of linear electron flow in absence of far red light, increase in the PQH2 pool and subsequent reactivation of the LHCII kinase. The phosphorylation of LHCII causes its dissociation from PSII correlated with a reduction in the cross-section of PSII antennae relative to PSI (State II, (8, 18)). Addition of excess nonmodulated far red light in this condition (Fig. 4, 4) does not significantly reduce the fluorescence emitted by PSII nor does the fluorescence increase when this light I is removed. The ratio ∆F/F in this case was only 0.073. The State I-State II transitions in Acetabularia have kinetics similar to those reported for intact leaves (8).

The Effect of Fe(CN)6 and Duroquinone on LHCII Phosphorylation—Since all steps of thylakoid preparation were carried out in room light prior to freezing and storage in the dark, one could consider that light exposure of the isolated thylakoids was sufficient to activate the kinase, thus enabling the enzyme to retain its activity during the subsequent dark incubation. The presence of a large plastoquinone pool which remains reduced in the dark could contribute to the persistence of the kinase activation in this case. To test this possibility, isolated thylakoids were preincubated in the dark at 25°C for up to 1 h prior to the assay of LHCII kinase in the dark, without apparent loss of activity (data not shown).

The size of the plastoquinone pool of isolated Acetabularia thylakoids estimated from fluorescence kinetics measurements was similar to that of thylakoids from higher plants (Lemma) or green algae (Chlamydomonas). The ratio of the area above the fluorescence induction curve in absence of DCMU to that in the presence of DCMU was about 8, in all cases. The light-reduced plastoquinone pool of isolated thylakoids was rapidly oxidized in the dark under aerobic conditions (t½, about 15 s).

The persistence of LHCII kinase activity in isolated thylakoids in the dark, in the absence of electron flow-dependent reduction of the PQ pool, or without addition of reduced PQ...
analogs could be explained if one assumes that reduced PQ might be bound to the Acetabularia LHCII kinase-activating system. In order to test this possibility, thylakoid membranes were incubated in presence of Fe(CN)₆⁻_{₄} under kinase assay conditions in the light or in the dark. It is clearly seen from Fig. 5B that in the presence of this oxidizing agent, LHCII phosphorylation in the light or in the dark is completely inhibited. The inhibition of LHCII kinase activity in the dark, following Fe(CN)₆⁻_{₄} treatment, was not reversed upon washing out the Fe(CN)₆⁻_{₄}. However, LHCII polypeptides could be phosphorylated after removal of Fe(CN)₆⁻_{₄} when the thylakoids were incubated in the light, or in the dark in presence of duroquinol (Fig. 5B, lanes L, D, DQH₂, FeCy washed). When a similar experiment was carried out in the presence of oxidized duroquinone the dark activation was abolished, while light-activated LHCII kinase was not affected. On the other hand, after attempted removal of duroquinone by extensive washings, the dark activation was significantly restored (Fig. 5A).

Inhibition of LHCII Kinase Activity by Various Quinone Analogs—To test the possibility that the secondary quinone-binding site of photosystem II, the Q₉ site, might be involved in the regulation of LHCII kinase activity in the dark, the effect of the Q₉ sitespecific inhibitors Atrazine and DCMU in the light, dark, or dark with addition of duroquinone was assayed. The results of these experiments (Fig. 6) show that Atrazine and DCMU inhibit the activity of the LHCII kinase in the light at concentrations similar to those required to inhibit light-dependent electron flow in Acetabularia (data not shown). However at least a 10-fold increase in the concentration of these herbicides was required to cause the inactivation of LHCII kinase activity in the dark. This activity could not be inhibited in the dark when tested in the presence of duroquinol unless DCMU and Atrazine concentration were further raised to the millimolar range.

Thus, the inhibitors of the Q₉ site of PSII do not appear to specifically inhibit the Acetabularia LHCII kinase system. The effect of DCMU or Atrazine in the light might simply arise from the light-dependent oxidation of the electron chain due to the activity of PSI while its reduction by PSII is inhibited.

The quinone analog DBMIB is known to inhibit photosynthetic electron flow at two different sites. At 0.1–0.5 μM DBMIB interacts directly with the Rieske protein of the cytochrome b₅₆₋f complex, thus blocking electron flow from PQH₂ to PSI (29). Indeed, addition of DBMIB at 2–5 × 10⁻⁷ M did not inhibit the light-dependent reduction of the plastoquinone pool in isolated Acetabularia thylakoids, whereas it inhibited electron flow from duroquinol to methyl viologen over 90%. At higher concentrations (5–10 μM), DBMIB blocked electron flow via PSI as well (30). Thus, it was of interest to assess the effect of DBMIB on LHCII phosphorylation under the various assay conditions.

Fig. 7A shows that indeed DBMIB inhibits LHCII kinase activity. Its inhibitory effect in the light (apparent KIₓₒ, 5 μM) corresponds with data previously reported for higher plants (2). However, the inhibition of LHCII kinase by DBMIB in the dark, with an apparent KIₓₒ, 0.1 μM, resembled its inhibitory effect on the cytochrome b₅₆₋f complex (29). In the presence of 1 mM duroquinol, the inhibition curve is shifted toward an apparent KIₓₒ of 10 μM (Fig. 7A). This shift could be due to a competition on the same quinone-binding site. Since DBMIB can be reduced by electron flow or by addition of duroquinol, the effect of chemically reduced DBMIB was also assayed. No difference could be detected in the inhibition curve of LHCII kinase when reduced DBMIB was used (data not shown).

The nonreducible quinone analog DNPINT, an inhibitor of cytochrome b₅₆₋f complex at the quinol oxidase site (29, 30) which interacts with the Rieske protein in a way apparently different from DBMIB (31) was tested as well. As opposed to DBMIB, DNPINT inhibited LHCII kinase activity under all experimental conditions with a similar apparent KIₓₒ of 8 μM.

The data so far presented indicated a similarity between the site(s) involved in the regulation of the LHCII kinase and the cytochrome b₅₆₋f complex. For further characterization of the activity of Acetabularia LHCII kinase system, the effect of other inhibitors known to interact with specific components of the cytochrome b₅₆₋f complex, as well as that of additional halogenated quinone analogs reported to inhibit electron flow via this complex to PSI (32) was also tested. The inhibition of LHCII kinase by all the above compounds was assessed at an inhibitor concentration of 1 μM. The results (Table I) show a strong inhibition of the kinase activity in the dark by the
the presence of an LHCII kinase system in Acetabularia thylakoids is inhibited if the electron transfer chain is interrupted, as shown in Fig. 6. Inhibition of LHCII phosphorylation by various inhibitors of the electron transfer chain is presented in Table I. In the dark, LHCII kinase activity was inhibited by DBMIB and other halogenated quinone analogs with K\textsubscript{D} values in the range of 0.1-1 \mu M (36). Thus, the light-driven reduction of the PQ pool for DBMIB and the reduced quinones for the same binding site. Duroquinol was reported to interact directly with the quinone oxidase site of the cytochrome b\textsubscript{6-f} complex (36). The activity of the Acetabularia kinase in the dark is in agreement with these findings.

**DISCUSSION**

The experimental results obtained in this work demonstrate the presence of an LHCII kinase system in Acetabularia thylakoids which, unlike other LHCII kinase systems of higher plants and algal thylakoids, retains its activity in vitro in the dark in absence of added reductants. However, the activity of this kinase like that of other LHCII kinases described can be inhibited if the electron transfer chain is oxidized in vitro by addition of relatively strong oxidants (Fe(CN)\textsubscript{6}) or in vivo by far red light. Furthermore, the Acetabularia kinase system is inactivated in the dark by inhibitors of the quinol oxidase (Q\textsubscript{o}) but not by those of the quinone reductase (Q\textsubscript{r}) sites of the cytochrome b\textsubscript{6-f} complex. Taken together, the above data and our recent findings showing that the LHCII kinase of L. perpusilla wild type thylakoids reacts in a similar way to cytochrome b\textsubscript{6-f} inhibitors while it is inactive in thylakoids of a mutant lacking the cytochrome b\textsubscript{6-f} complex (11) prompted us to reconsider the accepted schemes ascribing a direct role of activating the LHCII kinase to the reduced plastoquinone pool. We propose here as a working hypothesis that the activation of this kinase system is mediated by the redox state of a cytochrome b\textsubscript{6-f} complex component which in turn responds to the oxidation/reduction state of the plastoquinone pool (Scheme 1).

The inhibition of LHCII kinase by DBMIB while the plastoquinone pool is reduced by light-driven electron flow or in the presence of duroquinol is not consistent with a direct interaction between the FQH\textsubscript{o} pool and the LHCII kinase which, however, might be mediated via the cytochrome b\textsubscript{6-f} complex. The K\textsubscript{D} value for DBMIB under the above conditions was higher (5-10 \mu M) than that reported for the binding of this inhibitor to the quinol oxidase site of the cytochrome b\textsubscript{6-f} complex (0.1-1 \mu M (29)). This could be due to the competition between DBMIB and the reduced quinones for the same binding site. Duroquinol was reported to interact directly with the quinol oxidase site of the cytochrome b\textsubscript{6-f} complex (36). The activity of the Acetabularia kinase in the dark in absence of added reductants is indeed inhibited by DBMIB and other halogenated quinone analogs with an K\textsubscript{D} of 0.1-1 \mu M. Thus, the light-driven reduction of the PQ pool required for the activation of LHCII kinase may be needed to reduce the cytochrome b\textsubscript{6-f} complex. Inhibition of electron flow via the quinone reductase site by NQNO or Antimycin A (37) enhanced the activation of LHCII kinase in the dark (Table I), or in the dark with addition of DQH\textsubscript{2} (not shown).

Under these conditions both NQNO and Antimycin A can promote the reduction of the cytochrome b\textsubscript{6-f} by blocking the quinone-reducing site and thus its reoxidation (Scheme 1). It has been reported before that the mid-point potential

**TABLE I**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Activity, % of control</th>
<th>Reported site of interaction</th>
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<tbody>
<tr>
<td>Antimycin A</td>
<td>165 (Light) 100 (Dark)</td>
<td>Quinone reductase site</td>
</tr>
<tr>
<td>NQNO</td>
<td>140 (Light) 70 (Dark)</td>
<td>Quinone reductase site (low conc.)</td>
</tr>
<tr>
<td>Bromamid</td>
<td>40 (Light) 40 (Dark)</td>
<td>Rieske Fe-S protein</td>
</tr>
<tr>
<td>DIBB</td>
<td>34 (Light) 5 (Dark)</td>
<td>Not identified, possibly the Q\textsubscript{r} site</td>
</tr>
<tr>
<td>DDBB</td>
<td>40 (Light) 10 (Dark)</td>
<td>Possibly the Q\textsubscript{r} site</td>
</tr>
<tr>
<td>Stigmatellin</td>
<td>100 (Light) 75 (Dark)</td>
<td>Rieske Fe-S protein</td>
</tr>
</tbody>
</table>

(Ref. 33, 37)
Regulation of the LHCII Kinase Activity by Cytochrome b\(_6\).f

This activation cannot be ascribed to the plastoquinone pool since the pool is rapidly oxidized in the dark (20–40 s) while the active state is maintained in the dark for at least 1–2 h.

Preliminary results also indicate that the LHCII phosphorylation activity in the dark can be significantly reduced following preincubation of isolated *Acetabularia* thylakoids in 710 nm light, or under white light illumination with addition of methyl viologen and a low concentration of DCMU (not shown).

The inactivation of the LHCII kinase in the dark by DBMIB and other quinone analogs might arise from a reversible change in the interaction of the reduced putative component of the complex or its displacement when the quinol oxidase site is occupied by these inhibitors. This possibility is suggested by the observation that the inhibition of the kinase in the dark by duroquinone is reversed upon removal of the inhibitor without requirement for further addition of reductants or light-dependent reduction of the PQ pool. Thus, it is our contention that the results obtained with the *Acetabularia* LHCII kinase lend support to the view that the reduction of cytochrome b\(_6\).f complex and possibly its cytochrome b\(_6\) subunits is involved in the activation of the LHCII kinase as proposed in Scheme 1. The alternative possibility that the Rieske Fe-S center on cytochrome f might be the subunits involved in this process should also be considered. In our view this possibility is less favored since the \(E_m\) values of these components are too positive (±290 mv (37)). Moreover, the addition of NQNO or Antimycin A which should enhance the oxidation of these subunits in the light and thus decrease the kinase activity stimulates LHCII phosphorylation by 40–60%.

The 27-kDa polypeptide of LHCII, which is phosphorylated in the dark both in *vitro* and *in vivo*, is affected by the inhibitors of the cytochrome b\(_6\).f complex and appears to be involved in the state transition phenomenon. This conclusion is based on the specific dephosphorylation of this polypeptide in *vitro* following exposure of the cells to 710 nm light, under conditions which elicit state transition as demonstrated by the modulated fluorescence measurements. Differential phosphorylation of the LHCII polypeptides involved in state transition has also been reported in higher plants (42, 43).

The scheme involving the redox state of the cytochrome b\(_6\).f complex as a regulatory factor in the activation of the LHCII kinase and the resulting state transitions as proposed here has the advantage of relating these phenomena to the regulation of the cyclic/linear electron flow ascribed to the redox state of the cytochrome b\(_6\).f complex. The possibility that this regulation is mediated by this complex via a transition from a dimeric form supporting linear electron flow to a monomeric form favoring cyclic electron flow has been recently proposed by Cramer *et al.* (37).

Since the inhibitors used in this work are mostly quinone analogs one should also consider that the LHCII kinase might be activated by a direct interaction between a reduced quinone and a regulatory site (subunit) of the enzyme itself which is not a component of the cytochrome b\(_6\).f complex. While this possibility cannot be excluded, it does not seem likely in view of the similarity of the pattern of response to the various inhibitors of the cytochrome b\(_6\).f complex and the dark activity of the LHCII kinase. Furthermore, while this paper was being prepared similar results and conclusions concerning the effect of inhibitors of the quinol oxidase and quinone reduc- tase sites of the cytochrome b\(_6\).f complex on the redox-activated LHCII kinase of spinach have been communicated to us by J. Bennett and co-workers (44). These authors have also found that in a maize mutant lacking the cytochrome b\(_6\).f

**Scheme 1.** The LHCII kinase is activated when electron flow from photosystem II to the cytochrome b\(_6\).f complex exceeds the flow from this complex to Photosystem I (650 nm light ⇒ 710 nm light, State II). Under these conditions, the plastoquinone pool and the cytochrome b\(_6\).f are reduced. Excess electron flow from the cytochrome b\(_6\).f complex to PSI (710 nm light ⇒ 650 nm light, State I) causes oxidation of the plastoquinone and the cytochrome b\(_6\).f complex and results in the inactivation of the LHCII kinase; \(\uparrow\) sites of action of quinone reductase (Antimycin, NQNO) and quinol oxidase (DBMIB, DNPNT, Stigmatellin) inhibitors, respectively; \(\uparrow\) predominant electron flow; \(\downarrow\) light arrow, cyclic electron flow; \(\box up\) dashed box, plastoquinone pool.

Values for the activation of the LHCII kinase closely resemble those reported for the reduction of the PQ pool (\(a = 2, E_m = 0–80\) mv (38–40)). This could argue against the cytochrome b\(_6\).f complex as being involved in the kinase activation since all the subunits of this complex are one electron carriers and the \(E_m\) values of the cytochromes b\(_6\) and b\(_6\) are more negative while those of the Rieske Fe-S and cytochrome f are much more positive (±290 mv (37)). However, one should point out that the titration curve for the activation of LHCII kinase could fit also with a value of \(n = 1\) (40). In view of the fact that \(E_m\) values for the b\(_6\) cytochromes reported by various workers cover the range from −146 mv to +57 mv and values of \(n = 2\) have been observed (37), one cannot exclude these cytochromes as being involved in the process of LHCII kinase activation.

The involvement of cytochrome b\(_6\) subunits of the cytochrome b\(_6\).f complex in this process seems to be consistent with the data concerning the activation of LHCII kinases requiring addition of reductants or their generation in situ as depicted in the above scheme. The question arises however whether this scheme is also consistent with the observation concerning the activity of the *Acetabularia* LHCII kinase presented here.

The particular behavior of the *Acetabularia* LHCII kinase could be explained if one considers that the cytochrome b\(_6\).f complex of this organism once reduced retains this state for prolonged periods of time or binds in a stable way a reduced component, possibly a quinol molecule(s). Presence of bound quinone(s) in the cytochrome b\(_6\).f complex exhibiting \(E_m\) values of 40–60 mv has been considered (37, 41). The loss of *Acetabularia* thylakoids LHCII kinase activity in the dark following treatment with Fe(CN)\(_6\) is consistent with the presence of a reduced component involved in the activation of this enzyme in the dark.
f complex the LHCII kinase is inactive as previously reported by us for a * Lemma* mutant (11). Involvement of the cytochrome *b6* complex in the activation of LHCII kinase of *Chlamydomonas* was also reported by Wolman and co-workers (45). The finding of a permanently active LHCII kinase system which, however, responds to the alteration of the cytochrome *b6* complex redox state adds support to the concept that the regulation of LHCII kinase activity is mediated via the redox state of a cytochrome *b6* complex component, possibly one of the cytochrome *b6* subunits.

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