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Studies on kinase-controlled state transitions in Photosystem II and b\textsubscript{6}f mutants from Chlamydomonas reinhardtii which lack quinone-binding proteins

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We have studied kinase-dependent state transitions in vivo using photosynthetic mutants from the green alga Chlamydomonas reinhardtii lacking in quinone-binding proteins. The aim of our study was to identify proteins involved in the plastoquinone-dependent activation of the LHC-kinase. Whereas mutants totally devoid of the quinone-binding subunits D1 and D2 of Photosystem II showed unaltered state transitions, mutants lacking the b\textsubscript{6}f complexes were incapable of state transitions. These mutants were blocked in State 1, which is indicative of either the absence of the LHC-kinase responsible for the regulation, or of the loss of a component responsible for the activation of this enzyme. These two hypotheses are discussed in light of (i) the patterns of phosphorylation of the thylakoid membrane proteins observed in the b\textsubscript{6}f mutants and (ii) the characteristics of the kinase activities recovered from their thylakoids.

Introduction

Since the first report that state transitions in higher plants were associated with a reversible phosphorylation of some of the LHC subunits [1], several kinases have been identified and purified from spinach thylakoid membranes [2–5]. The LHC-kinase activity in higher plants as well as in green algae has been reported to be regulated in situ by the redox state of the plastoquinones [1,6] which carry electron transport from Photosystem II to cytochrome b\textsubscript{6}f complexes in the photosynthetic electron transfer chain. The molecular mechanism by which this regulation process occurs remains largely unknown and no plastoquinone-dependent activity has as yet been reported for any of the thylakoid kinases that have been purified.

A possible association between a kinase and an integral membrane protein containing plastoquinone-binding sites, with different affinities depending on the redox state of the molecule, would elicit an interaction between a kinase catalytic site and this regulation site. We investigated this possibility using photosynthetic mutants from the green algae Chlamydomonas reinhardtii, lacking in either of the quinone-binding proteins identified so far in the thylakoid membranes. These include PS II mutants totally devoid of the
quinone-binding subunits D1 or D2 [7,8] and mutants lacking in cytochrome b$_{6}$f complexes [9].

A preliminary report of this work has been published in Ref. 10.

**Material and Methods**

Wild-type and mutant strains of *C. reinhardtii* were grown in Tris-acetate/phosphate medium under continuous illumination (300 lx) using cool fluorescent light.

The F34 mutant lacks the PS II centres but still shows membrane insertion of D1 and D2 polypeptides [11]; FUD7 and FUD47 mutants lack the PS II centres and are totally devoid of D1 and D2 polypeptides, respectively [7,8]. F18 and FUD4 mutants lack cytochrome b$_{6}$f complexes [9]. ac21 and ac208 mutants lack the Rieske protein and plastocyanin, respectively [12,13].

ac21F34 and ac208F34 double mutants were obtained by crossing the corresponding nuclear single mutants according to Ref. 14. Tetrads were dissected and double mutant clones, screened by fluorescence as PS II mutants [15], were selected out of nonparental ditype tetrads. FUD7F18 double mutant was obtained by crossing the FUD7 chloroplast mutant, mt$^+$, by the F18 nuclear mutant, mt$^-$. Double mutants were selected after checking cytochrome deficiencies - by TMBZ staining after gel electrophoresis [16] - in whole cells taken from the four clones of a tetrad with a PS II-deficient phenotype.

A typical experiment devised to compare the amount of $^{32}$P incorporated in thylakoid polypeptides from cells having a plastoquinone pool oxidized (analogous to state 1) or reduced (analogous to state 2) was as follows: 200 ml of cells in midexponential phase (3.10$^6$ cells/ml) were resuspended in Tris-acetate at a chlorophyll concentration of 25/~g/ml and incubated in dim light for 120 min in the presence of $^{32}$P (200 $\mu$Ci/$\mu$mol) at 1 $\mu$Ci/ml. Cells were then washed in 5 mM Hepes/0.3 M sucrose/10 mM EDTA and resuspended in the same buffer at a chlorophyll concentration of 50 $\mu$g/ml. Half of the sample was placed in the anaerobic state by an incubation in a tightly capped vessel in the presence of 2 mg/ml glucose oxidase and 20 mM glucose. The other half was placed in a large flask under strong agitation in order to maximize the air/liquid medium interface. These anaerobic and aerobic treatments were performed in the dark for 40 min. Cells were then rapidly broken in a pre-cooled French press at 35 MPa in the presence of 10 mM NaF and 200 $\mu$M PMSF. Thylakoid membrane preparations, gel electrophoresis and autoradiography were then performed as previously described [6].

Fluorescence induction experiments at room temperature were performed as previously described [17] using intact cells pretreated as for $^{32}$P-labelling experiments, at a concentration of 0.4 $A_{680\text{ nm}}$.

Stationary labelling of whole cells was performed under 300 lx illumination by adding [14C]acetate (1 $\mu$Ci/ml) to cultures at $10^6$ cells/ml. Cultures were harvested after seven cell divisions (2 · $10^6$ cells/ml) and processed for thylakoid purification.

For $K_m$ determination, purified thylakoid membranes (200 $\mu$g chlorophyll/ml) were resuspended in a reaction mixture containing 20 mM Hepes (pH 7.5)/0.1 M sucrose/10 mM NaCl/10 mM MgCl$_2$/2.5–500 $\mu$M [$\gamma$-$^{32}$]ATP (50 $\mu$Ci/$\mu$mol). The reaction was continued for 2 min in the light at room temperature. The incubation time was chosen in the time-range of linear 32p incorporation in the thylakoid membranes. The reaction was terminated by a 10-fold dilution with a chilled Mg$^{2+}$-depleted Hepes buffer containing 10 mM NaF and 2 mM unlabelled ATP. After centrifugation, the membrane pellet was washed twice in the same buffer, resuspended in a small volume of Hepes buffer (500 $\mu$g chlorophyll/ml) and Cerenkov radioactivity was counted.

Preparation of kinase-enriched fractions was performed using a modification of the procedure for CF$_1$CF$_0$ extraction developed by Pick and Racker [18] which we have already applied to the preparation of b$_{6}$f complexes [9]. After octylglucoside/cholate membrane solubilization and ammonium sulphate fractionation of the crude extract, the 37%–48% fraction was subjected to sucrose gradient centrifugation. Gradient fractions (100 $\mu$l) were then assayed for kinase activity according to Lin et al. [2] in the presence of 100 $\mu$M [$\gamma$-$^{32}$P]ATP (200 cpm/pmol) using 500 $\mu$g casein or lysine-rich histone-III as a substrate.
The cytochrome content in the gradient fractions was estimated spectrophotometrically as previously described [9].

Results

We first compared the extent of fluorescence quenching induced, at room temperature, by the reduction of the plastoquinone pool in three strains deficient in PS II centres, but showing (F34) or not (FUD7 and FUD47) membrane insertion of either of the two PS II quinone-binding subunits, D1 and D2. These differences in polypeptide insertion have been described previously [7,8,11]. Independent of their content in D1 and D2, these PS II mutants had a lower fluorescence yield in the anaerobic state (Table I, columns 1–3), as expected for cells placed in State 2 [6].

Quinone-binding sites also exist on the \(b_{6}f\) complex. Therefore we looked for the redox control of the plastoquinone pool on the fluorescence yield at room temperature in strains lacking this complex. The comparison of the fluorescence yields at \(F_{\text{max}}\) under reducing conditions expressed as a function of that in oxidizing conditions in strains having or not the \(b_{6}f\) complex are shown in Table I, columns 3–6. The fluorescence decrease at \(F_{\text{max}}\) observed in the wild type in anaerobic conditions is not observed in the FUD4 chloroplast mutant lacking the \(b_{6}f\) complex. The \(F_{\text{max}}\) in the mutant remains at about the same level as it is in aerobic cells in the wild type (see values between brackets in Table I), which is characteristic of State 1. A similar observation arises from the comparison of the fluorescence yields in PS II mutants containing (FUD7) or not containing (FUD7F18) the \(b_{6}f\) complex.

The efficiency of reduction of the plastoquinones by the anaerobic treatment in \(b_{6}f\) mutants has been assessed previously [21] and causes a small increase at \(F_{\text{max}}\) (Table I, columns 4 and 5) due to the lower chemical quenching of the plastoquinones in their reduced form [20].

Thus, independent of the nuclear – in the case of F18 – or chloroplast – in the case of FUD4 – origin of the mutation, strains lacking the \(b_{6}f\) complex do not display the fluorescence changes at room temperature which were to be expected from State 1–State 2 transitions.

We then looked for a possible absence of LHC phosphorylation in vivo in \(b_{6}f\) mutants. The autoradiogram in Fig. 1 clearly shows that a number of thylakoid polypeptides were still phosphorylated in these mutants. The four PS II polypeptides which were phosphorylated in the wild-type cells [21] were still phosphorylated in the FUD4 mutant. These include the 47 kDa apoprotein of CPIV, analogous to the 43 kDa from PS II in higher plants, the quinone-binding polypeptide, D2, and the two small subunits, L5 and L6, in the 10 kDa region. These four bands were absent, as expected, in the two strains lacking PS II centres, strains FUD7 and FUD7F18. The other labelled bands in the wild type were as previously described [21], four LHC subunits (numbered 10, 11, 13 and 17 [22]) and a 40 kDa chlorophyll-binding polypeptide, numbered 9, the function of which is unknown. Two of these bands were no longer phosphorylated in the \(b_{6}f\) mutants (FUD4 and FUD7F18) and correspond to the two LHC subunits 13 and 17.

The increase in \(32\)P-labelling upon reduction of the PQ pool in cells from the wild-type or the FUD7 mutant was no longer observed in the two

| \(F_{\text{max}}\) IN THE ANAEROBIC STATE (STATE 2) IN STRAINS DEFICIENT IN PS II AND \(b_{6}f\) COMPLEXES |
|---|---|---|---|---|
| \(-\text{PS II}-\) | \(-\text{PS II}-\) | \(-b_{6}f\) | \(-b_{6}f\) | Wild type |
|  | \(F_{\text{max}}\) (100) in the aerobic state (State 1). |  |  |  |
| \(F_{34}\) | FUD47 | FUD7 | FUD7F18 | FUD4 | FUD7F18 |
| (1) | (2) | (3) | (4) | (5) | (6) |
| 54 | 66 | 52 | 108 | 104 | 70 |
| \(-\) | \(-\) | \(-\) | \(-\) | \((46/48)^{a}\) | \((46.5/32.5)^{a}\) |

\(a\) \(F_{\text{max}}\) values (arbitrary units) in (State 1/State 2) at a cell concentration of 0.4 \(A_{680nm}\) in wild type and FUD4 strains.
$b_6f$ mutants. The amount of labeling remained low under aerobic and anaerobic conditions. This is consistent with a block in State 1 in the latter mutants, as already suggested by their high fluorescence yield at room temperature.

That mutant strains lacking in $b_6f$ complexes were blocked in State 1 suggested either an absence of the LHC-kinase or the loss of its activation site.

In Fig. 2 is an autoradiogram of the polypeptide patterns from thylakoid membranes purified from FUD4 and wild-type cells grown in the presence of $[^{14}C]$acetate. Besides the deficiencies in $b_6f$ complex subunits previously reported in the FUD4 mutant [9], we noted a deficiency in a 40 kDa polypeptide (arrow on Fig. 2). We were unable to detect this deficiency using conventional staining procedures such as Coomassie blue staining or silver staining. The deficiency in a poor stainable 40 kDa polypeptide in $b_6f$ mutants might have been indicative of a possible association between a kinase and $b_6f$ complexes. We therefore looked
for kinase activity in b6f complex preparations. In Fig. 3 are shown the distributions of b6f complexes and kinase activities in the gradient used in the last step of the purification procedure. The fractions showing enrichment in kinase activity stood at lower sucrose density than the b6f complexes. Moreover, kinase activity was still recovered from similar gradient fractions in a preparation using thylakoid membranes from the FUD4 mutant lacking the b6f complex. In both cases the activity with histone-III as a substrate was only 10% that obtained with casein. The respective kinase activities extracted from the two strains were compared in several ways, using casein as a substrate (Table II); the activity recovered from FUD4 membranes was only 36% of that recovered from the wild type, on a chlorophyll basis. However this difference had to be corrected for possible variations in the yield of the preparation between the two strains. This was assessed by comparing the relative yields of recovery of CF1-CFo complexes which were in similar amounts in the starting membranes; the fractions containing the complex were pooled and run on an SDS-gel. We then scanned the Coomassie blue stained bands corresponding to the α and β subunits, and observed that the yield of recovery of CF1 was 1.7-times smaller in the FUD4 than in the wild-type preparation. The kinase activity recovered from the FUD4, when corrected by this yield parameter, measured up to 60% that recovered from the wild type.

Also shown in Table II is a comparison of the specific activities in fractions showing maximal kinase enrichment in the two strains. That from the FUD4 mutant was about 60% that from the wild type, an estimation which is consistent with the comparison of the total kinase activities extracted from the two strains. We note in Fig. 3 that the distribution of kinase activity in the b6f mutant was shifted slightly toward lower densities as compared to that in the wild type. When compared with the wild-type fraction of same density, the FUD4 fraction with maximal kinase enrichment displayed no significant decrease in specific activity. Therefore, the possibility that one type of kinase only (enriched in fractions 15–19 in the wild type on Fig. 3) would be missing from the FUD4 preparation is consistent with these mea-

<table>
<thead>
<tr>
<th>Strain</th>
<th>Specific activity (pmol P_i transferred/mg protein per min)</th>
<th>Kinase activity mg Chl solubilized (relative units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>max. fraction 20 uncorrected corrected</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>122 (100)* 79 (100)*</td>
<td>100 100</td>
</tr>
<tr>
<td>FUD4</td>
<td>69 (56)* 69 (87)*</td>
<td>36 62</td>
</tr>
</tbody>
</table>

* Relative units with respect to the wild-type specific activity (100).
measurements. However, the kinase activities in these fractions were highly unstable, and particularly sensitive to freezing (respective specific activities on a same wild-type sample before and after storage at \(-80^\circ\)C were 457 and 122 pmol P\(_i\)/mg protein per min). The 40% kinase deficiency in the mutant should then be taken as indicative only. The diversity in polypeptide content in the kinase-enriched fractions prevented a reliable attribution of the kinase activity to a particular silver-stained band (gels not shown).

We also studied the \(K_m\) for ATP of the total phosphorylation process in the FUD4 and wild-type thylakoid membranes. These experiments were performed using purified thylakoid membranes incubated in the light (PQ pool reduced) with different concentrations of \([\text{\textsuperscript{32}P}]\)ATP, from 2.5 to 500 \(\mu\)M, for 2 min. These experimental conditions aimed at activating the LHC-kinase in the wild type so as to produce maximal contrast in kinase activity with that in FUD4. The data plotted as \(V\) vs. \(V/S\) (Eadie-Hofstee representation) in Fig. 4 indicated more than one phosphorylation process in the thylakoid membranes from the two strains. These data could be fitted by two \(K_m\) values for ATP in each case. A 50 \(\mu\)M \(K_m\) was found in the wild-type as well as in the FUD4 thylakoid membranes. Computation of the value of the second \(K_m\) was highly dependent on the choice of graphic representation. Nevertheless, a small but significant difference was consistently observed, with the \(K_m\) computed for FUD4 mem-

**TABLE III**

\(F_{\text{max}}\) IN THE ANAEROBIC STATE (STATE 2) IN PS II MUTANTS WITH VARIOUS BLOCKS AT THE \(b_{6}/f\) LEVEL

\(F_{\text{max}}\) is expressed as a function of \(F_{\text{max}}\) (100) in the aerobic state (State 1).

<table>
<thead>
<tr>
<th>FUD7</th>
<th>ac21F34</th>
<th>ac208F34</th>
</tr>
</thead>
<tbody>
<tr>
<td>No block</td>
<td>(Q_{b}) blocked (^{a})</td>
<td>(Q_{c}) blocked (^{b})</td>
</tr>
<tr>
<td>52</td>
<td>50</td>
<td>62</td>
</tr>
</tbody>
</table>

\(^{a}\) Blocked with 30 \(\mu\)M DNP-INT.

\(^{b}\) Blocked with 2 \(\mu\)M NQNO.
We then investigated the possible involvement of the Rieske protein which is likely to participate in the \( Q_i \) site, and that of plastocyanin which binds to the \( b_{6f} \) complex. This was examined using mutants lacking in the Rieske protein, as in the F34ac21 strain [12], or lacking in plastocyanin, as in the F34ac208 strain [13]. We observed a quenching in fluorescence (Table III) and an increase in \(^{32}\)P-labelling (Fig. 5) in these strains upon aerobic to anaerobic transitions in darkness. These results clearly show that neither of the two proteins was responsible for the block observed in \( b_{6f} \)-deficient mutants.

**Discussion**

The respective abilities of photosynthetic mutants, blocked at different points of the electron transport chain, to perform the kinase-regulated changes in light energy distribution between the two photosystems, were studied in vivo in the green alga, *C. reinhardtii*. This process had to be studied using state transitions that were not to be driven by a photosynthetic electron flow. Therefore we compared aerobic (PQ pool oxidized) versus anaerobic (PQ pool reduced) conditions in darkness. The latter condition has been previously shown to induce State 2 in *C. reinhardtii* in vivo [6,7]. State 1, reached under aerobic conditions in darkness, compared well with that obtained previously by an illumination in the presence of DCMU [6,7], as shown by the amplitude of the changes in LHC phosphorylation and in fluorescence obtained in the present experimental conditions (about 30 and 50% quenching in State 2 in the wild-type and the F34 mutant, respectively).

Several reports have suggested a specific involvement of PS II centres in the regulation mediated by the LHC-kinase. These included the possibility that PS II centres would either diffuse laterally in association with phosphorylated LHC [25], control LHC movements through electrostatic repulsion due to a phosphorylated PS II component [26], contain a kinase [27] or its activation site on quinone-binding subunits as considered in the present paper. Previous studies using the F34 mutant from *C. reinhardtii*, lacking in PS II centres, gave evidence for an unaltered kinase-mediated regulation process [6,21]: this strain underwent a re-
versible association of LHC with PS I centres, depending on the phosphorylation state of LHC.

However, the F34 mutant still showed membrane insertion of the quinone-binding PS II subunits, D1 and D2 [11]. These could still be part of the kinase activation process observed in the mutant. The present analysis, showing identical behaviour in the F34 mutant and in two other PS II-deficient strains mutated respectively in the genes of D1 or D2 [7,8] and totally lacking in either of the two polypeptides, demonstrated that PS II has no part in the regulation mediated by the LHC-kinase. In particular, the absence, in such mutants, of the phosphorylated PS II subunits, L5 and L6, which are the probable counterparts of the 9–10 kDa phosphoprotein of PS II from higher plants, argues against the hypothesis of a specific involvement of this PS II subunit in the regulation process [26].

The absence of the b6f complex prevented the occurrence of State 1–State 2 transitions in vivo and greatly modified the phosphorylation pattern of thylakoid membrane polypeptides. These observations could be made irrespective of the presence (FUD4) or absence (F18F34) of PS II centres. We note that two different mutations, leading to the deficiency of the entire set of b6f subunits [9], but located on the nuclear (F18) and plastid (FUD4) chromosomes, respectively, similarly prevented state transitions. This excludes the possibility that this alteration could originate from the genetic background in a particular strain, or from a genetic link between either of the two mutations and another gene involved in the regulation process.

Kinase activity was still detected in the thylakoid membranes of the b6f mutants, although it was no longer regulated by the redox state of the PQ pool. When present, the PS II subunits showed unaltered phosphorylation, as did some of the other phosphoproteins currently observed in the wild-type or in PS II mutants. However, a group of two LHC subunits – polypeptides 13 and 17 – was no longer phosphorylated, whereas another group of polypeptides comprising polypeptide 9, which is a chlorophyll-binding protein (Bassi, R., personal communication), as well as two other LHC subunits – polypeptides 10 and 11 – still was. The former group was previously shown to undergo phosphorylation changes with kinetics different from those of the latter: only this second group of LHC subunits closely followed the kinetics of the state transitions [21]. The distinct phosphorylation characteristics of these two families of LHC subunits demonstrates a b6f-complex-dependent heterogeneity within LHC for the kinase system. This heterogeneity could involve two LHC-kinases with different specificities, a hypothesis which will be discussed below. Alternatively, it could result from a conformational change between two states of the LHC or from two different locations of the complex in the membrane. In these hypotheses, the phosphorylation sites on polypeptides 13 and 17 would be accessible to the kinase only upon an association between LHC and b6f complexes. Such an association has been suggested previously on the basis of the ultrastructural modifications observed in the thylakoid membranes of the b6f mutants [28]. We can rule out the possibility that the extra-membrane segments of polypeptides 13 and 17, which contain the phosphorylation sites and protrude at the outer thylakoid surface in the wild type, were buried in the membrane in the b6f mutants, since their trypsin sensitivity was conserved (not shown). However more subtle conformational changes may have occurred.

The question should then be raised of a possible link between the absence of redox control on the phosphorylation of thylakoid membrane polypeptides in the b6f mutants and the total lack of phosphorylation on polypeptides 13 and 17. Although phosphorylation of these two LHC subunits may be a prerequisite for an increase in phosphorylation of the other phosphoproteptides in vivo, this is not the case in vitro: we have occasionally observed similar phosphorylation patterns (not labelled bands corresponding to polypeptides 13 and 17) in wild-type thylakoid membranes incubated with [γ-32P]ATP (unpublished observation). However, these membranes still showed reversible phosphorylation of the other LHC subunits in a dark to light transition.

The low level of phosphorylation of the thylakoid membrane polypeptides in b6f mutants was consistent with their high level of fluorescence in reducing conditions, and was indicative of a block in State 1. If the low level of labelling of the
phosphopolypeptides in the \( b_{6f} \) mutants originated from a preexisting irreversible phosphorylation of the LHC subunits – for instance because of the absence of a phosphatase – the mutant cells would be blocked in State 2. Alternatively, enhanced phosphatase activity in the \( b_{6f} \) mutants could maintain the cells in State 1. It would not, however, prevent redox-controlled changes in the phosphorylation of the LHC subunits, but would only offset the levels of phosphorylation towards lower values. Contrary to this prediction, LHC phosphorylation in the \( b_{6f} \) mutants was not redox-controlled. Therefore, we considered two possible origins of their block in State 1: the absence of an LHC-kinase or the loss of its regulation site.

In the first hypothesis, one should take into account the multiple kinase activities that have been found in thylakoid membranes [3,21,29–32]. Two, maybe three, such enzymes have been purified [2–5]. Therefore, depending on their respective substrate specificities, the absence of the LHC-kinase in \( b_{6f} \) mutants could be partly masked by the ability of the other(s) to phosphorylate some LHC subunits. Consistent with the absence of an LHC-kinase would be the slight but significant shift towards lower densities in the distribution of kinase activity among the gradient fractions recovered from the \( b_{6f} \) mutant FUD4, as compared to that obtained with the wild type. The somewhat lower total kinase activity extracted from FUD4 thylakoids could also be indicative of a possible deficiency in one type of kinase, but the significance of this observation is severely limited by the variations in the level of kinase activity obtained with the wild type from one experiment to another.

The polypeptide of about 40 kDa lacking in the thylakoid membranes from \( b_{6f} \) mutants is a candidate for the particular LHC-kinase that would be missing in these strains. It has about the same molecular weight as CPK2, a putative kinase purified by Lin et al. [2] from spinach thylakoid membranes. The identity of CPK2 was, however, challenged by Coughlan and Hind [5] who attributed this polypeptide to ferredoxin-NADP reductase, an enzyme which may interact as well with \( b_{6f} \) complexes.

On the other hand, we noted some difference in our analysis of the \( K_m \) values for ATP associated with the total phosphorylation activity in thylakoid membranes from the \( b_{6f} \) mutants and from the wild type. However, out of the two \( K_m \) values which fitted the data from the two strains, the lower one around 50 \( \mu \text{M} \) – which is supposedly associated with the LHC-kinase responsible for the regulation [33,34,4] – was unchanged. Therefore, either the LHC-kinase, if missing in the mutant, has a higher \( K_m \) for ATP in the 100–150 \( \mu \text{M} \) range where we noted a difference between the wild-type and \( b_{6f} \) mutants, or it should have a similar \( K_m \) – in the 50 \( \mu \text{M} \) range – as one of the other kinases which remain in the mutants.

An unchanged 50 \( \mu \text{M} \) \( K_m \) is more consistent with an alteration of the activation process in the \( b_{6f} \) mutants. This could be due to the loss of the quinone-binding sites, \( Q_c \) or \( Q_z \), of the \( b_{6f} \) complexes. However, inhibitors of quinone binding on the \( Q_c \) site, such as NQNO, as well as on the \( Q_z \) site, such as DNP-INT, did not alter significantly the fluorescence transitions which accompany the reduction of the PQ pool in thylakoid membranes having a normal \( b_{6f} \) complement.

Alternatively, kinase activation could depend on the redox state of an electron carrier, other than the plastoquinones, but associated with the \( b_{6f} \) complex. Our experiments showed that neither plastocyanin nor the Rieske protein was part of the regulation process. However the redox carrier, G, which interacts with the \( b_{6f} \) complex [35] as well as the two \( b \) cytochromes, \( b_h \) and \( b_l \), become reduced upon the aerobic to anaerobic transitions in darkness used in the present study. The midpoint potential of \( G \) and \( b_h \) is at about 0 mV [35,36], which is consistent with the titration of kinase activation performed by Horton et al. [37]. In addition, changes in the redox state of these components may still occur via the \( Q_c \) site upon illumination of higher plant chloroplasts in the presence of DBMIB, an experiment which has been taken as indicative of the involvement of the PQ pool in the activation process [1].

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