Light and CO₂ Response of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Activation in Arabidopsis Leaves

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ABSTRACT

The requirements for activation of ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco) were investigated in leaves of Arabidopsis wild-type and a mutant incapable of light-activating rubisco in vivo. Upon illumination with saturating light intensities, the activation state of rubisco increased 2-fold in the wild-type and decreased in the mutant. Activation of fructose 1,6-bisphosphate phosphatase was unaffected by the mutation. Under low light, rubisco deactivated in both the wild-type and the mutant. Deactivation of rubisco in the mutant under high and low light led to the accumulation of high concentrations of ribulose 1,5-bisphosphate. Inhibiting photosynthesis with methyl viologen prevented ribulose 1,5-bisphosphate accumulation but was ineffective in restoring rubisco activation to the mutant. Net photosynthesis and the rubisco activation level were closely correlated and saturated at a lower light intensity in the mutant than in wild-type. At CO₂ concentrations between 100 and 2000 micromol per liter, the activation state was a function of the CO₂ concentration in the dark but was independent of CO₂ concentration in the light. High CO₂ concentration (1%) suppressed activation in the wild-type and deactivation in the mutant. These results support the concept that rubisco activation in vivo is not a spontaneous process but is catalyzed by a specific protein. The absence of this protein, rubisco activase, is responsible for the altered characteristics of rubisco activation in the mutant.

Ribulose-1,5-bisphosphate carboxylase/oxygenase catalyzes the first reactions of the photosynthetic carbon reduction and photorespiratory carbon oxidation pathways (14). Catalytic competence of the enzyme requires prior activation by carbamate formation on a lysine of the large subunit followed by the addition of Mg²⁺ (9, 12). Activation of rubisco² can be readily achieved in vitro by incubating the enzyme with CO₂ and Mg²⁺ prior to the initiation of catalysis with RuBP, but complete activation of rubisco by this spontaneous process requires CO₂ concentrations much greater than occur physiologically (7, 10, 12).

The activation state of rubisco increases upon illumination in isolated chloroplasts (2, 10, 26) and in leaves (11, 15, 16, 24). By analogy to the in vitro requirements, "light-activation" of rubisco has been attributed to increases in pH and Mg²⁺ (5) which occur in the chloroplast stroma upon illumination (18, 19). However, the response of rubisco activation to light intensity and CO₂ concentration is inconsistent with this suggested mechanism. The activation state of rubisco in vivo is a function of light intensity (11, 16), but the level of light required to saturate rubisco activation is considerably higher than that necessary for saturation of stromal alkalization and Mg²⁺ efflux (4). Furthermore, under saturating light, rubisco extracted from leaves is almost completely activated, even though the CO₂ concentration under ambient conditions is several-fold less than the Kₘ(CO₂) for activation of the isolated enzyme (7, 12). Under sub-saturating light, rubisco in leaves exists largely in an inactive form but is insensitive to CO₂ concentration (15, 21). These observations demonstrate that the requirements for light activation of rubisco in vivo differ substantially from those observed for the isolated enzyme.

Somerville et al. (24) presented genetic evidence for the involvement of a heritable factor in the light activation of rubisco. These investigators isolated a mutant of Arabidopsis (designated the rca mutant) which was incapable of activating rubisco upon illumination. Rubisco isolated from leaves of the mutant and wild-type plants, however, had identical activation kinetics and isoelectric characteristics. Thus, the lesion in the rca mutant did not affect rubisco itself. We have recently shown that the rca mutation affects the expression of a soluble chloroplast protein, rubisco activase, which promotes light activation of rubisco in a reconstituted light activation system (20). These data indicate that rubisco activation in vivo is not a spontaneous process but is catalyzed by a mechanism that is energetically coupled to the light reactions of photosynthesis in the thylakoid membrane. The present study compares the response of rubisco activation to light and CO₂ in Arabidopsis wild-type and the rca mutant. These investigations demonstrate that the properties of rubisco activation in leaves are consistent with the concept that the activation process in vivo is an enzyme-catalyzed reaction.

MATERIALS AND METHODS

Plant Material. Arabidopsis 'Columbia' wild-type and mutant strains were grown at 1% CO₂ as described (23), except that a 14 h light/10 h dark photoperiod (200–300 μmol photons/m²·s) was used instead of continuous illumination. Isolation of the mutant line CS2071 carrying the rca mutation has been described (24).

Labeling Studies. Intact plants were labeled with ¹⁴CO₂ and labeled products were separated into basic and nonbasic fractions by ion-exchange chromatography (23). The nonbasic fraction was further fractionated by HPLC (24).

Photosynthesis, Rubisco Activation, RubP Levels and FBPase Activation. IR gas analysis was performed on intact plants at 25°C (23). Light levels (PAR) were varied with neutral density filters. For measurements of rubisco activation and RuBP concentration (22) intact plants were flushed at 25°C with the desired
humidified gas stream, and at the designated time entire plants were rapidly plunged into liquid N₂. While frozen in N₂, roots were removed and the remainder of the plant was transferred to a Ten Broeck glass homogenizer at 4°C containing 100 mM N-(2 hydroxy-1,1-bis[hydroxymethyl]ethyl)-glycine (Tricine)-NaOH and 10 mM MgCl₂ at pH 8.1. Samples consisting of from two to four plants were homogenized for 30 s and aliquots were assayed immediately for activation level (initial RuBP carboxylase activity) or RuBP concentration. Total rubisco activity in wild-type and mutant Arabidopsis, measured after incubating the extract for 10 min with 10 mM NaHCO₃ and 10 mM MgCl₂ at 25°C, was not affected by light, dark, or low light treatment of the plants. Rubisco activity was determined in an assay system containing 100 mM Tricine, 10 mM MgCl₂, 0.4 mM RuBP, and 1.0 mM NaH¹⁴CO₃ at pH 8.1. Replicate assays were conducted at 25°C for 30 s and mean values were reported for each sample. For measurements of FBPase activity, plants were incubated under the conditions described in the text, frozen in liquid N₂, and then rapidly (30 s) extracted and assayed as described previously (8).

Methyl Viologen Treatments. Leaves were treated by immersing plants for two 3-min intervals in 0.05% Triton X-100 containing 0.2 mM methyl viologen prior to incubation in the dark. This treatment caused nearly complete inhibition of net photosynthesis as measured by IR gas analysis. Triton X-100 alone at this concentration had no effect on activation or net photosynthesis.

Chlorophyll Determination. Chl concentration was determined after extraction in 80% acetone (1).

RESULTS

The Arabidopsis rca mutant required a very high CO₂ concentration to saturate photosynthesis, accumulated a large percentage of photosynthetically fixed ¹⁴CO₂ into RuBP, and exhibited a decrease in RuBP activity upon exposure to saturating light at air levels of CO₂ (24). However, since rubisco activation is dependent on light intensity (11, 16) we examined the effects of light intensity on the in vivo activation level in Arabidopsis. Pretreatment of the wild-type with saturating light at air levels of CO₂ (350 μL/L) increased the activation state of rubisco 2-fold over the dark level (Table I). In contrast, this same light treatment caused deactivation below the dark level in the mutant. Under low light (30 μmol photons/m²·s) the activation state of rubisco decreased below the dark level in both wild-type and the mutant, and the decrease was greater in the mutant. To determine whether deactivation of rubisco in the rca mutant under high light might be caused by changes in the stromal pH or Mg²⁺ concentration, light activation of FBPase was examined. In both the dark and the light, the activation level of FBPase was similar in mutant and wild-type leaves (Table I). Thus, the lack of rubisco activation in the rca mutant was not caused by the absence of light-induced changes in pH and Mg²⁺ concentration. Under low light, FBPase activity in the wild-type decreased by 50%.

The time course of rubisco activation level in Arabidopsis wild-type and mutant was determined during illumination with high (near-saturating) and low light (Fig. 1). To examine activation under conditions where CO₂ fixation but not electron transport was inhibited, the activation level was also followed for plants that were illuminated with low light after pretreatment with methyl viologen (Fig. 1). In wild-type plants, rubisco activation state rapidly increased under high light and slowly decreased under low light. Slow deactivation occurred under both of these conditions in the mutant. The activation state in wild-type leaves treated with methyl viologen rapidly increased under low light, but methyl viologen treatment was not effective in stimulating activation of rubisco in the mutant. However, methyl viologen did reduce the extent of deactivation.

Table 1. Rubisco and FBPase Activation Levels in Leaves of Arabidopsis Wild-type and rca Mutant in the Dark and at Two Light Intensities

Plants were flushed continuously with 350 μL CO₂/L, 2% O₂, balance N₂ for 60 min prior to sampling.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rubisco</th>
<th>FBPase</th>
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<tbody>
<tr>
<td></td>
<td>Wild-type</td>
<td>rca mutant</td>
</tr>
<tr>
<td></td>
<td>μmol/mg Chl·h</td>
<td></td>
</tr>
<tr>
<td>Dark Light</td>
<td>54</td>
<td>37</td>
</tr>
<tr>
<td>350 μmol photons/m²·s</td>
<td>135</td>
<td>29</td>
</tr>
<tr>
<td>30 μmol photons/m²·s</td>
<td>15</td>
<td>6</td>
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FIG. 1. Time course of rubisco activation level (initial activity) in Arabidopsis wild-type (□, □, □) and rca mutant (●, ●, ●). Plants were flushed with 350 μL CO₂/L in 2% O₂, bal N₂ for 2 h in the dark prior to the onset of illumination (zero time) at 350 (□, □) or 30 (□, □) μmol photons/m²·s after pretreatment with methyl viologen (●, ●). Inset. Time course of RuBP levels in the rca mutant following the onset of illumination (zero time) at 350 (□, □) or 30 (●, ○) μmol photons/m²·s or at 30 μmol photons/m²·s after pretreatment with methyl viologen (●, ○).

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the wild-type (Fig. 2). The time course of the increase in labeled RuBP was inversely correlated with the time course of rubisco deactivation in the mutant (Fig. 1).

The light response of photosynthesis and rubisco activation in leaves of both wild-type Arabidopsis and the rca mutant demonstrated that the activation state of rubisco in leaves, measured after 1 h, closely matched the steady state photosynthetic rate of the plants (Fig. 3). A correlation between rubisco activation and photosynthetic rate has been reported by others (16). The activation level in leaves from wild-type plants at light intensities below 150 μmol photons/m²·s was lower than found in the dark, while in the mutant the activation level in light was always below the dark level regardless of the light intensity. The deactivation of rubisco which occurred at low light intensities followed a slow time course, requiring 60 min for complete deactivation (Fig. 1). In contrast, the lower photosynthetic rate that accompanied a reduction in light intensity occurred rapidly, usually reaching steady state within a few min (data not shown). Similar results have been reported by others (13).

The rca mutant requires high CO₂ (1%) for survival and becomes chlorotic in air (24). The effects of high CO₂ and air on the rubisco activation level were therefore examined in situ at a constant light intensity of 150 μmol photons/m²·s in wild-type and the mutant (Fig. 4). High CO₂ (zero time point) suppressed light deactivation in the rca mutant and inhibited light activation of rubisco in wild-type plants. This effect on wild-type plants may be due to a suppression of stromal alkalinization (27). Upon transfer to air levels of CO₂, rubisco slowly deactivated in the

![Figure 2](image2.png)

**Fig. 2.** Distribution of 14C among the photosynthetic intermediates in Arabidopsis wild-type and rca mutant. Plants were flushed with 350 μl CO₂/L in 2% O₂, bal N₂ and labeled for 10 min at various times after the onset of illumination (zero time) at 350 μmol photons/m²·s. Incorporation is expressed as a percentage of the total 14C fixed. Photosynthetic rates in wild-type were constant over the 2 h time course (130 μmol CO₂/mg Chl·h) but declined from 88 to 48 μmol CO₂/mg Chl·h in the rca mutant.

![Figure 3](image3.png)

**Fig. 3.** Response of net photosynthesis and rubisco activation level (initial activity) to light intensity in Arabidopsis wild-type (○) and rca mutant (□). Photosynthesis and activation were measured at 350 μl CO₂/L in 2% O₂, bal N₂ after 1 h at the indicated light intensities. Plants were preincubated for 30 min in the dark prior to the onset of illumination.

the wild-type and rapidly activated in the wild-type. RuBP concentration at 1% CO₂ was considerably higher in the rca mutant than in the wild-type (Fig. 4). At air levels of CO₂, a large increase in RuBP concentration occurred in the mutant. A comparatively minor increase in RuBP level occurred in the wild-type (Fig. 4). After 1 h in the dark, RuBP concentration and rubisco activation level were similar in the mutant and the wild-type.

These effects of 1% CO₂ prompted additional measurements of the response of rubisco activation state to the external CO₂ concentration. In the dark, the activation state of rubisco was similar in the mutant and wild-type plants, increasing as a function of CO₂ concentration over the range of 0 to 2000 μl CO₂/L (Fig. 5). A similar response of the activation state to CO₂ concentration in the dark was observed previously (21). In light, rubisco activation state in both the mutant and the wild-type was relatively insensitive to changes in the external CO₂ concentration between 100 and 2000 μl CO₂/L. The extent of light activation, however, differed substantially between the two strains. While the wild-type activation state in the light was considerably higher than in the dark at CO₂ concentrations below 2000 μl CO₂/L and in CO₂-free air, the activation state of the mutant was lower in the light than in the dark except in CO₂-free air, where activation states in the light and dark were similar (Fig. 5). At 2000 μl CO₂/L, rubisco activation level in the mutant in the light was only 30% of wild-type.

**DISCUSSION**

The results presented here support the conclusion of Somerville et al. (24) that the rca mutation specifically affected the light activation of rubisco. The changes in rubisco activation in the mutant at high light intensities resembled those which occurred in the wild-type at low light intensities. However, this similarity was specific for light activation of rubisco since activation of FBPase was not affected by the rca mutation. Portis et al. (18) and Purczeld et al. (19) showed that the activation states of FBPase and SBPase were affected by changes in the stromal pH and Mg²⁺ concentration, so it is unlikely that the rca mutation affects rubisco activation in such a manner. This conclusion is also supported by labeling data which demonstrated that RuBP
and PGA were the primary Calvin cycle intermediates affected by the mutation. Labeling patterns similar to those of the rca mutant have been obtained with chloroplasts following brief exposure to high temperatures (25), a treatment which specifically inhibited light activation of rubisco (26).

One possible explanation for the absence of rubisco activation in the rca mutant is that RuBP levels, which are increased by the mutation, might inhibit rubisco activation in vivo (24). This explanation is suggested by data from studies with isolated rubisco which demonstrated that RuBP can bind tightly to inactive rubisco and thus act as a potent inhibitor of activation (6). However, when RuBP accumulation was prevented by treating leaves with methyl viologen, light activation was not restored in the mutant. From these results we conclude that the lack of rubisco activation is the cause, not the effect, of high RuBP levels in the mutant.

The nuclear mutation which is responsible for the rca phenotype (24) affects the expression of two stromal polypeptides in Arabidopsis (20). We have presented genetic and biochemical evidence indicating that these polypeptides are subunits of a stromal protein which catalyzes rubisco activation in vivo (20). The revelation that rubisco activation is catalyzed by rubisco activase, a protein not expressed in the rca mutant, provides a mechanism to explain the lack of rubisco light activation in the mutant.

The occurrence of rubisco activase also provides a means to explain some characteristics of the in vivo activation process. For example, several investigations have demonstrated that the activation state of rubisco in vivo is a function of light intensity (11, 15, 16) and can limit photosynthesis under steady state conditions (16). The altered response of photosynthesis to light intensity in the mutant and its close correlation with activation state supports this latter point. The mechanism by which light intensity controls the activation state of rubisco is not known, but activation state of rubisco may be modulated by photon flux via rubisco activase. Studies with a reconstituted light activation system have shown that light (20), and specifically ΔpH (17) are required for in vivo activation of rubisco. Two PSI acceptors, methyl viologen and pyocyanine, promote the rubisco activation process both in vivo (Fig. 2) and in a reconstituted system (20). These compounds inhibit light activation of those photosynthetic enzymes which are activated via the ferredoxin-thioredoxin system (3), thus implying that light activation of rubisco is mechanistically distinct from other photosynthetic enzymes. The inability of methyl viologen to restore rubisco activation in the rca mutant suggests that the effect of these acceptors on rubisco activation is indirect and that rubisco activation is mediated by the activase.

Measurements of leaf RuBP indicate that under steady state conditions in the light, RuBP concentration is generally greater than rubisco binding site concentration regardless of the light intensity or CO₂ concentration (13, 15, 16). Since RuBP forms a tight complex with inactive rubisco (6), spontaneous activation...
of inactive rubisco by CO\textsubscript{2} and Mg\textsuperscript{2+} would largely be inhibited. Results from the previous study indicated that rubisco activase promotes activation of the inactive E-RuBP form of the enzyme (20). Thus, we suggest that inactive rubisco complexes with RuBP so that rubisco activation is determined primarily by the rate at which activase converts E-RuBP to active enzyme. The response of rubisco activation state to CO\textsubscript{2} concentration observed here and in other studies (15, 21) supports this conclusion since, unlike the isolated enzyme, the activation state in the light (but not in the dark, when RuBP levels are low) was largely independent of CO\textsubscript{2} concentration. In the case of the rca mutant, formation of the inactive E-RuBP complex in the absence of rubisco activase could be the cause of the low activation state in the light and might explain why activation was not increased by CO\textsubscript{2}.

The presence of rubisco activase in plants provides a mechanism to explain the coordinate regulation of photon flux rate through the electron transport chain with the activity of rubisco, which catalyzes the initial reaction of photosynthetic carbon reduction. Rubisco activation in leaves was stimulated by increasing light intensity but not by increasing CO\textsubscript{2} concentration. Thus, rubisco activation in vivo is controlled primarily by light intensity and not CO\textsubscript{2} concentration. By modulating the first reaction in carbon reduction, rubisco activase ultimately controls the balance between the utilization and regeneration of RuBP and hence the rate of net photosynthesis under steady state conditions.

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