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Regulation of Ribulose-1,5-Bisphosphate Carboxylase Activity in Response to Light Intensity and CO_2 in the C_3 Annuals *Chenopodium album* L. and *Phaseolus vulgaris* L.[1]

Rowan F. Sage*, Thomas D. Sharkey, and Jeffrey R. Seemann

Department of Botany, University of Georgia, Athens, Georgia 30602 (R.F.S.); Department of Botany, University of Wisconsin, Madison, Wisconsin 53706 (T.D.S.); and Department of Biochemistry, University of Nevada, Reno, Nevada 89557 (J.R.S.)

ABSTRACT

The light and CO_2 response of (a) photosynthesis, (b) the activation state and total catalytic efficiency (k_{cat}) of ribulose-1,5-bisphosphate carboxylase (rubisco), and (c) the pool sizes of ribulose 1,5-bisphosphate, (RuBP), ATP, and ADP were studied in the C_3 annuals *Chenopodium album* and *Phaseolus vulgaris* at 25°C. The initial slope of the photosynthetic CO_2 response curve was dependent on light intensity at reduced light levels only (less than 450 micromoles per square meter per second in *C. album* and below 200 micromoles per square meter per second in *P. vulgaris*). Modeled simulations indicated that the initial slope of the CO_2 response of photosynthesis exhibited light dependency when the rate of RuBP regeneration limited photosynthesis, but not when rubisco capacity limited photosynthesis. Measured observations closely matched modeled simulations. The activation state of rubisco was measured at three light intensities in *C. album* (1750, 550, and 150 micromoles per square meter per second) and at intercellular CO_2 partial pressures (C_i) between the CO_2 compensation point and 500 microbars. Above a C_i of 120 microbars, the activation state of rubisco was light dependent. At light intensities of 550 and 1750 micromoles per square meter per second, it was also dependent on C_i, decreasing as the C_i was elevated above 120 microbars at 550 micromoles per square meter per second and above 300 microbars at 1750 micromoles per square meter per second. The pool size of RuBP was independent of C_i only under conditions when the activation state of rubisco was dependent on C_i. Otherwise, RuBP pool sizes increased as C_i was reduced. ATP pools in *C. album* tended to increase as C_i was reduced. In *P. vulgaris*, decreasing C_i at a subsaturating light intensity of 190 micromoles per square meter per second increased the activation state of rubisco but had little effect on the k_{cat}. These results support modelled simulations of the rubisco response to light and CO_2, where rubisco is assumed to be down-regulated when photosynthesis is limited by the rate of RuBP regeneration.

It is well established that the activity of rubisco[2] is regulated downward in response to a decrease in light intensity below the photosynthetic light saturation point (for reviews see 1, 20, 26, 35). In addition, rubisco may be down-regulated in response to increased $p(CO_2)$ or decreased $p(O_2)$ (18, 19, 24, 30, 32). Two major mechanisms for regulating rubisco have been proposed: (a) carbamylation of a lysine residue in the active site of rubisco and (b) binding of the inhibitor CA1P to the active site (1, 9, 20, 26). While both regulatory mechanisms can be utilized following changes in light intensity, only modulation of the carbamylation state has been shown to regulate rubisco in response to changes in $p(CO_2)$ or $p(O_2)$ (18, 19, 30). Changes in the carbamylation state of rubisco are mediated by the enzyme rubisco activase and are related to the ATP status of the chloroplast (16). The mechanism of CA1P-dependent regulation is less clear, but may also involve rubisco activase (15, 20, 26).

The function of the regulation of rubisco activity is uncertain. Some have proposed that it acts to balance the rate of RuBP use with the rate of RuBP regeneration, and the rate of triose-phosphate use with the rate of triose phosphate production (11, 28). If this is true, then rubisco activity should be down-regulated whenever the rate of photosynthesis is limited by the capacity to regenerate RuBP. The regeneration of RuBP can reflect either a limitation in the rate at which light harvesting and electron transport make ATP and NADPH, or a limitation in the rate at which starch and sucrose synthesis consume triose phosphates and regenerate orthophosphate for photophosphorylation (28). The rate of RuBP consumption can become limiting when the $p(CO_2)$ falls below the atmospheric ambient, because decreasing CO_2 reduces the ability of rubisco to consume RuBP. However, the $p(CO_2)$ at which RuBP consumption just becomes limiting depends upon the capacity of RuBP regeneration relative to the rubisco capacity and should therefore depend upon light intensity. While the regulation of rubisco in response to light

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[2] Abbreviations: rubisco, ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39); A, net rate of CO_2 assimilation; CABP, carboxyarabinitol bisphosphate; CA1P, carboxyarabinitol 1-phosphate; C_i, intercellular partial pressure of CO_2; J_{max}, maximum rate of electron transport; IS, initial slope of the CO_2 response of photosynthesis; k_{cat}, catalytic turnover rate of rubisco; $p(CO_2)$, partial pressure of CO_2; PFD, photon flux density; $p(O_2)$, partial pressure of O_2; RuBP, ribulose 1,5-bisphosphate; V_{cmax}, maximum rate of carboxylation; Γ_*, CO_2 compensation point in the absence of nonphotorespiratory respiration.

intensity is well documented and generally conforms to the above interpretation, less is known concerning the regulation of rubisco in response to changes in $p(CO_2)$ and $p(O_2)$, particularly at physiologically relevant C_i (between 100 and 350 μbar). The interaction of light and CO_2 on the regulation of rubisco is poorly known. As predicted in a model presented in a companion paper (17), if photosynthesis is regulated to maintain a balance between the capacity of rubisco to consume RuBP and the rate of RuBP regeneration, there should be a strong interaction between light intensity and CO_2 on the activation state of rubisco. Thus, if RuBP regeneration is limiting photosynthesis, decreasing $p(CO_2)$ should reduce the degree to which RuBP regeneration is limiting and the activation state of rubisco should increase. In other words, reducing CO_2 at subsaturating light intensities should reactivate rubisco.

In the work described here, the regulatory relationship between rubisco, light and CO_2 was further examined to test the validity of the model presented by Sage (17) and to determine the degree to which rubisco is regulated to balance limitations on photosynthesis because of a limited RuBP regeneration capacity.

MATERIAL AND METHODS

Plant Material and Growth Conditions

Plants used in this study were *Chenopodium album* L. (lambsquarters), a rapidly growing weed with high photosynthetic capacity, and *Phaseolus vulgaris* L. cv Linden, the common red kidney bean. Plants were grown in a greenhouse in Reno, NV at 25 to 30°C, 40 to 60% RH from May to October 1987. Plants were fertilized daily with a 0.5 strength Hoagland solution (8). Light intensities regularly reached 1500 to 1600 μmol m^{-2} s^{-1} for much of the day.

Gas Exchange Measurements

In *C. album,* the response of photosynthesis to intercellular CO_2 of single leaves was characterized at a range of PFD between 80 and 1500 μmol m^{-2} s^{-1} using a null balance gas exchange measuring system previously described (30). Leaves were placed in a cuvette at 24 to 26°C and an ambient CO_2 of 340 μbar for 30 min prior to initiating measurements. Vapor pressure deficits between leaf and ambient air were kept at 6 to 12 mbar. Beginning at 1500 μmol photons m^{-2} s^{-1}, the CO_2 response of photosynthesis was determined by first lowering the ambient $p(CO_2)$ to 60 μbar, and subsequently increasing it in a series of steps back to 340 μbar. Measurements were made at each step and the initial CO_2 assimilation rate at 340 μbar was compared to the rate at 340 μbar determined after the CO_2 response was measured. Where initial and final CO_2 assimilation measurements at 340 μbar differed by more than 10%, the measurements were discontinued and restarted with a new leaf. After determining the CO_2 response of A at a PFD of 1500 μmol m^{-2} s^{-1}, the light intensity was reduced about 40% and the process repeated. Subsequently, the light intensity was again reduced about 40%. By the end of the measurement the CO_2-response of photosynthesis had been obtained at four to six light intensities for the same leaf.

In *P. vulgaris,* the CO_2 response of A was determined at four to five light intensities using the same procedure. The highest light intensity used was 1000 to 1200 μmol m^{-2} s^{-1}, which was just saturating for photosynthesis in this species.

The light response of A in *C. album* was measured at a C_i of 270 to 280 μbar and 120 to 130 μbar, and conditions as described above. Leaves were first equilibrated at about a PFD of 2000 μmol m^{-2} s^{-1} and a C_i of 270 μbar. Light intensity was then reduced in steps with steady-state measurements made at each step after a 10 to 20 min equilibration period. When finished, the light intensity was returned to near 2000 μmol m^{-2} s^{-1}, the C_i reduced to near 120 μbar and the light response redetermined.

All gas exchange measurements were calculated according to von Caemmerer and Farquhar (33). Light response curves were measured twice; CO_2 response curves were measured three times.

Leaf Sampling and Rubisco Assay

Leaf samples for the analysis of the activity ratio of rubisco, and the pool sizes of RuBP, ATP, and ADP were collected using a rapid-freeze cuvette and gas exchange system previously described (18). Young, fully expanded leaves were inserted into the cuvette at 24 to 26°C and allowed at least 30 min to equilibrate at an ambient $p(CO_2)$ ranging from 60 to 600 μbar and a light intensity of 1750 ± 50, 550 ± 50, or 150 ± 20 μmol m^{-2} s^{-1}. Gas-exchange parameters were measured at least 25 min after steady-state rates of photosynthesis were obtained. This portion of the leaf was then clamped between two copper heads prechilled in liquid N_2, freezing the tissue within 0.25 s. Two 1.75 cm^2 leaf discs were produced and stored in liquid N_2 until assay.

Rubisco activity was assayed on one-half of the leaf disc at 25°C (pH 8.2) as previously described (18). The activation state of rubisco was calculated as the activity of rubisco in extracts assayed within 90 s of extraction from the leaf, divided by the activity in extracts incubated for 10 to 15 min in 20 mM $MgCl_2$ and 12 mM $NaHCO_3$. This ratio primarily reflects the carbamylation state of the enzyme (1, 4). The content of rubisco active sites was estimated by determining the binding of radiolabeled ^{14}C-CABP in the crude leaf extract (5). Aliquots of the extract were incubated 3 h with ^{14}C-CABP at 37°C (pH 8.2), in the presence of 20 mM $MgCl_2$, 10 mM $NaHCO_3$, and antibodies to rubisco. The rubisco-antibody precipitate was filtered with a 0.45 μm polysulfone membrane filter (Supor 450; Gelman Sciences, Ann Arbor, MI), the filters were washed in a sodium phosphate buffer solution (10 mM sodium phosphate [pH 7.6], 0.85% NaCl, 10 mM $MgCl_2$), and the radiolabel retained on the filter was determined by scintillation counting. The ratio of the total rubisco activity to the content of rubisco active sites was used to estimate the catalytic turnover rate (k_{cat}) of the rubisco enzyme in mol CO_2 fixed mol^{-1} CABP binding site s^{-1}, assuming eight active sites per enzyme molecule. It should be noted that previous studies (for example 9, 18, 19, 30) reported k_{cat} values based on mol CO_2 fixed mol^{-1} rubisco s^{-1}, which is more appropriately termed molar activity.

RuBP, ATP, and ADP Assay

Metabolites were extracted as previously described (27) by grinding half of the leaf disk in 3.5% perchloric acid at subzero temperatures. After removing precipitated proteins by centrifugation and rapidly raising the pH to 7, the extract was stored in liquid N_2 until assay. RuBP was assayed by determining the amount of $^{14}CO_2$ incorporated into acid-stable products by partially purified rubisco (30). ATP and ADP pools were determined spectrophotometrically using a coupled-enzyme assay determining NADPH (for ATP) or NADH (for ADP) oxidation (3, 10). RuBP, ATP, and ADP pool sizes were expressed relative to the content of rubisco active sites determined in the other half of a leaf disc. This corrected for differences which may have existed between leaves in the size of the photosynthetic apparatus.

Modeled Response of Rubisco to Light and CO_2

The response of the rubisco activation state to light and CO_2 was modeled as described in a companion paper (17) for *C. album* only. Conditions were assumed to be 25°C; rubisco V_{cmax} = 120 μmol m^{-2} s^{-1} (similar to that measured in *C. album*; maximum electron transport rate (J_{max}) = 450 μmol m^{-2} s^{-1}; triose phosphate use rate = 25 μmol m^{-2} s^{-1}, and O_2 partial pressure = 180 mbar. All other conditions are as described in Sage (17).

RESULTS

CO_2 Response of Photosynthesis

The slope of the CO_2-response of photosynthesis at the CO_2 compensation point (referred here to as the initial slope) was dependent on light intensity below about 450 μmol m^{-2} s^{-1} in *Chenopodium album* and 250 μmol m^{-2} s^{-1} in *Phaseolus vulgaris* (Figs. 1, 2). Above these respective light intensities, the initial slope of the CO_2 response of photosynthesis was independent of light intensity, but as light intensity increased the C_i at which the CO_2 response of A noticeably deviated from the initial, linear portion also increased. This is similar to previously reported responses for spinach (2) and assorted other species (34).

According to equation 16.67 in Farquhar and von Caemmerer (7), when rubisco capacity limits photosynthesis, the initial slope of the CO_2 response of photosynthesis (IS) at Γ_* (the CO_2 compensation point in the absence of nonphotorespiratory respiration) is equal to

$$IS = \frac{V_{cmax}}{\Gamma_* + K_c(1 + O/K_o)} \quad (1)$$

where V_{cmax} equals the maximum activity of rubisco, K_c equals the K_m of rubisco for CO_2, O is the O_2 partial pressure, and K_o is the K_m of rubisco for O_2. Assuming all variables in Equation 1 are not affected by light intensity at the CO_2 compensation point, the initial slope of rubisco-limited photosynthesis will be independent of light intensity.

When RuBP regeneration limits photosynthesis at the CO_2 compensation point, the initial slope at Γ_* equals

$$IS = J/15\Gamma_* \quad (2)$$

where J is the electron transport rate (see Appendix 1 for the derivation). Since the electron transport rate is dependent upon light intensity, the initial slope will also depend upon light intensity.

Using Equations 1 and 2, we modeled the response of IS to light intensity for bean and *C. album*. The V_{cmax} of rubisco was assumed to be independent of light intensity. As shown in Figure 2, there is good agreement between modeled responses and measured values. Under conditions where RuBP regeneration was predicted to limit photosynthesis at the CO_2 compensation point, the initial slope responded to light intensity. When rubisco capacity was predicted to limit photosynthesis at the CO_2 compensation point, the initial slope was independent of light intensity. The light saturation point of the initial slope equals the light intensity at which the RuBP

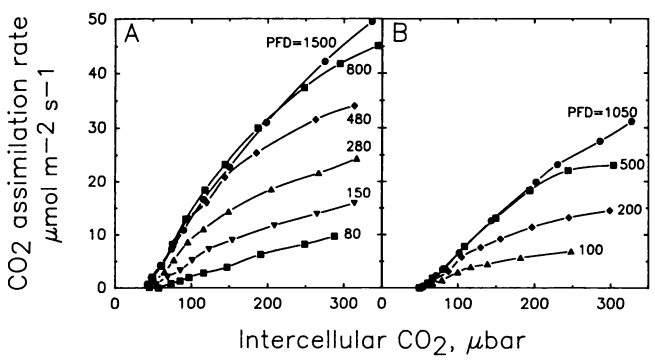

Figure 1. CO_2 response of photosynthesis as affected by photon flux density (PFD, in μmol m^{-2} s^{-1}) in (A *C. album* and (B *P. vulgaris*.

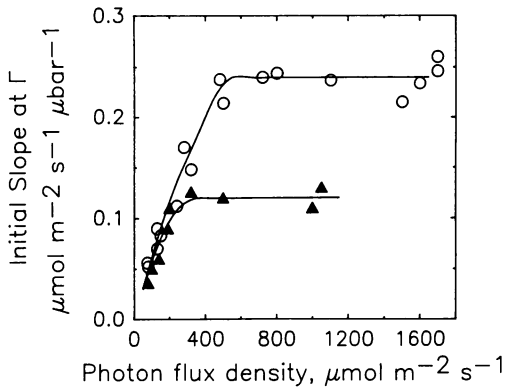

Figure 2. Relationship between photon flux density and the initial slope of the CO_2 response of photosynthesis at the CO_2 compensation point, Γ, in *C. album* (○) or *P. vulgaris* (▲). Initial slopes were calculated by fitting the first three to five points of the CO_2 response curve to a linear function. Lines indicate the modeled responses calculated at the CO_2 compensation point assuming the V_{cmax} of rubisco equals 120 μmol m^{-2} s^{-1} in *C. album* and 60 μmol m^{-2} s^{-1} for bean. These values are equivalent to V_{cmax} values measured *in vitro* for these species. The maximum electron transport rate, J_{max}, of rubisco was set at 400 μmol m^{-2} s^{-1} for *C. album* and 180 μmol m^{-2} s^{-1} for bean. All other modeled parameters are identical to those used by Sage (17).

regeneration capacity and the rubisco capacity are equal at the CO_2 compensation point, that is, the light intensity where RuBP regeneration first limits A at all C_i. This value is about 450 μmol m^{-2} s^{-1} in *C. album* and 250 μmol m^{-2} s^{-1} in *P. vulgaris*.

CO_2 Response of the Activation State of Rubisco in *C. album*

Modeled CO_2 responses of the activation state of rubisco are presented in Figure 3A and measured responses are presented in Figure 3B. At a PFD of 1750 μmol m^{-2} s^{-1}, the modeled activation state was 100% below a C_i of 300 μbar, and declined as C_i increased above 300 μbar. Similarly, the measured activation state was approximately 90% between 100 and 300 μbar, and declined to 76% as the C_i was increased from 300 to 500 μbar. At 550 μmol m^{-2} s^{-1}, the model predicted the activation state of rubisco to approach the value observed at 1750 μmol m^{-2} s^{-1} below a C_i of 100 μbar, but fell substantially as the C_i increased above 100 μbar so that at 500 μbar, it was well below that predicted at 1750 μmol m^{-2} s^{-1}. Measured responses confirmed this prediction. At a C_i of 100 μbar, the activation states measured at a PFD of 550 μmol m^{-2} s^{-1} and 1750 μmol m^{-2} s^{-1} were similar. In contrast, at 500 μbar, the activation state at 550 μmol m^{-2} s^{-1} was 50% compared to 76% at 1750 μmol m^{-2} s^{-1}.

The major difference between modeled and measured responses was that the model did not predict the observed drop in activation state below a C_i of 100 μbar. In addition, the maximum measured activation state of rubisco was 85 to 90%, never 100% as predicted, but this difference may have been due to characteristics of the rubisco assay which affect the maximum activity.

At a PFD of 150 μmol m^{-2} s^{-1}, the activation state of rubisco was not significantly dependent on C_i, ranging from 30 to 35% (Fig. 3A). In contrast, the model predicts a CO_2 dependence of the activation state of rubisco at this low light intensity and a lower absolute activation state (approaching 20%) above a C_i of 100 μbar.

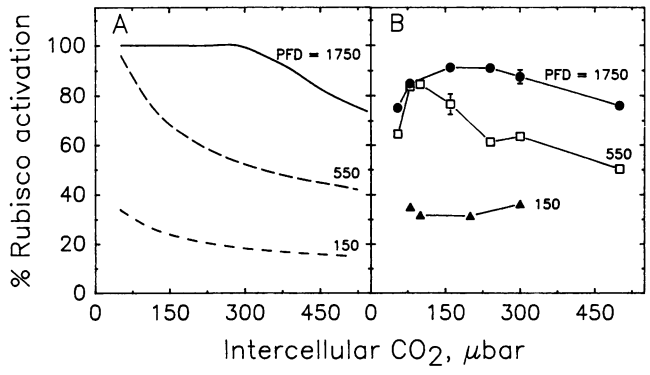

Figure 3. CO_2 response of the activation state of rubisco in *C. album* at three photon flux densities. A, Modeled responses; B, measured responses. Error bars equal SE; where not shown, error bars are smaller than the symbols; n = 4 to 6.

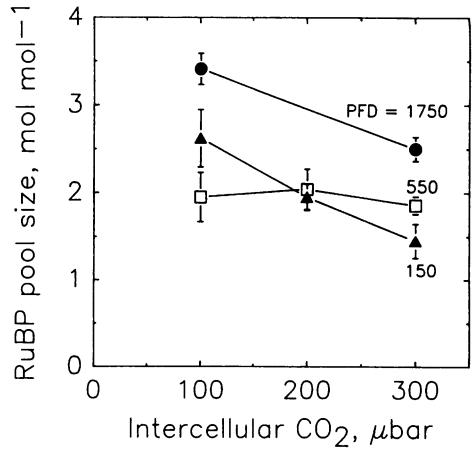

Figure 4. CO_2 response of the RuBP pool size (expressed as mol RuBP per mol CABP binding sites) in *C. album* at 25°C and three photon flux densities. Error bars equal SE; n = 4 to 6.

RuBP Pool Sizes in *C. album*

RuBP pool sizes were measured at a C_i between 100 and 300 μbar (Fig. 4). At a PFD of 1750 μmol m^{-2} s^{-1}, the pool size of RuBP decreased as C_i increased. At 150 μmol m^{-2} s^{-1}, RuBP pools also declined as C_i increased. However, at the intermediate light intensity, where changes in the activation state of rubisco were greatest, RuBP pool size was independent of C_i, being near 2 mol per mol rubisco binding sites, a pool size below which RuBP is suggested to be limiting *in vivo* (27, 32).

Light Response of Photosynthesis and the Activation State of Rubisco in *C. album*

In the modeled simulation in Figure 5A, reducing C_i from 275 to 120 μbar reduced the light saturation point of photosynthesis from above 1600 μmol photons m^{-2} s^{-1} to less than 1000 μmol m^{-2} s^{-1}. This is because in the model, A responds to light intensity when the RuBP regeneration capacity is limiting, but not when rubisco is limiting. Consequently, the light saturation point will occur where the RuBP regeneration and rubisco capacities are balanced. Decreasing C_i from 275 to 120 μbar reduces the capacity of rubisco to consume RuBP, and therefore reduces the light saturation point. As rubisco is predicted to be fully activated when it limits A, but partially deactivated when RuBP regeneration is limiting, the minimum PFD at which the activation state of rubisco is 100% will correspond to the light saturation point (Fig. 5C). Thus, decreasing C_i is predicted to reduce the PFD below which rubisco deactivates. In addition, at an intermediate light intensity, it is possible to reactivate rubisco by reducing C_i. These modeled predictions were generally confirmed in *C. album*. Measured photosynthetic light responses at a C_i of 275 and 120 μbar demonstrate that the light saturation point of A declines with decreasing C_i (Fig. 5B). Furthermore, the light intensity below which rubisco deactivates is reduced by a decrease in C_i (Fig. 5D), so that at a light intensity from 300 to 800 μmol m^{-2} s^{-1}, the activation state of rubisco is higher at a C_i of 120 μbar than at 275 μbar.

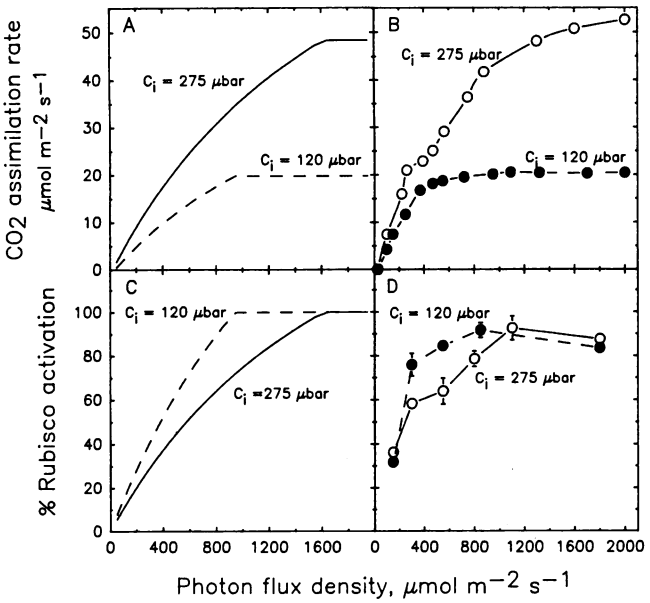

Figure 5. Light response of photosynthesis and the activation state of rubisco in *C. album* at an intercellular $p(CO_2)$ of 275 μbar (○) or 120 μbar (●). A and C are modeled responses. B and D are measured responses. Error bars are SE. Gas exchange responses in B are for single leaves. In panel D, $n = 4$ to 6.

ATP and ADP Pool Sizes in *C. album*

ATP pools have been correlated with the activation state of rubisco (16). In this study, changes in light and C_i had only a slight effect on the mean pool size of ATP and none on ADP (Table I). Although at a C_i of 300 and 100 μbar, mean ATP pools and the ATP to ADP ratios were consistently lower at 550 than 1750 μmol m^{-2} s^{-1} and tended to increase as C_i was reduced to 100 μbar, differences between means were generally not statistically different. Where different, trends in ATP pools and the ATP/ADP ratio are consistent with the observed changes in the activation state of rubisco, but the magnitude is well below that previously shown in spinach (16).

Light and CO_2 Response of the Catalytic Turnover Rate of Rubisco in *P. vulgaris*

In *P. vulgaris,* rubisco is regulated by a combination of CA1P binding and carbamylation control (9). At an ambient $p(CO_2)$ of 310 μbar, photosynthesis is typically light saturated at 1000 μmol photons m^{-2} s^{-1} under atmospheric gas concentrations, while CA1P inhibition of rubisco is substantial below a PFD of 300 μmol m^{-2} s^{-1} (9). We compared the CO_2 response of the k_{cat} and activation state of rubisco at a PFD of 1000 and 190 μmol m^{-2} s^{-1} (Table II). At a C_i of 300 μbar, lowering the PFD from 1000 to 190 μmol m^{-2} s^{-1} reduced the total k_{cat} of rubisco by 16% and the activation state of rubisco by 21%. Similar reductions in the k_{cat} of rubisco were observed at a C_i of 220 and 80 μbar following the same reduction in light intensity, but the degree to which rubisco deactivated following the drop in light intensity declined as the C_i was reduced. Thus, at a PFD of 190 μmol m^{-2} s^{-1}, reducing the C_i from 300 to 80 μbar had no effect on the total k_{cat} of rubisco while the activation state increased from 84.9 to 95.5%.

DISCUSSION

The initial slope of the CO_2 response of photosynthesis at light saturation has been correlated with the capacity of the rubisco-limited-rate of carboxylation (25, 33). However, as light intensities are reduced below saturating levels the C_i at which the potential rate of RuBP consumption equals the potential rate of RuBP regeneration declines (17). Above this "balancing" C_i where the potential rates of RuBP consumption and RuBP regeneration are equal, photosynthesis is light limited and the rate of CO_2 assimilation is dependent on light intensity, reflecting the limitation in the RuBP regeneration capacity. Below the balancing C_i, A is light saturated and the rate of photosynthesis reflects the rubisco-limited rate of carboxylation. Eventually, light intensity will be low enough so that the balancing C_i is equal to the CO_2 compensation point. At this and lower light intensities, RuBP regeneration is limiting at all C_i above the CO_2 compensation point and the initial slope of the CO_2 response of photosynthesis is light

Table I. *ATP and ADP Pool Sizes as a Function of Photosynthetic PFD and Intercellular $p(CO_2)$ in C. album at 24°C*

Means ± SE. Differences between means (by Duncan's multiple range test, P = 0.10) are indicated by different letters; $n = 4$ to 6.

C_i	PFD	ATP	ADP	ATP/ADP
μbar	μmol m^{-2} s^{-1}	mol mol^{-1} CABP binding sites		
300	1750	3.01_a ±0.11	3.22_a ±0.26	0.95_a ±0.08
	550	2.80_a ±0.27	3.25_a ±0.36	0.87_a ±0.06
200	550	2.74_a ±0.04	2.73_a ±0.50	1.04_{ab} ±0.10
100	1750	4.21_b ±0.27	3.49_a ±0.20	1.18_b ±0.08
	550	3.17_a ±0.10	2.92_a ±0.08	1.08_{ab} ±0.05

Table II. *Activation State and Catalytic Turnover Rate (k_{cat}) of Rubisco as a function of Photosynthetic PFD and Intercellular p(CO_2) in P. vulgaris*

Means ± SE. Differences between means (by Duncan's multiple range test, P = 0.05) are indicated by different letters; n = 5 to 6.

C_i	PFD	Activation	Total k_{cat}
μbar	μmol m^{-2} s^{-1}	%	mol mol^{-1} s^{-1}
300	1000	107.0_a	1.86_a
		±2.0	±0.05
	190	84.9_c	1.56_b
		±2.5	±0.03
220	1000	103.6_a	1.81_a
		±2.3	±0.07
	190	86.6_c	1.64_b
		±2.3	±0.04
80	1000	102.4_{ab}	1.86_a
		±3.0	±0.04
	190	95.5_b	1.56_b
		±2.4	±0.06

dependent, reflecting the RuBP regeneration capacity. Above this PFD, the initial slope reflects the rubisco limited rate of carboxylation and is independent of light intensity. Based on this interpretation, the light intensity where the balance between the RuBP regeneration and consumption capacities equalled the CO_2 compensation point occurred near 450 μmol photons m^{-2} s^{-1} in *C. album* and near 250 μmol m^{-2} s^{-1} in *P. vulgaris*.

If rubisco is regulated so that the capacity to consume RuBP balances the RuBP regeneration capacity, then its activation state will depend on both C_i and light intensity. At light saturation when rubisco is limiting, its activation state should be maximum, with all active sites fully functional. As C_i is increased, the increased availability of CO_2 will increase the rate of RuBP consumption by rubisco. Eventually, with a large enough increase in CO_2, RuBP will be consumed faster than it is produced and the RuBP consumption capacity will be in excess. Regulatory processes would then turn off the excess catalytic capacity of rubisco and a balance will be reestablished. With further increases in CO_2, more active sites would need be switched off to maintain the balance, as the additional CO_2 will enhance the rate of RuBP consumption by the remaining functional active sites. Hence, when RuBP regeneration limits A, increases in C_i should deactivate rubisco. Data presented here and elsewhere (12, 18, 32) demonstrate a reduction in the activation state of rubisco as C_i is increased, in support of this hypothesis. According to the modeled simulation, at a given C_i, reducing the light intensity will reduce the RuBP regeneration capacity and induce rubisco deactivation at subsaturating light intensities. Subsequently reducing C_i will lead to a reactivation of rubisco, and if the balance point between the capacity of RuBP regeneration and RuBP consumption is greater than the CO_2 compensation point, the activation state of rubisco should be able to reach 100%. If the balance point is less than the CO_2 compensation point, then the activation state will not fully recover. Observations with *C. album* demonstrate, in agreement with the model, that at 550 μmol m^{-2} s^{-1}, reducing C_i from 275 to 100 μbar did reactivate rubisco to the level measured at high light. Based on the initial slope data in Figure 2, it should be possible to fully activate rubisco by decreasing C_i at light intensities above 450 μmol m^{-2} s^{-1}, while below this PFD, RuBP regeneration will limit A at all C_i and it should not be possible to fully activate rubisco by decreasing C_i.

Below a C_i of 100 μbar, rubisco deactivated as C_i was reduced to the compensation point. The degree of deactivation was dependent on light intensity. This deviation from the model indicates that the ability to regulate rubisco is reduced below 100 μbar, possibly because carbamylation of rubisco by rubisco activase is CO_2 limited. Low CO_2-deactivation of rubisco is important for at least three reasons. First, if rubisco is limiting photosynthesis at low CO_2, the value of the initial slope of the CO_2 response of A will reflect the activity of rubisco at a decreased activation state, and not V_{cmax}. Second, low-CO_2 deactivation of rubisco, if extensive enough, could reduce the catalytic capacity of rubisco to the point where rubisco could become limiting at low light intensities. This probably occurs at 2% O_2 (data not shown). However, the close match between the modeled and observed initial slopes indicates that rubisco deactivation at low light was not severe enough to limit photosynthesis in the initial slope region. At 2% O_2, modeled initial slopes were substantially greater than measured, indicating that rubisco deactivation has a major effect on the initial slope (RF Sage, unpublished data). Third, under conditions where stomata close completely in the light, such as during nonuniform stomatal closure induced by water stress or following ABA treatment (6, 29, 31), deactivation of rubisco may be a secondary effect resulting from low C_i and not a direct effect of water stress or ABA on the stromal biochemistry.

Regulatory control of rubisco by the carbamylation state may also be reduced at low light, around 150 μmol m^{-2} s^{-1} and below. In *C. album*, this is supported by (a) the failure of the rubisco activation state to respond to C_i at 150 μmol m^{-2} s^{-1} in contrast to the modeled predictions, (b) the high activation state at 150 μmol m^{-2} s^{-1} relative to that modeled (30–35% instead of the predicted 20–25%), and (c) the increase in RuBP pool size with decreasing C_i at 150 μmol m^{-2} s^{-1}. At 550 μmol m^{-2} s^{-1}, where the rubisco activation state responds to C_i, RuBP pools are constant, as would be expected in a well regulated system. The minimum activation state seen here (30%) is similar to minimum activation states of rubisco (20–40%) reported for other species, even in darkness (1, 3, 9, 13, 32). Carbamylation control may become ineffective at low light because at air levels of CO_2, a certain fraction of active sites will be carbamylated, even in the absence of functioning rubisco activase (14, 22). In plants adapted to low light, or with low photosynthetic capacity, carbamylation control may be enhanced at low light. Alternatively, a supplemental regulatory mechanism may be operating. The binding of CA1P may be such supplemental mechanism of regulation. In *P. vulgaris*, the inhibition of rubisco by CA1P becomes significant at about 300 μmol m^{-2} s^{-1} (when the carbamylation state is near its minimum), and continues to increase as the light intensity is reduced to darkness (9).

If the regulatory control of rubisco by CA1P and carbamylation respond to the same biochemical parameters, then lowering $p(CO_2)$ at subsaturating PFD should reduce the

degree of CA1P binding, resulting in an increase in the total k_{cat} of rubisco. This was tested in *P. vulgaris* and the total k_{cat} was observed to be independent of C_i. All changes in the regulatory state of rubisco with decreasing C_i were because of changes in activation state. As indicated by the changes in total k_{cat}, CA1P binding occurred only in response to light intensity, indicating that the biochemical parameters controlling activation state are not identical to those controlling CA1P binding. Consistent with this, other studies link the control of the activation state to ATP status, while metabolism of CA1P is dependent on NADPH levels (21, 23).

In summary, the data presented here support the qualitative predictions of the regulation model presented in a companion paper (17). Differences between modeled predictions and measured results were minor except at low $p(CO_2)$ and low light. Improved prediction by the model would result from better estimates of the kinetic parameters of the photosynthetic biochemistry in *C. album* plus a better accounting of the heterogeneous nature of the leaf. However, the substantial deviation from modeled predictions observed at low $p(CO_2)$ indicate that the regulation of rubisco was less effective. In the extreme, ineffective regulation may lead to a limitation by a poorly regulated component even though its potential capacity is well in excess. Such a situation would be a significant waste of resources and could lead to damage such as photoinhibition. By combining the use of theoretical models with empirical tests, it should be possible to identify conditions where the components of photosynthesis are regulated to balance each other as opposed to situations where regulation is lost and the control of photosynthesis is dominated by a single process.

APPENDIX 1

Following is a way to estimate the initial slope of the CO_2 response of photosynthesis at Γ_* when the rate of electron transport limits photosynthesis.

When the rate of electron transport limits photosynthesis, the rate of photosynthesis can be described by

$$A = \frac{J(C - \Gamma_*)}{4.5C + 10.5\Gamma_*} - R_d \qquad \text{(A1)}$$

where C is the partial pressure of CO_2 and R_d is the rate of nonphotorespiratory respiration (7). By the quotient rule of calculus

$$\frac{dA}{dC} = J\left(\frac{(4.5C + 10.5\Gamma_*) - 4.5(C - \Gamma_*)}{(4.5C + 10.5R_*)^2}\right) \qquad \text{(A2)}$$

This simplifies to

$$\frac{dA}{dC} = J\left(\frac{15\Gamma_*}{(4.5C + 10.5\Gamma_*)^2}\right) \qquad \text{(A3)}$$

At $C = \Gamma_*$, dA/dC equals the initial slope of the CO_2 response of photosynthesis (IS), substituting and simplifying

$$\text{IS} = \frac{J}{15\Gamma_*} \qquad \text{(A4)}$$

which is presented as Equation 2 in the "Results" section.

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