Plant Physiol. (1989) 89, 918–924
0032-0889/89/89/0918/07/$01.00/0

Received for publication February 26, 1988
and in revised form November 7, 1988

Regulation of Ribulose-1,5-Bisphosphate Carboxylase Activity in Response to Diurnal Changes in Irradiance[1]

John Kobza and Jeffrey R. Seemann*

Department of Biochemistry, University of Nevada, Reno, Nevada 89577

ABSTRACT

The regulation of ribulose-1,5-bisphosphate (RuBP) carboxylase (Rubisco) activity and metabolite pool sizes in response to natural diurnal changes in photon flux density (PFD) was examined in three species (*Phaseolus vulgaris*, *Beta vulgaris*, and *Spinacia oleracea*) known to differ in the mechanisms used for this regulation. Diurnal regulation of Rubisco activity in *P. vulgaris* was primarily the result of metabolism of the naturally occurring tight-binding inhibitor of Rubisco, 2-carboxyarabinitol 1-phosphate (CA1P). In *B. vulgaris*, the regulation of Rubisco activity was the result of both changes in activation state and CA1P metabolism. In *S. oleracea*, Rubisco activity was regulated by a combination of changes in activation state and the binding/release of another tight binding inhibitor, probably RuBP. Despite these different mechanisms for the light regulation of Rubisco activity, the relationship between the *in vivo* activity of Rubisco and the PFD was the same for all three species. Rates of CA1P metabolism were thus sufficient to allow this mechanism to participate in the diurnal regulation of Rubisco activity as PFD changed at its normal rate. Furthermore, under natural conditions this regulatory mechanism was found to be important in controlling Rubisco activity over approximately the same range of PFD as did changes in activation state of the enzyme. Finally, this regulation of Rubisco activity resulted in relatively similar and saturating RuBP pool sizes for photosynthesis at all but the lowest PFD values in all three species.

The light-dependent regulation of Rubisco[2] activity involves a number of components, including carbamylation/decarbamylation of a specific lysine residue (13), synthesis/breakdown of a tight-binding inhibitor of catalysis, CA1P (2, 7, 10, 21, 26), and a protein factor, Rubisco activase, involved in the release of noncatalytically bound RuBP from the enzyme (17). Regulation of Rubisco activity by these mechanisms is important in matching the leaf's capacity for RuBP regeneration (light harvesting, electron transport, photophosphorylation) with its capacity for RuBP carboxylation and starch and sucrose synthesis (28). Different species of C_3 plants do not necessarily use these regulatory mechanisms in the same proportions, however, (10, 23). In a previous study, we examined the light regulation of Rubisco activity and photosynthesis under steady-state light conditions in three species which differed in the mechanisms used for Rubisco regulation. In this study, we have examined how Rubisco activity is regulated in these same three species under a natural diurnal irradiance pattern, the mechanisms of this regulation, and the consequences of this regulation on the pool size of RuBP.

[1] This research was supported by the National Science Foundation under grant DCB 8796314 to J. R. S.

[2] Abbreviations: Rubisco, RuBP carboxylase (EC 4.1.1.39); CA1P, 2-carboxyarabinitol 1-phosphate; k_{cat}, catalytic constant; PFD, photon flux density; RuBP, ribulose 1,5-bisphosphate.

MATERIALS AND METHODS

Plant Growth and Termination of Metabolism

Plants were grown from seed in 4 L pots in a mixture of compost:sand:perlite (2:1:1, v:v:v). *Phaseolus vulgaris* L. var Linden, *Beta vulgaris* L. var SSBN1, and *Spinacia oleracea* were moved to a common greenhouse for the diurnal experiment. Throughout the day, leaf samples were obtained using a hand-held freeze-clamp with copper heads (designed in our laboratory) cooled to the temperature of liquid N_2. This ensured rapid termination of the leaf's metabolism. The leaf samples were also bisected into two equal halves by the freeze clamp. Samples were stored in liquid N_2 until further processing could occur. At each sampling point during the d 3 samples per species were generated and the mean is reported here. The PFD was monitored using a model LI-185B quantum-radiometer-photometer (LiCor, Inc.).

Metabolite and Rubisco Analyses

RuBP assays were carried out with $HClO_4$ acid extracts of one-half of the leaf disc, as described by Seemann and Sharkey (24). CA1P content in the acid extract was determined by the inhibition of purified and activated spinach Rubisco produced by the metabolite extract in comparison to a standard curve of activity produced in the absence of any inhibitor (21).

The second half of the leaf sample was extracted for enzyme analysis in 4 mL of a CO_2-free, ice-cold buffer containing 100 mM Bicine (pH 7.8), 5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM DTT, and 1.5% (w/v) polyvinylpolypyrrolidone. The homogenate was clarified by a 10 s spin (total time) in an Eppendorf model 5414 microfuge and duplicate aliquots were immediately assayed for Rubisco activity. This activity is referred to as the 'initial activity'. The initial activity is dependent upon the state of activation of the enzyme and the concentration of tight-binding inhibitors in the leaf (23) and represents the substrate saturated *in vivo* activity as affected by these regulatory mechanisms. Another aliquot of the leaf homogenate was made 10 mM and 20 mM with HCO_3^- and Mg^{2+}, respectively (10% dilution), and allowed to incubate on ice for 10 min. We have previously demonstrated that this procedure

fully activates the enzyme (23) but maintains an inhibitor other than CA1P (apparently RuBP) bound to the enzyme (10, 23). At the end of the incubation period, duplicate aliquots were measured for enzyme activity and this activity is referred to as the 'total activity'. This total activity is dependent on the concentration of tight-binding inhibitors in the leaf and not on the activation state. The percent activation of the enzyme is then [(initial activity/total activity) × 100]. However, it should be noted that the activation percentage does not include any catalytic sites bound with an inhibitor such as CA1P.

The activity of Rubisco was measured as the rate of incorporation of $^{14}CO_2$ into acid stable products in a 30 s assay at 25°C. The assay consisted of 100 mM Bicine (pH 8.2), 20 mM $MgCl_2$, 1 mM EDTA, 5 mM DTT, and 1.5 mM RuBP (generated immediately prior to assay from ribose 5-P).

The k_{cat} (specific activity) of Rubisco was determined by obtaining the concentration of the enzyme in the extract by a radioimmunoprecipitation technique as described by Kobza and Seemann (10). Aliquots of the activated enzyme extract were allowed to incubate in the presence of 2[^{14}C]carboxyarabinitol 1,5-bisphosphate and rabbit serum antibody raised against purified spinach Rubisco. The precipitated protein was filtered using a GA6-S modified polysulfone filter (Gelman Sciences, Ann Arbor, MI) and washed to remove excess [^{14}C]carboxyarabinitol 1,5-bisphosphate. The bound radiolabel was then determined by liquid scintillation counting. The Rubisco k_{cat} was calculated by dividing the initial and total activities by the Rubisco concentration. The resulting data are the apparent substrate saturated specific activity *in vivo* (initial k_{cat}) and the fully carbamylated specific activity (total k_{cat}) in units of mol $CO_2 \cdot mol^{-1}$ Rubisco $\cdot s^{-1}$.

RESULTS

Rubisco activity and metabolite levels were monitored in leaves of *P. vulgaris*, *B. vulgaris*, and *S. oleracea* throughout a normal daily light regime. Two separate experiments were conducted on 5/6/87 (open symbols) and 10/15/87 (closed symbols). PFD as a function of time of day is shown for each species (Figs. 1A, 2A, and 3A). The major difference between the two data sets is that during the late afternoon of 5/6/87 clouds obscured the sun at approximately 1600 h and the PFD decreased relatively quickly from 700 to 200 μmol quanta m^{-2} s^{-1} and remained constant for the remainder of the sampling period. This difference between the data sets in the diurnal light course was probably the cause of the differences in enzyme regulation observed between the two data sets during the late afternoon (see below). The later and slower increase in PFD on 10/15/87 also produced a lag in metabolic responses relative to 5/6/87.

Rubisco Activation Levels

At the very low PFD values prior to sunrise, the activation percentage of Rubisco in *P. vulgaris* exceeded 100% (Fig. 1B). This phenomenon was also observed in extracts from darkened leaves (10). We believe this is an artifact due to the binding of free CA1P in the extract to sites carbamylated *in vitro* (23). As the PFD increased in the morning hours the

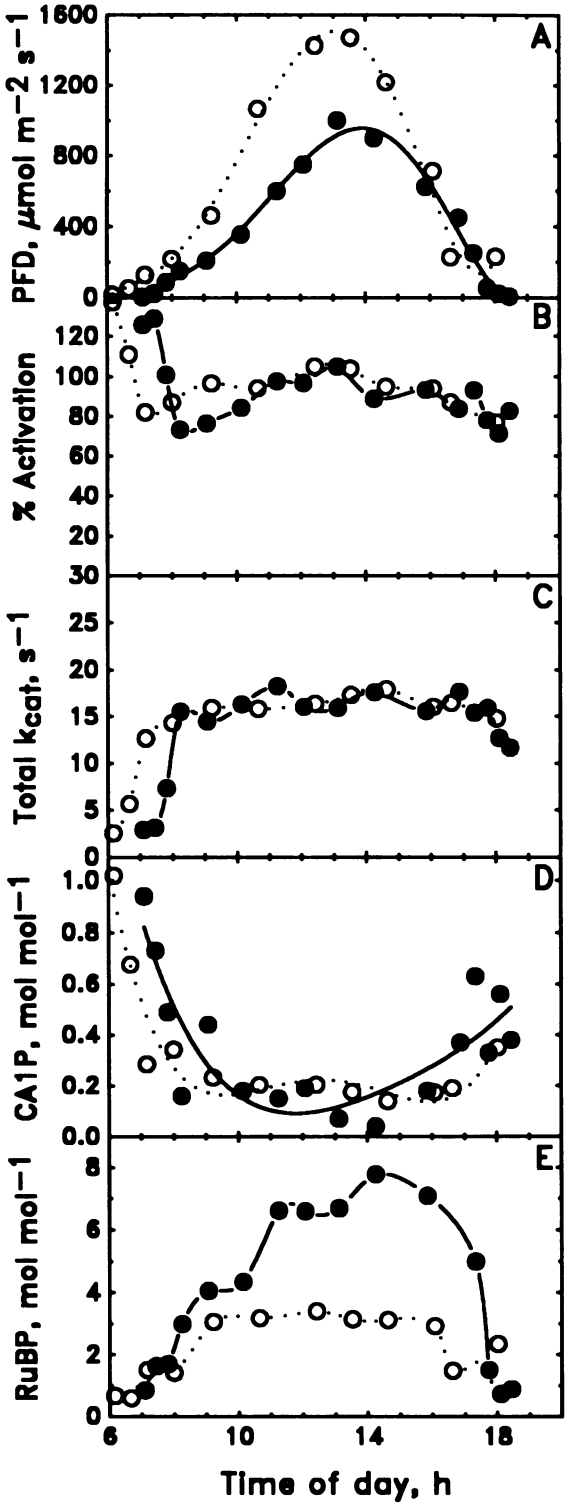

Figure 1. Diurnal responses in *P. vulgaris*. (A) Diurnal response of PFD. (B) Diurnal response of percentage activation of Rubisco ([initial activity/total activity] × 100). (C) Diurnal response of the total k_{cat} of Rubisco (mol CO_2/mol Rubisco·s). (D) Diurnal response of the level of CA1P (mol CA1P/mol Rubisco catalytic sites). (E) Diurnal response of the level of RuBP (mol RuBP/mol Rubisco catalytic sites). Open circles represent the responses observed on 5/6/87 and the closed circles represent the responses observed on 10/15/87.

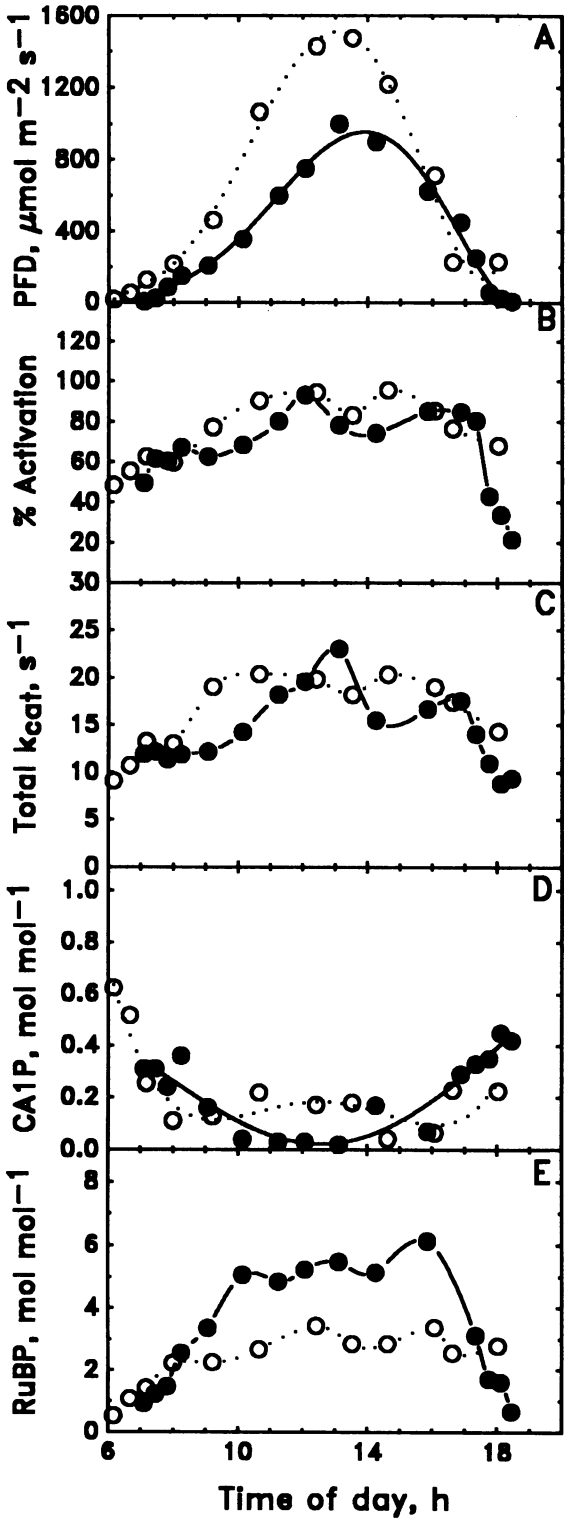

Figure 2. Diurnal responses in *B. vulgaris*. Panels A to E are as in Figure 1.

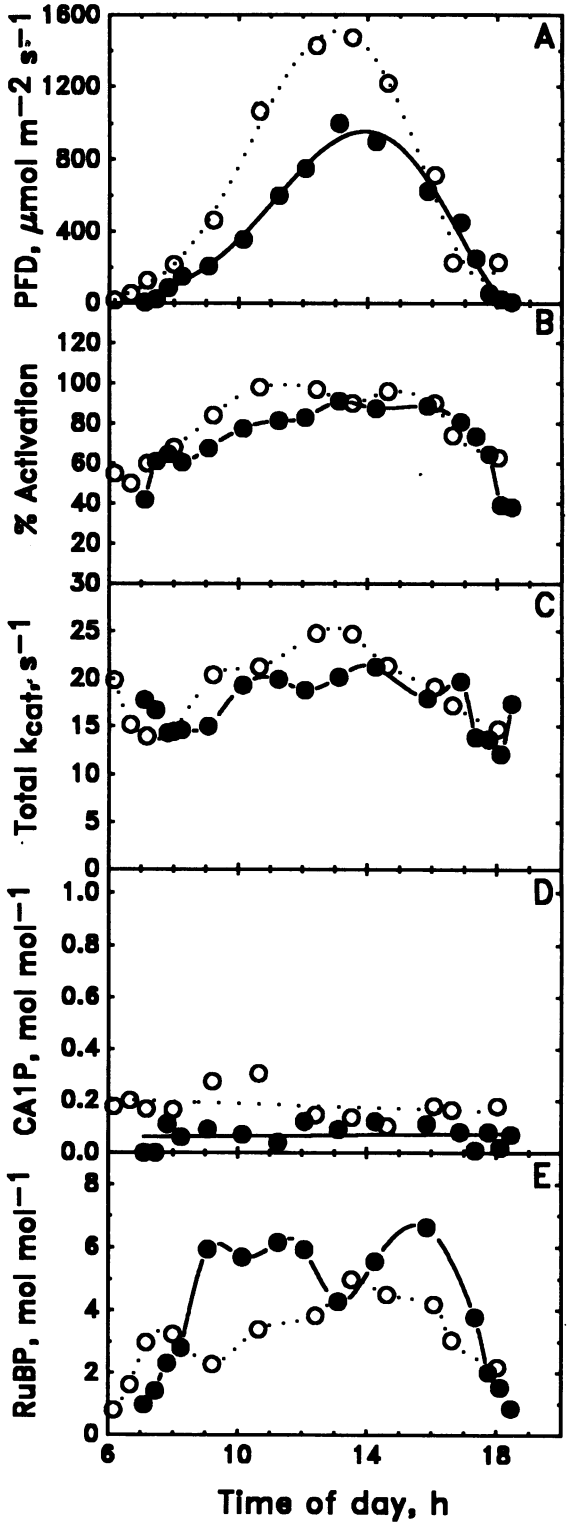

Figure 3. Diurnal responses in *S. oleracea*. Panels A to E are as in Figure 1.

percentage activation of Rubisco decreased in *P. vulgaris* to about 75% at a PFD of 150 μmol quanta m^{-2} s^{-1}, but thereafter increased to almost 100% as the PFD reached its daily maximum (Fig. 1B). Subsequently, the percentage activation decreased slightly as the PFD decreased in the late afternoon. There was little variation between the two data

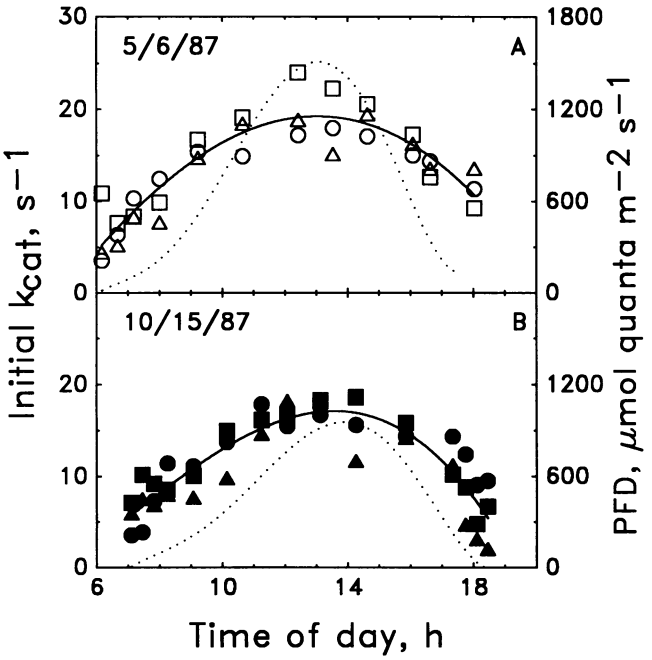

Figure 4. Diurnal response of initial k_{cat} (mol CO_2/mol Rubisco·s) in *P. vulgaris*, *B. vulgaris*, and *S. oleracea*. (A) Data collected on 5/6/87 (open symbols). (B) Data collected on 10/15/87 (closed symbols). *P. vulgaris*, circles; *B. vulgaris*, triangles; *S. oleracea*, squares. The solid lines are the regressions generated from the initial k_{cat} data of all three species on the given day. The dotted lines are the regressions generated from the PFD data given in Figures 1A, 2A, and 3A.

sets except for the early morning offset associated with the later sunrise on 10/15/87.

Changes in the percentage activation of Rubisco in *B. vulgaris* (Fig. 2B) and *S. oleracea* (Fig. 3B) through the day were much more substantial than in *P. vulgaris* (Fig. 1B). At low PFD values in the early morning the percentage activation was approximately 50% for both *B. vulgaris* and *S. oleracea*. The percentage activation increased with PFD, reaching a maximum of 80 to 90% in both species at a PFD of 400 to 600 μmol quanta m^{-2} s^{-1}, and remained relatively constant through the midday until the PFD decreased below 400 to 600 μmol quanta m^{-2} s^{-1}. In the late afternoon on 5/6/87, the percentage activation of Rubisco did not decrease to predawn levels in *B. vulgaris* and *S. oleracea*. This was most likely due to the fact that the PFD did not decrease below 200 μmol quanta m^{-2} s^{-1} in the late afternoon during the 5/6/87 sampling period. As PFD decreased during the late afternoon hours on 10/15/87, the percentage activation of Rubisco decreased to its predawn value in *S. oleracea*. In *B. vulgaris* Rubisco activation percentage in the late afternoon of 10/15/87 decreased below that observed in the morning at comparable levels of PFD (*e.g.* the percentage activation in the morning at 5 μmol quanta m^{-2} s^{-1} was 50%, while in the afternoon the percentage activation was 22% at 6 μmol quanta m^{-2} s^{-1}).

Rubisco Total k_{cat}

The total k_{cat} of Rubisco in *P. vulgaris* was extremely low at the low PFD values of the early morning (*e.g.* 3.5 s^{-1} at 5 μmol quanta m^{-2} s^{-1}, Fig. 1C). With increasing PFD the total k_{cat} reached a maximum at 200 to 400 μmol quanta m^{-2} s^{-1} and remained relatively constant throughout the afternoon as PFD decreased. In the late afternoon when the PFD had decreased below 400 μmol quanta m^{-2} s^{-1}, the Rubisco total k_{cat} also began to decrease. However, there was some degree of hysteresis in the response, as the total k_{cat} in the afternoon was higher than that observed at comparable light levels in the morning when the PFD was increasing. The total k_{cat} on 5/6/87 did not decrease to as low a level as was observed on 10/15/87, probably again due to the fact that the PFD did not decrease below 200 μmol quanta m^{-2} s^{-1} during our sampling period on 5/6/87.

The total k_{cat} of Rubisco in *B. vulgaris* increased with increasing PFD from a morning minimum of 12 s^{-1} to a maximum at 500 to 700 μmol quanta m^{-2} s^{-1} of approximately 20 s^{-1} (Fig. 2C). The total k_{cat} then decreased as PFD decreased below 400 μmol quanta m^{-2} s^{-1} in the afternoon. As was observed for *P. vulgaris*, the decrease in total k_{cat} in *B. vulgaris* on 5/6/87 was not as large as observed on 10/15/87.

The total k_{cat} of Rubisco in *S. oleracea* was initially high (approximately 20 s^{-1}) at the low PFD values of the early morning (Fig. 3C). As the PFD increased in the morning, in contrast to the other species the total k_{cat} of *S. oleracea* Rubisco decreased, reaching a minimum of 13 s^{-1} at approximately 100 μmol quanta m^{-2} s^{-1}. As PFD increased further the total k_{cat} then increased, attaining a maximum of approximately 20 s^{-1} at 400 μmol quanta m^{-2} s^{-1}. The Rubisco total k_{cat} of *S. oleracea* then remained relatively constant until the middle of the afternoon. Then, as light levels decreased below 400 to 500 μmol quanta m^{-2} s^{-1}, the total k_{cat} decreased. On 5/6/87, the total k_{cat} decreased until the PFD reached 200 μmol quanta m^{-2} s^{-1}. On 10/15/87, the total k_{cat} declined to a minimum at 22 μmol quanta m^{-2} s^{-1} and then, as the PFD continued to decrease, the total k_{cat} again increased, mirroring the morning response.

CA1P Levels

In the early morning hours, when the PFD was 5 to 6 μmol quanta m^{-2} s^{-1}, the level of CA1P in *P. vulgaris* was equal to the level of CA1P found in the dark in previous studies (1.3–1.5 CA1P mol mol^{-1} Rubisco catalytic sites) (10, 21) (Fig. 1D). As the PFD increased the level of CA1P decreased until a minimum close to zero was reached. As the PFD decreased below 400 to 600 μmol quanta m^{-2} s^{-1} in the afternoon, the level of CA1P increased, although not to levels which would be predicted from morning PFD values. Changes in the CA1P pool size mirrored changes in the Rubisco total k_{cat} (Fig. 1C). The levels of CA1P in the late afternoon on 5/6/87 were lower than those observed on 10/15/87, probably due to the higher light levels observed on 5/6/87 during this time period.

The response of the CA1P level in *B. vulgaris* to the diurnal changes in PFD was very similar to the response observed in *P. vulgaris* except that the absolute level of CA1P in *B. vulgaris* was lower than for *P. vulgaris* at any particular PFD, with the exception of midday (Fig. 2D). Changes in the CA1P pool size in this species also mirrored changes in the total k_{cat} of Rubisco (Fig. 2C).

No CA1P above the constant background of our assay was detected in *S. oleracea* (Fig. 3D) even though changes in the total k_{cat} of Rubisco were observed. This background represents nonspecific inhibition in the assay (21).

Rubisco initial k_{cat}

Figure 4 shows the Rubisco initial k_{cat} as a function of time of day for the three species on the 2 separate days. The diurnal pattern of *in vivo* Rubisco activity was the same for all three species on both sampling days. Rubisco activity, as regulated by activation and tight-binding inhibitors, tended to track the diurnal change in PFD, regardless of the regulatory mechanism(s) employed by a particular species.

RuBP Levels

The diurnal patterns of RuBP pool size were relatively similar between the three species, but significant differences existed between the patterns observed on 5/6/87 and 10/15/87. During the morning hours in *P. vulgaris*, the levels of RuBP on both days increased similarly until the PFD reached 400 μmol quanta m^{-2} s^{-1} (Fig. 1E). On 5/6/87, the levels of RuBP in this species remained relatively constant near 3 mol RuBP mol^{-1} Rubisco catalytic sites, decreasing only when PFD decreased suddenly to 200 μmol quanta m^{-2} s^{-1} as a consequence of clouds. On 10/15/87, the level of RuBP in *P. vulgaris* did not reach its maximum until 600 μmol quanta m^{-2} s^{-1} was reached and began to decrease when PFD decreased to 250 μmol quanta m^{-2} s^{-1} in the afternoon (Fig. 1E). The maximum level of RuBP attained on 10/15/87 in *P. vulgaris* was significantly higher than that observed on 5/6/87.

The levels of RuBP in *B. vulgaris* increased in the morning, reaching a maximum at 200 μmol quanta m^{-2} s^{-1} and 350 μmol quanta m^{-2} s^{-1} on 5/6/87 and 10/15/87, respectively (Fig. 2E). On 5/6/87 the level of RuBP in *B. vulgaris* remained constant at its maximum of 2.5 mol mol^{-1} Rubisco catalytic sites for the remainder of the day. During the afternoon of 10/15/87, the level of RuBP in *B. vulgaris* began to decrease from a maximum of 5 mol mol^{-1} when PFD reached 250 μmol quanta m^{-2} s^{-1}.

The level of RuBP in *S. oleracea* on 5/6/87 initially increased to a maximum of 3 mol mol^{-1} at 75 μmol quanta m^{-2} s^{-1} (Fig. 3E). The level was relatively constant over the remainder of the day with a minor increase observed in mid-afternoon. The level of RuBP in *S. oleracea* on 10/15/87 increased with PFD in the morning hours until the maximum (6 mol mol^{-1}) was reached at 200 μmol quanta m^{-2} s^{-1}. The level of RuBP had begun to decrease as PFD decreased to 250 μmol quanta m^{-2} s^{-1} in the afternoon.

DISCUSSION

Light-dependent control of Rubisco activity by changes in activation state (carbamylation) and the level of tight binding inhibitors has been well characterized in intact leaves under conditions which lead to steady-state gas exchange at a constant PFD (10). In the real world, however, plants generally do not experience such conditions. Rather, the incident PFD is constantly changing throughout the day. In studies with soybean (*Glycine max*), the maximum extractable activity of Rubisco varied as PFD changed during a normal daily light course (27, 29). We now know this to be a consequence of the metabolism of CA1P. We have expanded upon these previous studies by selecting three species representative of the different mechanisms by which Rubisco is light regulated and analyzing the responses of activation state and inhibitor metabolism to changing PFD.

In *P. vulgaris*, most of the regulation of Rubisco in response to changing PFD was accomplished by changes in the levels of CA1P (Fig. 1D), which alters the total k_{cat} of Rubisco (Fig. 1C). However, in contrast to the result observed under steady state conditions (10), a minor contribution to the regulation of Rubisco in this species in response to PFD was a reduction in the activation state of the enzyme at low PFD. In *B. vulgaris*, regulation of Rubisco in response to changes in PFD over the day was the result of a combination of changes in both the activation state of Rubisco and the level of CA1P (Fig. 2, B, C, and D), similar to our previous results under steady-state conditions (10). In *S. oleracea*, the regulation of Rubisco throughout the day resulted from changes in both activation state and binding of some tight-binding inhibitor other than CA1P (Fig. 3, B and C). The presence of such an inhibitor is indicated by the decrease in the total k_{cat} at low PFD. The regulation of Rubisco in *B. vulgaris* may also involve binding of this second tight-binding inhibitor, since the level of CA1P can be below the level required to produce the observed inhibition of Rubisco total k_{cat} (*e.g.* on 10/15/87 at 400 μmol quanta m^{-2} s^{-1} (1000 h), the CA1P level was negligible and yet the total k_{cat} was approximately 75% of its maximum). We have previously speculated that this tight-binding inhibitor is RuBP (10, 23). The noncatalytic binding of RuBP to the decarbamylated enzyme has been demonstrated *in vitro* a number of times (*e.g.* Refs. 6, 8, and 12), and results from the high affinity of RuBP for the decarbamylated catalytic site (k_D = 20 nM) (8). More recently, it has been demonstrated that RuBP can be bound in a noncatalytic fashion to Rubisco in leaves which were exposed to conditions that caused deactivation of the enzyme (3, 5, 10, 23). This bound RuBP is presumably removed by another light-dependent enzyme, Rubisco activase (17). Bound CA1P in *P. vulgaris* and *B. vulgaris* is presumably removed by an NADPH-dependent chloroplastic enzyme (20).

The data presented here suggest that activation and inhibitor control of Rubisco activity operate over approximately the same PFD range, in agreement with the results of Kobza and Seemann (10) and Salvucci and Anderson (19). Furthermore, the changes in CA1P pool size through the day (and the resultant change in the Rubisco total k_{cat}) indicate that rates of synthesis and degradation of this compound, as driven by diurnal changes in PFD, are sufficient to allow this mechanism to participate in light-dependent Rubisco regulation. We have noted, however, that the apparent rate of CA1P synthesis in the afternoon in *P. vulgaris* (Fig. 1, C and D) is less than that required for afternoon total k_{cat} values to match morning values at similar PFD values (see also Refs. 27 and 29). However, under certain conditions, synthesis of CA1P in *Phaseolus* is extremely rapid, causing changes in Rubisco

activity at rates equal to those which occur in other species during rapid deactivation of Rubisco (11). In contrast, under similar conditions other species may have rates of CA1P synthesis which are substantially slower than the rate of deactivation (*e.g. Alocasia macrorrhiza* [22] and *Nicotiana rustica* [19]).

The changes in the initial k_{cat} of Rubisco, as brought about by inhibitor metabolism and activation state changes, followed changes in PFD (Fig. 4) except when PFD approached levels which are known to be saturating for CO_2 assimilation in these species (10). This result was not only observed as PFD increased in the morning but also as PFD decreased in the afternoon. We have previously demonstrated that the rate of whole leaf CO_2 assimilation is highly correlated with the initial k_{cat} of Rubisco (10). Furthermore, the *in vivo* activity of Rubisco (initial k_{cat}) was independent of the species and the mechanism used to regulate the activity of this enzyme (Fig. 4 and see also Ref. 10). This result is consistent with previous suggestions that Rubisco activity is coupled to a common factor such as ATP pool size (16, 25).

Data collected from leaves at steady state CO_2 assimilation rates at various light levels have indicated that one consequence of the regulation of Rubisco activity in response to changes in PFD is to maintain levels of RuBP at constant and saturating levels with respect to the Rubisco catalytic site concentration at all but the lowest PFD values (10, 14, 15). These relatively constant levels of RuBP have been suggested as a means of preventing metabolite imbalances which could lead to a depletion of P_i and a resultant triose-P utilization limitation on photosynthesis (18, 28). The RuBP data collected on 5/6/87 for all three species is consistent with this hypothesis. Levels of RuBP remained constant and saturating relative to the Rubisco catalytic site concentration (approximately 3 mol RuBP mol^{-1} Rubisco catalytic sites) during most of the day (Figs. 1E, 2E, and 3E). The data collected on 10/15/87 is not so easily interpreted within this hypothesis. The light levels at which the RuBP pool size remained constant were in a considerably narrower range, similar to the results of Vu *et al.* (29). Also, levels of RuBP were 5- to 8-fold higher than the Rubisco catalytic site concentration in all species at some point during the day. From data gathered under steady-state gas exchange conditions at various light levels (10), it would be predicted that RuBP levels would saturate at a maximum of 2 to 2.5 mol mol^{-1} at relatively low light levels (*i.e.* from 75 to 300 μmol quanta m^{-2} s^{-1}), as was observed on 5/6/87. The reasons for the very high RuBP levels on 10/15/87 are not clear from the data presented here. However, two possible explanations for such high levels of RuBP may be found in data collected by other investigators. Badger *et al.* (1) and Caemmerer and Edmondson (4) observed high levels of RuBP at low intercellular CO_2 partial pressures. These investigators found that the ratio of RuBP/PGA could exceed one under such conditions. In the present study, not only were the levels of RuBP high but the ratio of RuBP/PGA exceeded one during midday (data not shown). It is therefore possible that the high levels of RuBP were due to a reduction in stomatal conductance such that the intercellular CO_2 partial pressure decreased during midday on 10/15/87. An alternative explanation is that leaf temperatures could have increased sufficiently during the midday to produce the elevated levels of RuBP. Kobza and Edwards (9) found that as the leaf temperature of wheat increased to supraoptimal temperatures for photosynthesis, the level of RuBP increased to extremely high levels and was well in excess of the assumed catalytic site concentration of Rubisco. The RuBP/PGA ratio also approached or exceeded one.

ACKNOWLEDGMENT

We thank Judy Miles for her expert technical assistance.

LITERATURE CITED

1. **Badger MR, Sharkey TD, von Caemmerer S** (1984) The relationship between steady-state gas exchange of bean leaves and the levels of carbon reduction cycle intermediates. Planta **160:** 305–313
2. **Berry JA, Lorimer GH, Pierce J, Seemann JR, Meeks J, Freas S** (1987) Isolation, identification, and synthesis of 2-carboxyarabinitol 1-phosphate, a diurnal regulator of ribulose-bisphosphate carboxylase activity. Proc Natl Acad Sci USA **84:** 734–738
3. **Brooks A, Portis AR** (1988) Protein-bound ribulose bisphosphate correlates with deactivation of ribulose bisphosphate carboxylase in leaves. Plant Physiol **87:** 244–249
4. **von Caemmerer S, Edmondson DL** (1986) The relationship between steady-state gas exchange, *in vivo* ribulose bisphosphate carboxylase activity and intermediates in *Raphanus sativus*. Aust J Plant Physiol **13:** 669–688
5. **Cardon ZG, Mott KA** (1989) Evidence that ribulose 1,5-bisphosphate (RuBP) binds to inactive sites of RuBP carboxylase *in vivo* and an estimate of the rate constant of dissociation. Plant Physiol **89:** (in press)
6. **Chu DK, Bassham JA** (1975) Regulation of ribulose 1,5-diphosphate carboxylase by substrates and other metabolites. Further evidence for several types of binding sites. Plant Physiol **55:** 720–726
7. **Gutteridge S, Parry MAJ, Burton S, Keys AJ, Mudd A, Feeney J, Servaites JC, Pierce J** (1986) A nocturnal inhibitor of carboxylation in leaves. Nature **324:** 274–276
8. **Jordan DB, Chollet R** (1983) Inhibition of ribulose bisphosphate carboxylase by substrate ribulose-1,5-bisphosphate. J Biol Chem **258:** 13752–13758
9. **Kobza J, Edwards GE** (1987) Influences of leaf temperature on photosynthetic carbon metabolism in wheat. Plant Physiol **83:** 69–74
10. **Kobza J, Seemann JR** (1988) Mechanisms for the light regulation of ribulose-1,5-bisphosphate carboxylase activity and photosynthesis in intact leaves. Proc Natl Acad Sci USA **85:** 3815–3819
11. **Kobza J, Seemann JR** (1989) Light-dependent kinetics of 2-carboxyarabinitol 1-phosphate metabolism and ribulose-1,5-bisphosphate carboxylase activity *in vivo*. Plant Physiol **89:** 174–179
12. **Laing WA, Christeller JT** (1976) A model for the kinetics of activation and catalysis of ribulose 1,5-bisphosphate carboxylase. Biochem J **159:** 563–570
13. **Miziorko HM, Lorimer GH** (1983) Ribulose-1,5-bisphosphate carboxylase-oxygenase. Annu Rev Biochem **52:** 507–535
14. **Mott KA, Jensen RG, O'Leary JW, Berry JA** (1984) Photosynthesis and ribulose 1,5-bisphosphate concentrations in intact leaves of *Xanthium strumarium* L. Plant Physiol **76:** 968–971
15. **Perchorowicz JT, Raynes DA, Jensen RG** (1981) Light limitation of photosynthesis and activation of ribulose bisphosphate carboxylase in wheat seedlings. Proc Natl Acad Sci USA **78:** 2985–2989
16. **Robinson SP, Portis AR** (1988) Involvement of stromal ATP in the light activation of ribulose 1,5-bisphosphate carboxylase/oxygenase in intact chloroplasts. Plant Physiol **86:** 293–298

17. **Robinson SP, Streusand VJ, Chatfield JM, Portis AR** (1988) Purification and assay of rubisco activase from leaves. Plant Physiol **88:** 1008–1014
18. **Sage RF, Sharkey TD, Seemann JR** (1988) The *in vivo* response of ribulose-1,5-bisphosphate carboxylase activation state and pool sizes of photosynthetic metabolites to elevated CO_2. Planta **174:** 407–416
19. **Salvucci ME, Anderson JC** (1987) Factors affecting the activation state and the level of total activity of ribulose bisphosphate carboxylase in tobacco protoplasts. Plant Physiol **85:** 66–71
20. **Salvucci ME, Holbrook GP, Anderson JC, Bowes G** (1988) NADPH-dependent metabolism of the ribulose bisphosphate carboxylase/oxygenase inhibitor 2-carboxyarabinitol 1-phosphate by a chloroplast protein. FEBS Lett **231:** 197–201
21. **Seemann JR, Berry JA, Freas SM, Krump MA** (1985) Regulation of ribulose carboxylase activity *in vivo* by a light-modulated inhibitor of catalysis. Proc Natl Acad Sci USA **82:** 8024–8028
22. **Seemann JR, Kirschbaum MUF, Sharkey TD, Pearcy RW** (1988) Regulation of ribulose-1,5-bisphosphate carboxylase activity in *Alocasia macrorrhiza* in response to step changes in irradiance. Plant Physiol **88:** 148–152
23. **Seemann JR, Kobza J** (1988) Genetic variation in the regulation of ribulose-1,5-bisphosphate carboxylase activity. Plant Physiol Biochem **26:** 461–471
24. **Seemann JR, Sharkey TD** (1986) Salinity and nitrogen effects on photosynthesis, ribulose-1,5-bisphosphate carboxylase and metabolite pool sizes in *Phaseolus vulgaris*. Plant Physiol **82:** 555–560
25. **Seemann JR, Sharkey TD** (1987) The effect of abscisic acid and other inhibitors on photosynthetic capacity and the biochemistry of CO_2 assimilation. Plant Physiol **84:** 696–700
26. **Servaites JC** (1985) Binding of a phosphorylated inhibitor to ribulose bisphosphate carboxylase/oxygenase during the night. Plant Physiol **78:** 839–843
27. **Servaites JC, Torisky RS, Chao SF** (1984) Diurnal changes in ribulose-1,5-bisphosphate carboxylase activity and activation state in leaves of field grown soybeans. Plant Sci Lett **35:** 115–121
28. **Sharkey TD** (1985) Photosynthesis in intact leaves of C_3 plants: physics, physiology and rate limitations. Bot Rev **51:** 53–105
29. **Vu CV, Allen LH, Bowes G** (1983) Effects of light and elevated atmospheric CO_2 on the ribulose bisphosphate carboxylase activity and ribulose bisphosphate level of soybean leaves. Plant Physiol **73:** 729–734