Plant Physiol. (1989) 89, 174–179
0032-0889/89/89/0174/06/$01.00/0

Received for publication June 9, 1988
and in revised form August 22, 1988

Light-Dependent Kinetics of 2-Carboxyarabinitol 1-Phosphate Metabolism and Ribulose-1,5-Bisphosphate Carboxylase Activity *in Vivo*[1]

John Kobza and Jeffrey R. Seemann*

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

ABSTRACT

The light-dependent kinetics of the apparent *in vivo* synthesis and degradation of 2-carboxyarabinitol 1-phosphate (CA1P) were studied in three species of higher plants which differ in the extent to which this compound is involved in the light-dependent regulation of ribulose-1,5-bisphosphate carboxylase (Rubisco) activity. Detailed studies with *Phaseolus vulgaris* indicate that both the degradation and synthesis of this compound are light-stimulated, although light is absolutely required only for CA1P degradation. We hypothesize that the steady state level of CAIP at any particular photon flux density (PFD) represents a pseudo-steady state balance between ongoing synthesis and degradation of this compound. The rate of CA1P synthesis in *P. vulgaris* and the resultant reduction in the total catalytic constant of Rubisco were maximal at 200 micromoles quanta per square meter per second following a step decrease from a saturating PFD, and substantially faster than the rate of synthesis in the dark. Under these conditions an amount of CA1P equivalent to approximately 25% of the Rubisco catalytic site content was synthesized in less than 1 minute. The rate of synthesis was reduced at higher or lower PFDs. In *Beta vulgaris*, the rate of CA1P synthesis at 200 micromoles quanta per square meter per second was substantially slower than in *P. vulgaris*. In *Spinacea oleracea*, an apparent noncatalytic tight-binding of RuBP to deactivated sites on the enzyme was found to occur following a step decrease in PFD. When dark acclimated leaves of *P. vulgaris* were exposed to a step increase in PFD, the initial rate of CA1P degradation was also found to be dependent on PFD up to a maximum of approximately 300 to 400 micromoles quanta per square meter per second. The rate of degradation of this compound was similar in *B. vulgaris*. In *S. oleracea*, a step increase in PFD resulted in noncatalytic RuBP binding to Rubisco followed by an apparent release of RuBP and activation of the enzyme. The *in vivo* rate of change of Rubisco activity in response to an increase or decrease in PFD was similar between species despite the differences between species in the mechanisms used for the regulation of this enzyme's activity.

CA1P[2] is an important component in the light-dependent regulation of Rubisco activity *in vivo* (1, 6, 8, 11, 13–15, 17, 21) in many species of higher plants (13, 18, 22). This tight-binding inhibitor of Rubisco is synthesized in the dark or at low PFDs and serves, in combination with changes in the extent of enzyme carbamylation, to match the *in vivo* activity of Rubisco with the capacity for RuBP regeneration. At higher PFDs, CA1P is degraded in a light-dependent process that involves photosynthetic electron transport (11, 13), a NADPH dependent chloroplast protein (12) and possibly Rubisco activase (10). Species differ in the extent to which CA1P is used to regulate Rubisco activity as PFD changes (8). A few species, such as *Phaseolus vulgaris*, use this mechanism almost exclusively for the light regulation of Rubisco activity. In a much larger number of species, such as *Beta vulgaris* and *Glycine max*, the light-dependent regulation of Rubisco activity is shared between CA1P metabolism and changes in enzyme activation (carbamylation) state. In another group of species which apparently lack the capacity to synthesize CA1P, such as *Spinacea oleracea* and *Arabidopsis thaliana*, an apparent noncatalytic tight-binding of RuBP to deactivated sites *in vivo* appears to be involved in the light-dependent regulation of Rubisco activity along with changes in carbamylation state of the enzyme (2, 3, 8).

We have investigated the light dependence and kinetics of CA1P synthesis and degradation *in vivo*. We have concentrated on *P. vulgaris* (beans) because of its substantial capacity for CA1P metabolism, but we have also included comparative data for *B. vulgaris* (sugar beet) and *S. oleracea* (spinach), species with less or no apparent capacity for CA1P metabolism, respectively. Rubisco activity and levels of CA1P were followed in leaves during transitions from high to low PFDs to determine apparent CA1P synthetic rates *in vivo*. Time courses of apparent CA1P degradation and Rubisco activity were also determined during the induction of photosynthesis following a step increase in PFD from the dark in these same three species.

MATERIALS AND METHODS

Plant Material and Termination of Metabolism

Phaseolus vulgaris L. var Linden, and *Beta vulgaris* L. var SSBN1 were grown from seed in a greenhouse as described in Sharkey *et al.* (19). *Spinacea oleracea* var Polka was grown from seed in a growth chamber as described by Kobza and Seemann (8). In all experiments leaf metabolism was terminated at a specific time by freeze-clamping the leaf between two copper heads cooled to the temperature of liquid N_2. The copper heads were milled such that the frozen circular leaf section which was produced was bisected during the harvest. One-half of the leaf section was used for the determination of Rubisco activities and content and the other half was used for

[1] Supported by the National Science Foundation under grant No. DMB 86–08004 to J. R. S.

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

the determination of CA1P levels. Samples were stored in liquid N_2 until this processing could occur.

Light Transients—*P. vulgaris*

In order to study the *in vivo* synthesis of CA1P and the resultant regulation of Rubisco activity in *P. vulgaris*, fully expanded attached leaves were equilibrated at 1600 μmol quanta m^{-2} s^{-1}. The PFD was then reduced in a single step to either 320, 200, 100, or 0 μmol quanta m^{-2} s^{-1}. Leaf samples were taken immediately prior to the decrease in PFD (zero time in the figures) and at 1, 5, 10, and 60 min following the decrease in PFD.

In order to study the degradation of CA1P *in vivo* and the resultant regulation of Rubisco activity in *P. vulgaris*, plants were kept in the dark overnight, and then fully expanded attached leaves were exposed to either 100, 200, 300, or 1100 μmol quanta m^{-2} s^{-1} in a single step change from the dark. Samples were taken immediately prior to illumination (zero time in the figures) or at 1, 5, 10, or 30 min following the increase in PFD.

Light Transients—*B. vulgaris* and *S. oleracea*

For these two species, attached leaves which had been in the dark overnight were exposed to a step increase in PFD to 1100 μmol quanta m^{-2} s^{-1}. Alternatively, leaves which had been in the light were equilibrated at 1100 μmol quanta m^{-2} s^{-1} for 30 min prior to decreasing the PFD to 200 μmol quanta m^{-2} s^{-1}. In both cases, samples were taken using the hand-held freeze clamp at 0, 1, 5, 10, or 30 min after the step change in PFD.

Metabolite and Rubisco Analyses

$HClO_4$ acid extracts were prepared from one-half of the leaf disc (16). CA1P content in the acid extract was determined by the inhibition of activity 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 (13, 15).

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 to yield an initial activity. Another aliquot of the leaf homogenate was made 10 and 20 mM with HCO_3^- and Mg^{2+}, respectively (10% dilution), and allowed to incubate on ice for 10 min. This procedure fully activates the enzyme but apparently maintains any RuBP bound to decarbamylated sites (8, 15). At the end of the incubation period, duplicate aliquots were measured for enzyme activity to yield a total activity. 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 inhibitors such as CA1P or RuBP.

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) (16).

The k_{cat} (specific activity) of Rubisco was obtained by determining the enzyme content in the extract by a radioimmunoprecipitation technique described by Collatz *et al.* (4) and Evans and Seemann (5). 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}CABP. The bound radiolabel was then determined by liquid scintillation counting. The initial and total Rubisco k_{cat} (mol $CO_2 \cdot mol^{-1}$ Rubisco $\cdot s^{-1}$) were calculated by dividing the initial and total activities by the Rubisco content. The k_{cat} is an intrinsic property of the enzyme; *i.e.* the rate which the enzyme turns over substrate. Our present usage takes into account various metabolic means of regulation which change the apparent k_{cat} without affecting the intrinsic capacity of the enzyme. The value of the initial k_{cat} is dependent upon the state of activation of the enzyme and the concentration of tight-binding inhibitors in the leaf and represents the substrate saturated *in vivo* activity as affected by these regulatory mechanisms. The value of the total k_{cat} is dependent on the concentration of tight-binding inhibitors in the leaf and not on the activation state (15).

RESULTS

We use the terms synthesis and degradation to refer to the changes we observed in whole leaf levels of CA1P (increases and decreases, respectively) following changes in PFD. As we discuss below, changes in CA1P levels observed at the physiological (whole leaf) level provide a measure of the apparent rates of synthesis and degradation of this compound. In fact, these apparent rates are the net result of the specific biochemical reactions which either synthesize or degrade CA1P, *and which may occur simultaneously.* Furthermore, we recognize that what we refer to as CA1P degradation may, in fact, involve a separate release step from the enzyme catalytic site prior to the degradative step (10).

Kinetics of CA1P Synthesis

For *P. vulgaris*, a step decrease in PFD from 1600 μmol quanta m^{-2} s^{-1} to 320, 200, 100, or 0 μmol quanta m^{-2} s^{-1} in all cases resulted in a reduction in the Rubisco total k_{cat} (Fig. 1A). The reductions in total k_{cat} were accompanied by a proportional increase in the pool size of CA1P (Fig. 1B). The initial rate of CA1P synthesis and rate of total k_{cat} decrease in the first min following a step decrease in PFD was not proportional to the final PFD, however. Maximal rates of synthesis occurred when PFD was decreased to 200 μmol quanta m^{-2} s^{-1}. This rate was approximately 4 mmol CA1P mol^{-1} Rubisco catalytic sites s^{-1}, resulting in an amount of CA1P equivalent to approximately one-quarter of the Rubisco catalytic site content being synthesized in less than 1 min, and a steady state pool size being reached in approximately 10 min. At both higher and lower PFDs, the initial rate of CA1P synthesis was lower. Most notably, the rate of CA1P synthesis at 200 μmol quanta m^{-2} s^{-1} was substantially faster than the rate in the dark. However, given sufficient time, the steady state pool size of CA1P will be greater at PFDs below 200 μmol quanta m^{-2} s^{-1} (8, 13). At steady state, the CA1P pool size is maximal in the dark (approximately 1–1.4 mol

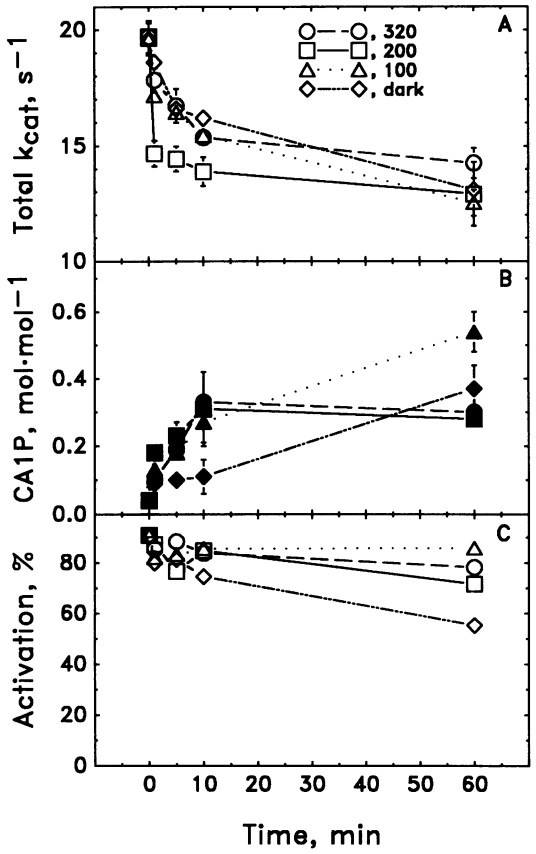

Figure 1. Responses of *P. vulgaris* to a step reduction in PFD from 1600 μmol quanta m^{-2} s^{-1} to either 320, 200, 100 μmol quanta m^{-2} s^{-1} or dark. A, Response of the Rubisco total k_{cat} (mol CO_2 mol^{-1} Rubisco s^{-1}); B, response of the CA1P pool size (mol CA1P mol^{-1} Rubisco catalytic sites); C, response of the Rubisco activation state ([initial activity/total activity]× 100). Each point represents the mean ± SE of 4 to 6 samples taken from separate leaves. In all figures, error bars smaller than the point size are not shown.

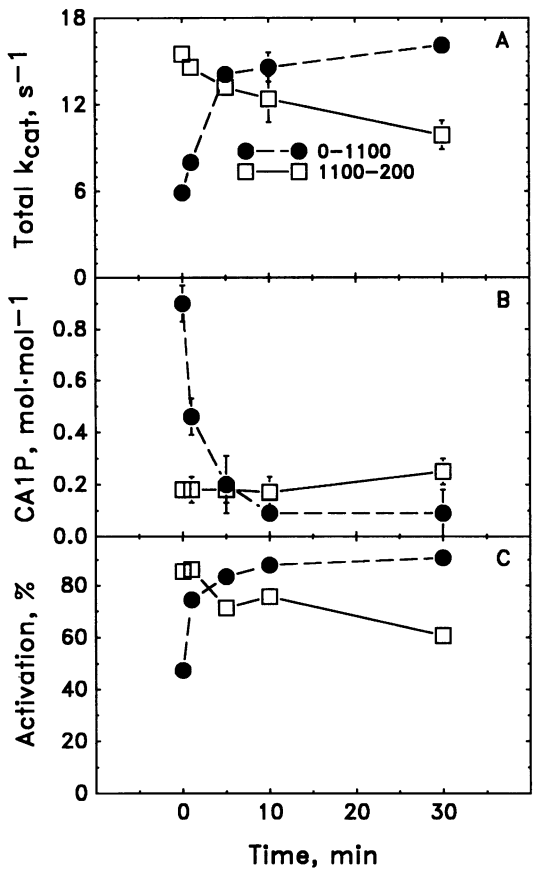

Figure 2. Responses of *B. vulgaris* to either an increase (dark to 1100 μmol quanta m^{-2} s^{-1}) (closed symbols) or a decrease (1100 μmol quanta m^{-2} s^{-1} to 200 μmol quanta m^{-2} s^{-1}) (open symbols) in PFD. A, Response of the Rubisco total k_{cat} (mol CO_2 mol^{-1} Rubisco s^{-1}); B, response of the CA1P pool size (mol CA1P mol^{-1} Rubisco catalytic sites); C, response of the Rubisco activation state ([initial activity/total activity]× 100). Each point represents the mean ± SE of at least three replicates taken from separate leaves.

mol^{-1}) and the Rubisco total k_{cat} is at a minimum (1–2 s^{-1}). At 100 μmol quanta m^{-2} s^{-1}, the CA1P pool size will be approximately 0.5 to 0.7 mol mol^{-1} and the Rubisco total k_{cat} will be approximately 7.5 s^{-1} (see Ref. 8 and Fig. 4). The data in Figure 1 thus indicates that after 60 min in the dark or 100 μmol quanta m^{-2} s^{-1}, the total k_{cat} and CA1P pool size were far from steady state.

The activation state of *P. vulgaris* Rubisco remained relatively constant following the decrease in PFD to 100 μmol quanta m^{-2} s^{-1} during the 60 min time course, and decreased only slightly at 320 and 200 μmol quanta m^{-2} s^{-1} (Fig. 1C). However, the % activation did decline to 55% within 60 min following the complete darkening of the leaf (Fig. 2B). This decline in % activation is an atypical response to PFD changes for *P. vulgaris*. We have previously demonstrated that the enzyme is almost completely activated following an overnight dark period (approximately 12 h) (8, 13). We assume that as CA1P levels increase to steady state dark levels following the darkening of the leaf, the enzyme becomes fully activated again. This activation presumably occurs because CA1P acts as a positive effector for activation as a consequence of its higher affinity for the carbamylated *versus* decarbamylated catalytic site. It should be noted that the activation state of Rubisco in *P. vulgaris* at high light is reduced when photosynthesis is limited by the capacity for starch and sucrose synthesis (19).

In leaves of *Beta vulgaris*, a decrease in PFD from 1100 to 200 μmol quanta m^{-2} s^{-1} resulted in a relatively slow decrease in the total k_{cat} over the subsequent 30 min time period (Fig. 2A) when compared to the rate of change of the total k_{cat} observed in *P. vulgaris* under similar conditions (Fig. 1A). The levels of CA1P increased only slightly over the same time period in *B. vulgaris* (Fig. 2B). The decrease in PFD to 200 μmol quanta m^{-2} s^{-1} also produced a decline in the activation state of Rubisco from 85 to 61% in this species (Fig. 2C), in contrast to *P. vulgaris*.

In *Spinacea oleracea*, a step decrease in PFD from 1100 to 200 μmol quanta m^{-2} s^{-1} resulted in a decrease in the total k_{cat} during the 30 min following the change in PFD (Fig. 3A) at a rate similar to *B. vulgaris*. No CA1P (over the standard background of the assay) could be detected (Fig. 3B). The activation state of Rubisco also declined from 80 to 62% following the step reduction in PFD (Fig. 3C). This deactivation was significantly slower than the rate of CA1P synthesis

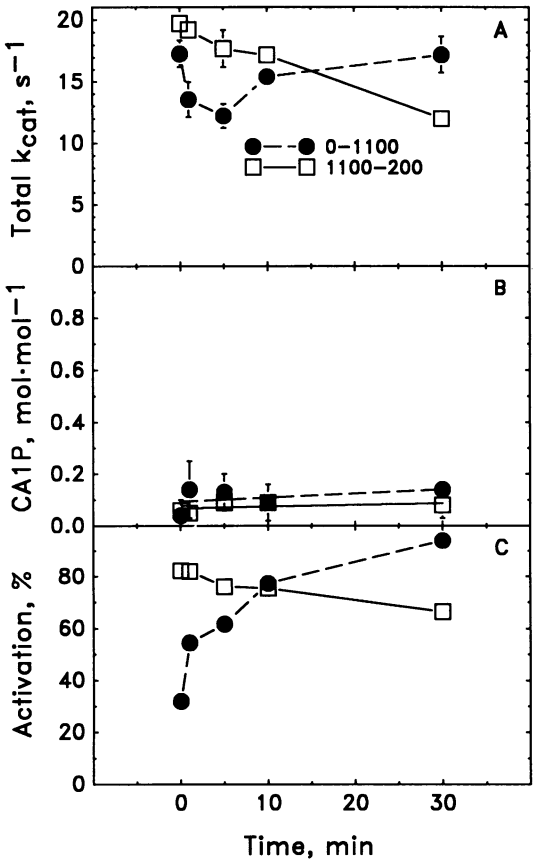

Figure 3. Responses of *S. oleracea* to either an increase (dark to 1100 μmol quanta m^{-2} s^{-1}) (closed symbols) or a decrease (1100 μmol quanta m^{-2} s^{-1} to 200 μmol quanta m^{-2} s^{-1}) (open symbols) in PFD. A, Response of the Rubisco total k_{cat} (mol CO_2 mol^{-1} Rubisco s^{-1}); B, response of the CA1P pool size (mol CA1P mol^{-1} Rubisco catalytic sites); C, response of the Rubisco activation state ([initial activity/total activity]×100). Each point represents the mean ± SE of at least three replicates taken from separate leaves.

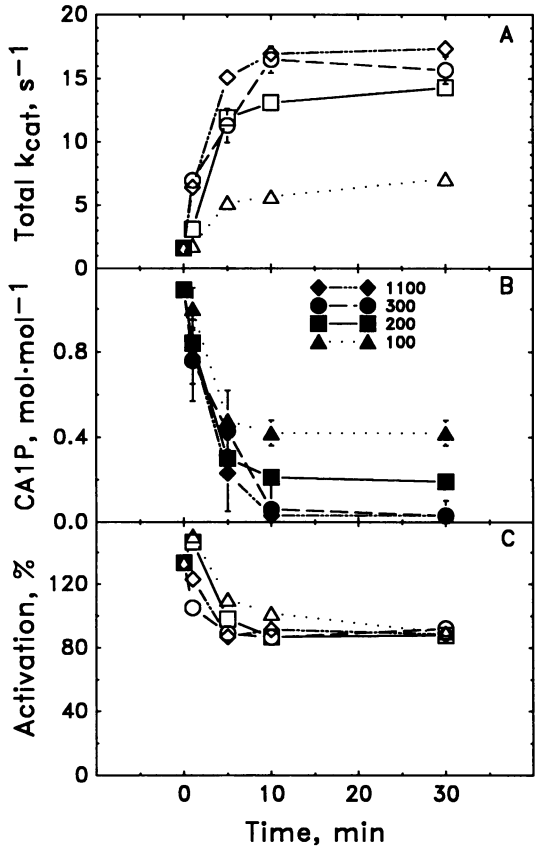

Figure 4. Responses of *P. vulgaris* to a step increase in PFD from the dark to either 100, 200, 300, or 1100 μmol quanta m^{-2} s^{-1}. A, Rubisco total k_{cat} (mol CO_2 mol^{-1} Rubisco s^{-1}); B, response of the CA1P pool size (mol CA1P mol^{-1} Rubisco catalytic sites); C, response of the Rubisco activation state ([initial activity/total activity]×100). Each point represents the mean ± SE of at least three samples taken from separate leaves.

in *P. vulgaris* under similar conditions, not reaching a steady state value until 30 min after the decrease in PFD.

Kinetics of CA1P Degradation

In *P. vulgaris*, a step increase in PFD from dark to a specific PFD resulted in a rapid increase in the total k_{cat} of Rubisco (Fig. 4A) and a proportional decrease in the CA1P pool size (Fig. 4B). At all final PFDs, a new steady state total k_{cat} and CA1P pool size were reached within 10 min following the increase in PFD. The rate of increase of the total k_{cat} in the first min of the response was proportional to PFD from 100 μmol quanta m^{-2} s^{-1} to 320 μmol quanta m^{-2} s^{-1}. The maximal rate of CA1P degradation during the first minute was approximately 6.0 to 6.5 mmol CA1P mol^{-1} Rubisco catalytic sites s^{-1}. Steady state total k_{cat} values and CA1P pool sizes reached after 10 min were also PFD-dependent. The activation percent of *P. vulgaris* Rubisco (Fig. 4C) was above 100% in the dark (an artifact of the assay; see Ref. 15) and decreased following the increase in PFD, reaching a steady state level of 90 to 100% by 10 min at all PFDs.

In *B. vulgaris*, a step increase from dark to 1100 μmol quanta m^{-2} s^{-1} produced a three-fold increase in total k_{cat} values and a concomitant decrease in the level of CA1P after 10 min of illumination (Fig. 2, A and B). The initial rate of CA1P degradation in this species was similar to *P. vulgaris*. The Rubisco activation state increased from 47 to 91% over the same time period (Fig. 2C), in contrast to *P. vulgaris*.

In *S. oleracea*, a step increase in PFD from dark to 1100 μmol quanta m^{-2} s^{-1} resulted in a decrease in the Rubisco total k_{cat} during the first 5 min, from approximately 17 s^{-1} to 12 s^{-1} (Fig. 3A). The total k_{cat} then rose slowly, reaching a steady state level after 30 min at high light that was equal to the dark value. We could detect no significant change above the standard background of our assay in the pool size of a tight binding inhibitor during this same time period (Fig. 3B). The Rubisco activation state was approximately 32% in the dark and increased continuously to 94% following illumination (Fig. 3C).

The initial k_{cat} of Rubisco is representative of the *in vivo* activity of the enzyme as influenced by all regulatory mechanisms. The similar patterns of the initial k_{cat} in all three species following the step decrease in PFD from high to 200 μmol quanta m^{-2} s^{-1} (Fig. 5A) and the step increase from dark to 1100 μmol quanta m^{-2} s^{-1} (Fig. 5B) demonstrates the similarity of the regulation of Rubisco activity to changing

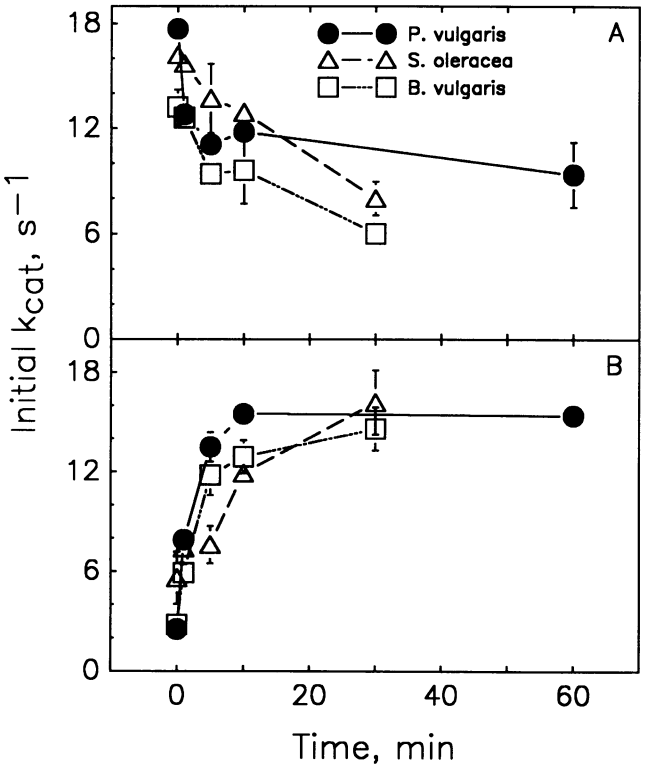

Figure 5. Response of the Rubisco initial k_{cat} in *P. vulgaris*, *B. vulgaris*, and *S. oleracea* to either a step decrease or increase in PFD. A, response of the Rubisco initial k_{cat} (mol CO_2 mol^{-1} Rubisco s^{-1}) for all three species following a step decrease in PFD (1100 to 200 μmol quanta m^{-2} s^{-1}); B, response of the Rubisco initial k_{cat} for all three species to a step increase in PFD (dark to 1100 μmol quanta m^{-2} s^{-1}). Each point represents the mean ± SE of at least three replicates taken from separate leaves.

PFD regardless of the mechanisms involved in its regulation. The regulation of Rubisco activity by CA1P in *P. vulgaris* was at least as fast as the regulation of the enzyme in the other species, which possess a reduced or absent capacity for regulation of Rubisco activity by CA1P. Similar results have been observed both under steady state gas exchange conditions (8) and during natural diurnal changes in PFD (7).

DISCUSSION

We provide evidence here that the *in vivo* synthesis of CA1P is light stimulated (Fig. 1). However, light is not absolutely required for synthesis, since it is well known that CA1P can be produced in leaves in the dark. These results suggest that there is either an energy requirement for CA1P synthesis that can also be met in the dark (*i.e.* nonphotosynthetic) or that a light regulated enzyme may be involved in the synthetic pathway. In *Phaseolus vulgaris*, the rate of CA1P synthesis was maximal at 200 μmol quanta m^{-2} s^{-1}, and was apparently reduced at lower (and higher) PFDs. A detailed analysis of the kinetics of CA1P metabolism is difficult, however, since the individual reactions of synthesis and degradation cannot be separated in our *in vivo* studies (Figs. 1 and 4). We hypothesize that both the rate of CA1P synthesis and its final level at any particular PFD are the net result of the competing energy-dependent reactions of CA1P synthesis and degradation, with steady state levels of CA1P a consequence of a pseudo-steady state balance of the two reactions. We hypothesize that CA1P synthesis proceeds at all PFDs, but that this compound cannot be detected at PFDs in excess of 500 μmol quanta m^{-2} s^{-1} (8) because of a substantially higher rate of CA1P degradation than synthesis at these PFDs.

The rates at which the total k_{cat} decreased and the CA1P pool size increased in *Beta vulgaris* following the transition from 1100 to 200 μmol quanta m^{-2} s^{-1} (Fig. 2, A and B) were markedly slower than in *P. vulgaris* under similar conditions (Fig. 1, A and B). This slow rate of Rubisco regulation by CA1P has been observed in other species (*e.g. Nicotiana rustica, Alocasia macrorrhiza*) which also lack the capacity to synthesize as high a dark level of CA1P as does *P. vulgaris* (8, 11, 14). The fact that the increase in the CA1P pool size was not sufficient to account for the decrease in total k_{cat} in *B. vulgaris* suggests that noncatalytic binding of RuBP to the inactive form of the enzyme may also have been contributing to the reduced catalytic capacity of Rubisco (see below and Ref. 8).

In *Spinacea oleracea*, a step decrease in PFD from 1100 to 200 μmol quanta m^{-2} s^{-1} produced a depression in the total k_{cat} of Rubisco (Fig. 3A), indicating some effect on the catalytic capacity of Rubisco. However, this loss of activity could not be attributed to CA1P since this species apparently does not synthesize this compound (Fig. 3B). There is increasing evidence that this reduction in the total k_{cat} was the result of noncatalytic RuBP binding to deactivated sites *in vivo* (2, 3, 8, 15). This inhibition of Rubisco activity by RuBP is consistent with the facts that the enzyme was deactivating and RuBP levels were increasing during this time period (Fig. 3C; and data not shown), as was the case with *B. vulgaris*. Therefore, changes in both the activation state of Rubisco and possibly the extent of noncatalytic RuBP binding contributed to the reduction of Rubisco activity in *S. oleracea* during the period following the decrease in PFD. The extent of noncatalytic RuBP binding in *B. vulgaris* and *S. oleracea* was presumably controlled by the activity of Rubisco activase (20).

An important question is whether RuBP is acting as an effector of Rubisco activity when bound noncatalytically to the enzyme. Noncatalytic tight-binding of RuBP must occur only after deactivation of the enzyme has taken place, and is thus in a sense redundant. However, it is possible that RuBP, once bound to the deactivated catalytic site, acts to stabilize the enzyme in the inactive form against a pressure toward activation provided by Rubisco activase (albeit, a reduced pressure at lower PFDs compared to high PFDs). The noncatalytic binding of RuBP could therefore be important in the maintenance of the lower activation state of the enzyme at lower PFDs.

The mechanism by which inhibition of Rubisco activity by CA1P is relieved and this compound modified to a noninhibitory form is becoming clearer. The process is light dependent (13, 17, 21) and can be inhibited by compounds which interfere with electron transport (11, 13). Rubisco activase has been shown to promote the ATP-dependent displacement of CA1P from Rubisco *in vitro* (10). A second chloroplast protein can promote the NADPH-dependent conversion of CA1P to a noninhibitory form *in vitro* (12). Data presented

in Figure 4 for *P. vulgaris* indicates that light-dependent relief of inhibition of Rubisco activity by CA1P is dependent not only upon the release of CA1P from the enzyme, but also upon the breakdown (or modification) of CA1P, since a good correlation exists between enzyme activity and the level of CA1P.

Upon illumination of leaves of *P. vulgaris* which had been in the dark for an extended time period, there was a rapid rise in the catalytic capacity of Rubisco when leaves were illuminated. This increase was due almost exclusively to degradation of CA1P in this species (Fig. 4). During the induction response in *B. vulgaris* the catalytic capacity of Rubisco increased during the first 10 min at a rate similar to *P. vulgaris*. However, in contrast to *P. vulgaris*, the change in catalytic capacity of Rubisco in *B. vulgaris* was due to both CA1P degradation and activation of the enzyme (Fig. 2). During the induction response in *S. oleracea*, a transient depression in the total k_{cat} (Fig. 3A) was observed that was not related to the presence of CA1P, but rather was presumably due to the noncatalytic binding of RuBP to the deactivated sites on the enzyme, as described above. The enzyme was substantially deactivated in the dark (time zero) (Fig. 3C), but the total k_{cat} was high since no RuBP was available for noncatalytic binding. Upon illumination, the RuBP pool size increased (data not shown) and noncatalytic RuBP binding apparently occurred, as indicated by the decrease in the total k_{cat} (Fig. 3A). After 10 min, the activation state had risen substantially and noncatalytic RuBP binding had been substantially relieved, presumably by Rubisco activase. After 30 min, we could detect no inhibition by RuBP. *S. oleracea* also contains low levels (relative to tobacco, which produces moderate levels of CA1P) of the chloroplast protein involved in the conversion of CA1P to a noninhibitory form (12). The significance of this finding is as yet unclear.

The similarity of response of Rubisco activity to changes in PFD among species which differ in the mechanisms used for this regulation (Fig. 5; Refs. 7, 8) strongly suggests a common element in all plants as the cue for determining the appropriate activity of Rubisco at a particular PFD. This light-dependent control of Rubisco activity involves at least two regulatory systems, Rubisco activase and CA1P metabolism, which may be interlinked. These regulatory systems control the degree of carbamylation of Rubisco and the extent of binding of inhibitors. Evidence suggests that these regulatory systems may respond to a common metabolic signal which reflects the light-dependent energy status of the cell (9, 11–13, 19) and is independent of the specific mechanism(s) used for the light-dependent regulation of Rubisco activity.

ACKNOWLEDGMENTS

We thank Judy Miles and Dianne Stortz-Lintz for their expert technical assistance.

LITERATURE CITED

1. **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
2. **Brooks A, Portis AR** (1988) Protein-bound ribulose bisphosphate correlates with deactivation of ribulose bisphosphate carboxylase in leaves. Plant Physiol **87**: 244–249
3. **Cardon ZG, Mott KA** (1989) Evidence that RuBP binds to inactive sites of RuBP carboxylase *in vivo* and an estimate of the rate constant of dissociation. Plant Physiol (in press)
4. **Collatz GJ, Badger MR, Smith C, Berry JA** (1979) A radioimmune assay for RuP_2 carboxylase protein. Carnegie Inst Wash Year Book **78**: 171–175
5. **Evans JR, Seemann JR** (1984) Differences between wheat genotypes in specific activity of RuBP carboxylase and the relationship to photosynthesis. Plant Physiol **74**: 759–765
6. **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
7. **Kobza J, Seemann JR** (1989) Regulation of ribulose-1,5-bisphosphate carboxylase activity in response to diurnal changes in irradiance. Plant Physiol (in press)
8. **Kobza J, Seemann JR** (1988) Mechanisms for the light-dependent regulation of ribulose-1,5-bisphosphate carboxylase activity and photosynthesis in intact leaves. Proc Natl Acad Sci USA **85**: 3815–3819
9. **Robinson SP, Portis AR** (1988) Involvement of stromal ATP in the light activation of ribulose-1,5-bisphosphate carboxylase/oxygenase in intact isolated chloroplasts. Plant Physiol **86**: 293–298
10. **Robinson SP, Portis AR** (1988) Release of the nocturnal inhibitor, carboxyarabinitol-1-phosphate, from ribulose bisphosphate carboxylase/oxygenase by rubisco activase. FEBS Lett **233**: 413–416
11. **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
12. **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
13. **Seemann JR, Berry JA, Freas SM, Krump MA** (1985) Regulation of ribulose bisphosphate carboxylase activity *in vivo* by a light-modulated inhibitor of catalysis. Proc Natl Acad Sci USA **82**: 8024–8028
14. **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
15. **Seemann JR, Kobza J** (1988) Genetic variation in the regulation of ribulose-1,5-bisphosphate carboxylase activity. Plant Physiol Biochem **26**: 461–471
16. **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
17. **Servaites JC** (1985) Binding of a phosphorylated inhibitor to ribulose bisphosphate carboxylase/oxygenase during the night. Plant Physiol **78**: 839–843
18. **Servaites JC, Parry MAJ, Gutteridge S, Keys AJ** (1986) Species variation in the predawn inhibition of ribulose-1,5-bisphosphate carboxylase/oxygenase. Plant Physiol **82**: 1161–1163
19. **Sharkey TD, Seemann JR, Berry JA** (1986) Regulation of ribulose-1,5-bisphosphate carboxylase activity in response to changing partial pressure of O_2 and light in *Phaseolus vulgaris*. Plant Physiol **81**: 788–791
20. **Streusand VJ, Portis AR** (1987) Rubisco activase mediates ATP-dependent activation of ribulose bisphosphate carboxylase. Plant Physiol **85**: 152–154
21. **Vu JCV, 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
22. **Vu JCV, Allen LH, Bowes G** (1984) Dark/light modulation of ribulose bisphosphate carboxylase activity in plants from different photosynthetic categories. Plant Physiol **76**: 843–845