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Biphasic Activation of Ribulose Bisphosphate Carboxylase in Spinach Leaves as Determined from Nonsteady-State CO_2 Exchange[1]

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ABSTRACT

The activation kinetics of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) following an increase in photon flux density (PFD) were studied by analyzing CO_2 assimilation time courses in spinach leaves (*Spinacia oleracea*). When leaves were exposed to 45 minutes of darkness before illumination at 690 micromoles per square meter per second, Rubisco activation followed apparent first-order kinetics with a relaxation time of about 3.8 minutes. But when leaves were illuminated for 45 minutes at 160 micromoles per square meter per second prior to illumination at 690 micromoles per square meter per second the relaxation time for Rubisco activation was only 2.1 minutes. The kinetics of this change in relaxation times were investigated by exposing dark-adapted leaves to 160 micromoles per square meter per second for different periods before increasing the PFD to 690 micromoles per square meter per second. It was found that the apparent relaxation time for Rubisco activation changed from 3.8 to 2.1 minutes slowly, requiring at least 8 minutes for completion. This result indicates that at least two sequential, slow processes are involved in light-mediated activation of Rubisco in spinach leaves and that the relaxation times characterizing these two processes are about 4 and 2 minutes, respectively. The kinetics of the first process in the reverse direction and the dependence of the relaxation time for the second process on the magnitude of the increase in PFD were also determined. Evidence that the first slow process is activation of the enzyme Rubisco activase and that the second slow process is the catalytic activation of Rubisco by activase is discussed.

It is well established that the activity of the enzyme Rubisco is the principal "biochemical" limitation to the steady-state rate of CO_2 assimilation in C_3 plants at relatively high PFD[2] values and ambient or lower-than-ambient CO_2 concentrations (see review by Woodrow and Berry [18]). There are two basic lines of evidence that support this view. First, under high PFD conditions, Rubisco appears to operate at the maximum rate allowed by the prevailing CO_2 and O_2 concentrations: Rubisco is generally fully activated (16, 18), there is little if any of the inhibitor CA1P present (15), and most of the enzyme is at any time in a complex with the substrate RuBP (18). Second, at relatively high PFD values the response of photosynthetic rate to changes in the partial pressures of CO_2 and O_2 is consistent with the kinetic parameters of Rubisco for CO_2 and O_2 as determined using purified enzyme. Assuming that the principal effect of these gases on the photosynthetic system is on the activity of Rubisco, this effect indicates that the rate of photosynthesis is sensitive to changes in Rubisco activity (5, 7). This assumption has been used to calculate that, in C_3 plants at relatively high PFD values, the rate of photosynthesis is approximately proportional to the carboxylase activity of Rubisco (17, 19, 21).

Similar lines of evidence substantiate the hypothesis that Rubisco activity is important in determining the rate of nonsteady-state photosynthesis in C_3 leaves following a rapid change from darkness to a relatively high PFD (10). First, during much of the time course for nonsteady-state photosynthesis, the RuBP concentration in spinach leaves is high enough that most of the active Rubisco is at any time in a complex with this substrate (15, 20). Second, in contrast to steady-state photosynthesis, the proportion of active Rubisco increases with time during the nonsteady state, and this increase occurs in parallel with the increase in photosynthesis during a mathematically resolvable, slow phase of the time course for photosynthesis (20, see also refs. 11, 15). Finally, by varying the length of the dark period preceding the illumination of the spinach leaf, Woodrow and Mott (20) poised the amount of active Rubisco present at the onset of illumination at different levels. When Rubisco was almost fully activated at the time of illumination, the increase in photosynthesis rate during the slow phase contributed very little to the total increase in photosynthesis after illumination. But when the initial dark period was relatively long and Rubisco was largely inactive prior to illumination, the rise in assimilation rate during the slow phase was proportionally greater. In terms of our current understanding of the regulation of Rubisco activity, these observations lead logically to the conclusion that Rubisco activity largely determines the rate of photosynthesis during the slow phase of nonsteady-state photosynthesis defined by Woodrow and Mott (20). The kinetics of the slow phase in the time course for photosynthesis are

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[2] Abbreviations: PFD, photon flux density; RuBP, ribulose 1,5-bisphosphate; c_i, intercellular CO_2 concentration; τ, relaxation time; ER, inactive Rubisco:RuBP complex; ECMR, active Rubisco:CO_2:Mg^{2+}:RuBP complex.

therefore a unique indicator of the activation of Rubisco *in vivo*.

Jackson *et al.* (3) used the kinetics of the slow phase to investigate the effect of initial PFD on the rate of Rubisco activation following an increase in PFD. They showed that the relaxation time[3] characteristic of Rubisco activation following an increase in PFD was approximately 3 min for leaves exposed to initial PFD values below 130 μmol m^{-2} s^{-1} but changed in a roughly sigmoidal fashion to about 1.5 min for initial PFD values higher than 130 μmol m^{-2} s^{-1}. These workers proposed that Rubisco activation *in vivo* may proceed by a process that involves two relatively slow steps. They also proposed that the 3 min relaxation during the dark to high light transition reflects the relaxation of both processes, whereas the 1.5 min relaxation reflects just the second slow step (see "Appendix").

In this study, we use the mathematically resolvable slow phase in the assimilation time course of spinach leaves following a rapid rise in PFD as a probe of the activation kinetics of Rubisco. We examine the kinetics of the two proposed phases of Rubisco activation following an increase in PFD in spinach leaves. We then derive relaxation times for both phases and present equations that can be used to predict the overall apparent relaxation time for Rubisco activation following an increase in PFD from any initial PFD. The kinetics of one of the phases shows remarkable similarity to those of the activation of Rubisco activase (6). Evidence is discussed that activase activation together with activase-mediated activation of Rubisco are reflected in the assimilation time course following illumination.

MATERIALS AND METHODS

Plants of spinach (*Spinacia oleracea* L.) were grown in controlled-environment growth chambers in aerated, half-strength Hoagland solution. The day and night temperatures were 25 and 20°C, respectively, the photoperiod was 10 h, and the PFD during the photoperiod was 350 μmol m^{-2} s^{-1}. Gas exchange measurements were made with the single-pass system described elsewhere (9, 20). The rate of CO_2 assimilation was normalized to a c_i of 250 ppm by assuming that the relationship between the photosynthetic rate and c_i was linear and passed through the compensation point (20). This procedure compensated for the effect of changing c_i on photosynthesis. Leaf temperature was 25°C in all gas-exchange experiments.

RESULTS

When spinach leaves were held in darkness for 1 h before illumination, there was an initial, rapid increase in the rate of CO_2 assimilation followed by a slower increase to the steady-state value (Fig. 1). As discussed in the introduction (see also ref. 10), this slow increase in assimilation rate appears to be largely due to the activation of Rubisco and to changes in stomatal conductance, which both result in a change in c_i. Because the plants used in this study were grown hydroponically and because of the high humidity in the leaf chamber, stomatal conductance in darkness was relatively high, and it increased very slowly following an increase in PFD (data not shown, but see refs. 3 and 20). Therefore, most of the change in c_i during the first 10 min after the increase in PFD was caused by the increase in photosynthesis. The effect of changes in c_i on photosynthesis was removed, however, by normalizing the photosynthesis rate to a constant c_i of 250 ppm as described in "Materials and Methods." This normalization allows a clear resolution of the change in assimilation rate due to Rubisco activation (20).

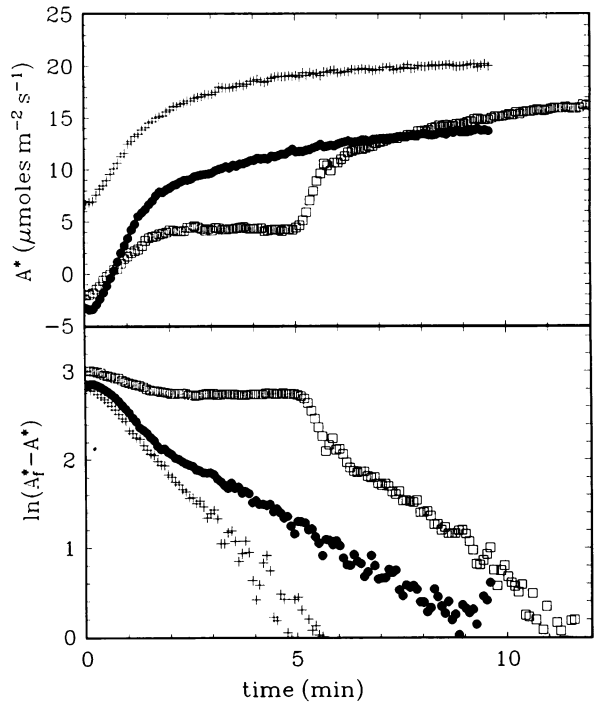

Figure 1. Time courses for photosynthesis following an increase in PFD. The upper panel shows normalized photosynthesis (A^*) time courses for the same leaf but for three different treatments. The solid circles show the time course when the leaf was placed in darkness for 45 min before increasing the PFD to 690 μmol m^{-2} s^{-1}. The crosses show time course when the leaf was exposed to a PFD of 160 μmol m^{-2} s^{-1} for 45 min before increasing the PFD to 690 μmol m^{-2} s^{-1}. The open squares show data for the leaf when it was first placed in darkness for 45 min and then exposed to 160 μmol m^{-2} s^{-1} for 5 min before increasing the PFD to 690 μmol m^{-2} s^{-1}. The lower panel shows the same data plotted as the natural logarithm of the difference between the final steady-state normalized photosynthesis rate and the rate at each time. The slope of the linear phase occurring approximately 1 min after the increase to 690 μmol m^{-2} s^{-1} equals $(-1/\tau)$ for that treatment. The solid circles allow calculation of τ_{s0}; the crosses, τ_{s2}; and the open squares, τ_s for a leaf illuminated for 5 min at 160 μmol m^{-2} s^{-1}.

To resolve and characterize the component of the increase in photosynthetic rate attributable to increases in Rubisco activity, $ln(A_f^* - A_t^*)$, was plotted *versus* time, where A_f^* and A_t^* are the normalized rates of CO_2 assimilation in the final steady state and at any time, t, respectively (20) (Fig. 1, lower panel). The absolute slope of the linear portion of this semi-

[3] Relaxation time is defined as the inverse of the rate constant describing the transition from one steady state to another.

logarithmic plot (m) is equal to a rate constant (k_a), which is characteristic of Rubisco activation (20). However, we routinely describe Rubisco activation using a relaxation time (τ_s) defined as follows:

$$\tau_s = \frac{-1}{m}, \quad (1)$$

where the subscript s indicates that the process is slow (*i.e.* several minutes) compared with the response times of most of the processes in the photosynthetic system. The intercept of the line of slope m with the ordinate axis is $ln(A_f^* - A_i^*)$, where A_i^* is the rate of CO_2 assimilation that would have occurred had Rubisco not been activated beyond the initial level (20).

When a spinach leaf was initially placed in darkness for at least 45 min and then illuminated at a PFD of 690 μmol m^{-2} s^{-1}, the assimilation time course reflected slow activation of Rubisco with a τ_s value of about 3.8 min (henceforth called τ_{s0}) (Fig. 1). However, when leaves were initially illuminated at a PFD value of 160 μmol m^{-2} s^{-1} for 45 min, Rubisco activated relatively quickly following the change in PFD to 690 μmol m^{-2} s^{-1}. A relaxation time of about 2 min (henceforth called τ_{s2}) characterized Rubisco activation under these conditions (Fig. 1). Previous studies (3) have shown that the change between τ_{s0} and τ_{s2} occurs at an initial PFD of about 135 μmol m^{-2} s^{-1}, and this suggests that there is a change in the photosynthetic system occurring at about 135 μmol m^{-2} s^{-1}, which allows Rubisco to activate twice as quickly as it does in dark-adapted leaves.

To clarify the processes responsible for these differences in the rate of Rubisco activation, we examined the kinetics of the transition from τ_{s0} (characteristic of the dark- to high-PFD transition) to τ_{s2} (characteristic of the low- to high-PFD transition) using a two-step increase in PFD. We placed leaves in darkness for 45 min, then exposed them to 160 μmol m^{-2} s^{-1} for various times before increasing the PFD to 690 μmol m^{-2} s^{-1}. We compared the resulting time course to time courses for leaves that had been (a) held in darkness for 45 min before an increase to 690 μmol m^{-2} s^{-1} (to determine τ_{s0}), or (b) held at 160 μmol m^{-2} s^{-1} for 45 min before an increase to 690 μmol m^{-2} s^{-1} (to determine τ_{s2}). The result of one such experiment, in which the leaf was held at 160 μmol m^{-2} s^{-1} for 5 min before being exposed to 690 μmol m^{-2} s^{-1}, is shown in Figure 1. In this experiment, τ_{s2} (160–690 μmol m^{-2} s^{-1}) was 2.1 min, whereas τ_{s0} (dark to high PFD) was 3.8 min, and the τ_s for the leaf exposed to 5 min at a PFD of 160 μmol m^{-2} s^{-1} was 3.1 min.

A series of these two-step experiments was done to characterize the kinetics of the process or processes responsible for the change in the rate of activation of Rubisco. It was found that the τ_s value changed slowly between τ_{s0} and τ_{s2} (*i.e.* between 3.8 and 2.1 min) as the time of illumination at 160 μmol m^{-2} s^{-1} was increased (Fig. 2). Because the scatter in the data made it impossible to accurately resolve the kinetics of the relaxation from τ_{s0} to τ_{s2}, we used the simplest possible model for the process—a single slow step characterized by a first-order rate constant. The curve drawn through the data in Figure 2 reflects a first-order rate constant of about 0.259 min^{-1} for the slow transition from τ_{s0} to τ_{s2}; it is not a "best fit" to the data. The inverse of this rate constant, the relaxation time (see Eq. 1), will henceforth be termed τ_{s1}.

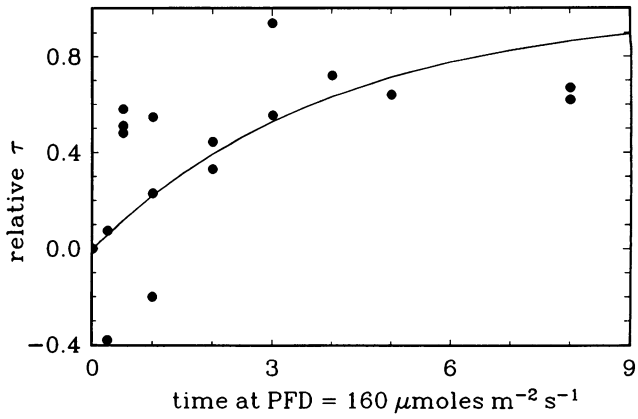

Figure 2. The transition from τ_{s0} to τ_{s2}. Leaves were treated as described in Figure 1 except that they were held for various times at 160 μmol m^{-2} s^{-1} before being illuminated at 690 μmol m^{-2} s^{-1}. The apparent relaxation time of the slow phase for each treatment τ_s was determined from the semi-logarithmic plot of the photosynthesis time course (*e.g.* see Fig. 1) and was expressed relative to τ_{s0} (0–690 μmol m^{-2} s^{-1}) τ_{s2} (160–690 μmol m^{-2} s^{-1}) for that leaf as follows: relative $\tau = (\tau_{s0} - \tau_s)/(\tau_{s0} - \tau_{s2})$. The solid line shows an exponential function (relative $\tau = 1 - e^{-t/\tau}$, where t is the time at 160 μmol m^{-2} s^{-1}) with a relaxation time (τ) of 3.9 min (SE = 2.9 min), which was determined by a nonlinear regression in which the relative τ was constrained to vary between 0 and 1.

To examine the kinetics of the transition from fast to slow Rubisco activation (*i.e.* τ_{s2}–τ_{s0}), a second series of two-step experiments was conducted in which a leaf was first illuminated at 160 μmol m^{-2} s^{-1} for 45 min, then exposed to a period of darkness before reillumination at 690 μmol m^{-2} s^{-1}. The relaxation time of the slow phase depended upon the length of the preceding dark period (Fig. 3); the τ_s value changed from τ_{s2} to τ_{s0} as the length of the dark period increased. Due to the scatter in the data, we also chose to fit an exponential curve to these data (see Fig. 3); the relaxation time in this case was about 28 min.

Last, the effect of the final PFD on the kinetics of Rubisco activation was determined by analyzing the kinetics of the slow phase for PFD transitions from 160 μmol m^{-2} s^{-1} to various higher PFD values. Both the relaxation time for the slow phase (τ_{s2}) and the final assimilation rate (A_f^*) increased with the final PFD. Moreover, the overall change in the assimilation rate relative to A_i^*, which is directly proportional to the change in the proportion of active Rubisco (20), and the τ_{s2} value were approximately proportional (Fig. 4).

DISCUSSION

These results indicate that at least two relatively slow processes are involved in the activation of Rubisco following an increase in PFD. The relaxation of the first slow process was observed indirectly by illuminating dark-adapted leaves at 160 μmol m^{-2} s^{-1} for different periods (Figs. 1 and 2). In these experiments, the amount of fully active Rubisco changed very little from darkness to 160 μmol m^{-2} s^{-1}, but the first slow

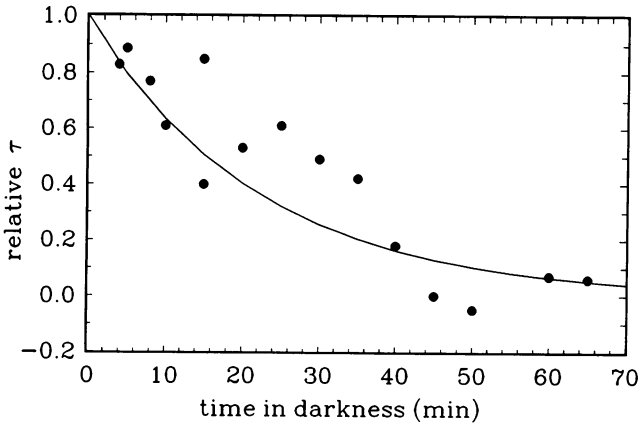

Figure 3. The transition from τ_{s2} to τ_{s0}. Leaves were placed at 160 μmol m^{-2} s^{-1} for 45 min and then exposed to darkness for different times before increasing the PFD to 690 μmol m^{-2} s^{-1}. The apparent relaxation time of the slow phase for each treatment τ_s was determined from the semi-logarithmic plot of the photosynthesis time course (*e.g.* see Fig. 1) and was expressed relative to the τ_{s0} (0–690 μmol m^{-2} s^{-1}) and τ_{s2} (160–690 μmol m^{-2} s^{-1}) for that leaf as follows: relative $\tau = (\tau_{s0} - \tau_s)/(\tau_{s0} - \tau_{s2})$. The solid line shows an exponential function (relative $\tau = e^{-t/\tau}$, where t is the time in darkness) with a relaxation time of 28.4 min (SE = 6.8 min), which was determined by a nonlinear regression in which the relative τ was constrained to vary between 0 and 1.

process apparently proceeded to completion with a relaxation time (τ_{s1}) of about 3.9 min (Fig. 2). This procedure also allowed us to resolve a relaxation time of approximately 2 min for the second slow process from the rate of Rubisco activation following illumination of a leaf at 160 μmol m^{-2} s^{-1} for at least 45 min. Although both processes are apparently light dependent, the first process appears light-saturated at a PFD value of approximately 135 μmol m^{-2} s^{-1} (3), and the second saturates at a much higher PFD. Furthermore, the two processes must be in series, such that the second process depends on the first.

The possibility that the first slow phase represents simply the autocatalytic buildup of RuBP can be ruled out on two grounds. First, direct measurement of the RuBP concentration in leaves showed that the maximum (and quasi-steady-state) concentration of this metabolite is reached within 1 min of illumination (15, 20). Second, when leaves were illuminated at 160 μmol m^{-2} s^{-1} for 45 min and then either (a) placed in darkness for 2 min before reillumination at 690 μmol m^{-2} s^{-1} or (b) illuminated at 690 μmol m^{-2} s^{-1}, the slow phase in the assimilation time course was the same in both cases and was described by τ_{s2}. The difference between the two treatments was that in the first case the RuBP concentration would have been close to zero upon illumination at 690 μmol m^{-2} s^{-1} (2, 4), whereas in the second case the RuBP concentration would have been relatively high. Therefore, these results demonstrate that the buildup of RuBP and the first slow phase (characterized by τ_{s1}) are distinct processes.

There are two basic types of mechanism that are consistent with these data and those of Jackson *et al.* (3). The first mechanism involves the slow interconversion of Rubisco or activase, or both enzymes, between structurally and kinetically distinct forms. This interconversion would occur in addition to the slow production of ECMR from ER. The second possible mechanism involves the slow modification of the kinetic properties of Rubisco or activase in response to a slow change in the concentration of effectors of these enzymes. There are, however, three reasons why the kinetics described in this study may best be interpreted in terms of the first of the above mechanisms. (a) As Rubisco is largely saturated with RuBP under the conditions in which both slow relaxations occur (15, 20), it is highly unlikely that a slow change in the level of any competitive effector of Rubisco activity could cause the first slow relaxation (characterized by τ_{s1}). (b) Although slow activation of other photosynthetic carbon reduction cycle enzymes (*e.g.* sedoheptulose 1,7-bisphosphatase, fructose 1,6-bisphosphatase, and ribulose 5-phosphate kinase) could conceivably cause slow changes to the ATP/ADP ratio, which could in turn affect activase activity, there is no evidence that such changes in the adenylate ratio are sufficient to cause the slow phase observed in this study (see ref. 18). (c) Recent studies by Campbell and Ogren (1) have shown that illuminated thylakoid membranes may influence the activation of Rubisco in a manner that is quite independent of the provision of ATP for the activase reaction. Moreover, Lan *et al.* (6) have shown that Rubisco activase undergoes a light-dependent increase in activity requiring a relatively low PFD and about 10 min for completion. It is this process that we propose is reflected by τ_{s1}. It should be emphasized, however, that the ability of the model presented in the "Appendix" to predict assimilation time courses following a rapid rise in PFD does not depend upon the identity of the mechanism underlying the first slow relaxation.

If we accept the hypothesis that the first slow phase represents the activation of activase, then our data show that this process is characterized by a τ of about 3.9 min and that the inactivation of this enzyme in darkness proceeds very slowly indeed (τ = 28.4 min) (Fig. 3). It is noteworthy that the latter

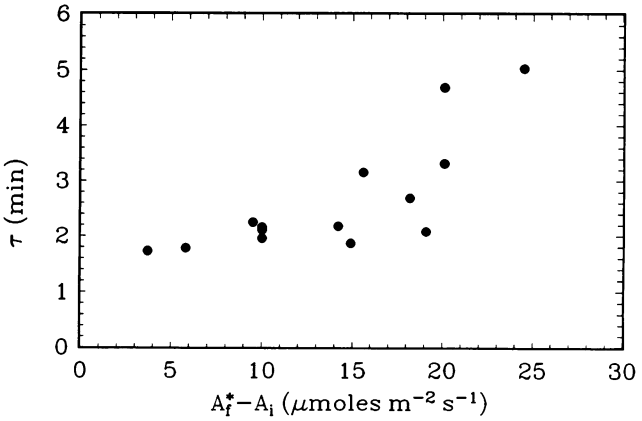

Figure 4. The relaxation time for the slow phase (τ) as a function of the increase in assimilation due to the slow phase ($A_f^* - A_i^*$). Leaves were held at 160 μmol m^{-2} s^{-1} for 45 min before increasing the PFD to various levels. The relaxation time for the slow phase (τ) and value of $A_f^* - A_i^*$ were determined from the semi-logarithmic plots of the photosynthesis time courses as discussed in the text.

relaxation time is very similar to that characterizing Rubisco inactivation in darkness (20). It is unlikely, however, that continuing activase activity in darkness is responsible for the slow inactivation of Rubisco. Cardon and Mott (2), for example, demonstrated that the RuBP pool is depleted within 1 min of the cessation of illumination, which indicates that the ER, a substrate of Rubisco activase (12), will be absent for much of the inactivation process (see ref. 2). Slow inactivation of Rubisco, therefore, most probably reflects uncatalyzed decarbamylation of the active enzyme (8).

The second slow phase in our experiments most probably reflects activase-catalyzed activation of Rubisco. This can be deduced from considerations of the thermodynamics of the Rubisco regulatory mechanism, which indicate that, shortly after illumination, most Rubisco will be as ER (18). The ER complex can be readily converted into the active ECMR complex by activase (12).

Variations in the final PFD to which the leaves were exposed also influenced the value of τ_{s2}. As the final PFD was increased, and with it the final, steady-state activation state of Rubisco, the relaxation time for Rubisco activation also increased beyond the τ_{s2} value of 2.1 min observed for a final PFD of 690 μmol m^{-2} s^{-1}. These changes can be interpreted by considering the equation describing the rate of change in the corrected assimilation rate following a rise in PFD (20):

$$\frac{dA^*}{dt} = \left(\frac{A_f^* - A_i^*}{\tau}\right) e^{-t/\tau} \quad (2)$$

When $t = 0$, the rate of increase in A^* ($A'_{t=0}$) is given by ($A_f^* - A_i^*/\tau$. The data in Figure 4 indicate, therefore, that this initial rate of increase in A^* (which is proportional to the initial velocity of Rubisco activation) may be independent of the final PFD, and the data indicate that the $A'_{t=0}$ value is approximately 7.2 μmol m^{-2} s^{-1}. This approximate constancy in initial Rubisco activation rate is convenient for modeling the activation of Rubisco because it allows τ_{s2} to be predicted from A_f^* and A_i^* values. These values can be calculated from the steady-state relationship between assimilation rate and PFD curve when the initial and final PFD values are known. If, however, the initial PFD is less than about 135 μmol m^{-2} s^{-1} (and therefore the relaxation time for Rubisco activation is greater than τ_{s2}), then a correction for τ_{s1} must be made.

Simulations of Rubisco activation using Equations 11 and 12 (see "Appendix") showed that assuming 100% activation of activase at 300 μmol m^{-2} s^{-1} and above (6), the dark level of active activase must be between 0 and 30% to account for the relaxation times presented in this study. This hypothesis is consistent with the data of Lan *et al.* (6) who showed that, relative to the fully active activase pool, activase is approximately 20% active in darkness. The simulations also showed that when activase was more than 70% active before an increase in PFD, the rate of Rubisco activation following the increase in PFD was indistinguishable from the maximum rate of Rubisco activation. This explains why the change in Rubisco activation rate occurs at a PFD of approximately 135 μmol m^{-2} s^{-1} (3) and activase activity appears to saturate at approximately 300 μmol m^{-2} s^{-1} (6). Finally, the simulations showed that the semilogarithmic plot of $ln([ECMR]_f - [ECMR]_t)$ *versus* time will not be linear, but it appears linear for the τ values for the two processes discussed in this study.

In summary, notwithstanding our lack of a complete picture of the mechanisms underlying the regulation of Rubisco activity, we have sufficient information to devise an empirical model of the kinetics of Rubisco activation following a rapid rise in PFD. For any given initial and final PFD, the initial and final proportions of active Rubisco can be predicted from a curve relating the steady-state activation state of Rubisco to PFD. The relaxation time for movement between these two steady-state values is described by the equations presented in the "Appendix" and the empirical data of Jackson *et al.* (3). The data of Lan *et al.* (6) suggest that the first slow process (characterized by τ_{s1}) reflects the light-dependent activation of Rubisco activase. Further experimentation is necessary to test this hypothesis and characterize the processes involved in activase activation if we are to develop a truly mechanistic model describing the kinetics of Rubisco activation following an increase in PFD.

APPENDIX

Activation of an Activating Enzyme

Consider a mechanism for Rubisco activation in which the enzyme (*Act*) catalyzing the activation process is activated relatively slowly itself before being able to catalyze the reaction. We assume that this initial step is described as follows:

$$P' + Act \underset{k_{-p}}{\overset{k_p}{\rightleftharpoons}} Act' + P \quad (3)$$

where Act' is the catalytically active form of *Act* and P and P' are the substrate and product of the activating process, respectively. It is also assumed that the rates of the forward and reverse reactions are given by the products of the rate constants (k_p and k_{-p}) and the two concentration terms. Thus, at equilibrium

$$k_p[P'][Act] = k_{-p}[Act'][P]. \quad (4)$$

Once activated, we assume that Act' catalyzes the reaction that activates the ER as follows (12–14):

$$CO_2 + Mg^{2+} + nATP + ER \underset{k_{-act}[Act']}{\overset{k_{act}[Act']}{\rightleftharpoons}} ECMR + nADP + nPi \quad (5)$$

where $k_{act}[Act']$ and $k_{-act}[Act']$ are the apparent rate constants for the activation and inactivation reactions, respectively, ECMR is the fully activated Rubisco:RuBP complex (8), and n is the number of ATP molecules required to produce each ECMR.

It is assumed that Act' is not structurally modified during the activation reaction. Therefore, the activation of *Act* can be described by solving two equations:

$$\frac{d[Act']}{dt} = k_p[P'][Act] - k_{-p}[P][Act']; \quad (6)$$

$$[Act]_t = [Act] + [Act']. \quad (7)$$

Act' as a function of time is, therefore, given by

$$[Act'] = [Act']_f - ([Act']_f - [Act']_i)e^{-t/\tau_p} \quad (8)$$

where $[Act']_i$ and $[Act']_f$ are the initial ($t = 0$) and final ($t = \infty$) concentrations of Act', respectively, and

$$\tau_p = (k_p[P'] + k_{-p}[P])^{-1}. \quad (9)$$

τ_p is the relaxation time for activation of activase.

We can describe the activation of Rubisco by solving Equation 8 and an equation describing the rate of change of [ECMR]:

$$\frac{d[\text{ECMR}]}{dt} = k_{act}[Act'][CO_2][Mg^{2+}][ATP]^n - k_{-act}[Act'][ADP]^n[Pi]^n \quad (10)$$

The concentration of ECMR as a function of time, therefore, is given by

$$[\text{ECMR}] = [\text{ECMR}]_f - ([\text{ECMR}]_f - [\text{ECMR}]_i)e^{-t/\tau_{app}} \quad (11)$$

where $[\text{ECMR}]_f$ and $[\text{ECMR}]_i$ are the final ($t = \infty$) and initial ($t = 0$) concentrations of ECMR, respectively. The apparent relaxation time for the process, τ_{app}, is given by:

$$\tau_{app}^{-1} = \tau_\alpha^{-1}\left(1 + \frac{([Act']_f - [Act']_i)\tau_p}{[Act']_t}[e^{-t/\tau_{app}} - 1]\right) \quad (12)$$

where

$$\tau_a = [k_{act}[Act']_t[CO_2][Mg^{2+}][ATP]^n + k_{-act}[Act']_t[ADP]^n[Pi]^n]^{-1}. \quad (13)$$

τ_a is the relaxation time for Rubisco activation in the presence of fully activated activase.

Equation 11 predicts that the activation of Rubisco is a function of both τ_a and τ_p, and that the slope of a *log* ([ECMR]$_f$ − [ECMR]) *versus* time plot would not be linear, although such a plot may appear linear given certain values of τ_a and τ_p.

In this study, we propose that τ_p is τ_{s1} and τ_a is τ_{s2}.

ACKNOWLEDGMENT

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