Plant Physiol. (1992) 99, 1348–1353
0032-0889/92/99/1348/06/$01.00/0

Received for publication November 26, 1991
Accepted February 21, 1992

Dissociation of Ribulose-1,5-Bisphosphate Bound to Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase and Its Enhancement by Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Activase-Mediated Hydrolysis of ATP[1]

Zhen Yuan Wang[2] and Archie R. Portis, Jr.*

Department of Agronomy, University of Illinois (Z.Y.W., A.R.P.), and Photosynthesis Research Unit, Agricultural Research Service, U.S. Department of Agriculture (A.R.P.), Urbana, Illinois 61801

ABSTRACT

Ribulose bisphosphate (RuBP), a substrate of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), is an inhibitor of Rubisco activation by carbamylation if bound to the inactive, noncarbamylated form of the enzyme. The effect of Rubisco activase on the dissociation kinetics of RuBP bound to this form of the enzyme was examined and characterized with the use of ^{3}H-labeled RuBP and proteins purified from spinach (*Spinacia oleracea* L.) In the absence of Rubisco activase and in the presence of a large excess of unlabeled RuBP, the dissociation rate of bound [1-^{3}H]RuBP was much faster after a short (30 second) incubation than after an extended incubation (1 hour). After 1 hour of incubation, the dissociation rate constant (K_{off}) of the bound RuBP was 4.8×10^{-4} per second, equal to a half-time of about 35 minutes, whereas the rate after only 30 seconds was too fast to be accurately measured. This time-dependent change in the dissociation rate was reflected in the subsequent activation kinetics of Rubisco in the presence of RuBP, CO_2, and Mg^{2+}, and in both the absence or presence of Rubisco activase. However, the activation of Rubisco also proceeded relatively rapidly without Rubisco activase if the RuBP level decreased below the estimated catalytic site concentration. High pH (pH 8.5) and the presence of Mg^{2+} in the medium also enhanced the dissociation of the bound RuBP from Rubisco in the presence of RuBP. In the presence of Rubisco activase, Mg^{2+}, ATP (but not the nonhydrolyzable analog, adenosine-5′-*O*-[3-thiotriphosphate]), excess RuBP, and an ATP-regenerating system, the dissociation of [1-^{3}H]RuBP from Rubisco was increased in proportion to the amount of Rubisco activase added. This result indicates that Rubisco activase-mediated hydrolysis of ATP is required for promotion of the enhanced dissociation of the bound RuBP from Rubisco. Furthermore, product analysis by ion-exchange chromatography demonstrated that the release of the bound RuBP, in an unchanged form, was considerably faster than the observed increase in Rubisco activity. Thus, RuBP dissociation was experimentally separated from activation and precedes the subsequent formation of active, carbamylated Rubisco during activation of Rubisco by Rubisco activase.

The initial reactions in photosynthetic CO_2 reduction and photorespiratory carbon oxidation are catalyzed by Rubisco (EC 4.1.1.39), which has to be activated to be catalytically competent. *In vitro,* Rubisco activation is achieved spontaneously by incubation with CO_2 and Mg^{2+}, resulting in the formation of a carbamate in the active site (12). However, the carbamylation reaction is severely inhibited by RuBP[3], the substrate of the active enzyme (6).

In vivo, the activation state of Rubisco varies with irradiance. The enzyme is fully activated at high light intensity, whereas the total amount of RuBP in the chloroplast does not vary much with irradiance (14). Recently, it has been shown that there is a significant correlation between RuBP tightly bound to Rubisco and the estimated number of inactive Rubisco sites at various irradiances (3, 4), implying that the kinetic characteristics of Rubisco with tightly bound RuBP are important in the regulation of Rubisco in leaves.

A soluble chloroplast protein, Rubisco activase, has been shown (16) to promote a rapid conversion of the inactive Rubisco-RuBP complex to an active form, which is also carbamylated (22), in the presence of otherwise inhibitory concentrations of RuBP. *In vitro,* activation of Rubisco by this protein requires ATP and Mg^{2+} and is inhibited by ADP (19, 21). However, many of the details of how Rubisco activase mediates the activation of RuBP-bound Rubisco are still unclear.

In this study, the dissociation kinetics of Rubisco-bound RuBP were examined in the presence and absence of Rubisco activase by using ^{3}H-labeled RuBP and purified spinach (*Spinacia oleracea* L.) Rubisco and Rubisco activase proteins. We obtained evidence that (a) binding of RuBP induced an isomerization of inactive Rubisco to a form that bound RuBP more tightly; (b) a dissociation of protein-bound RuBP from Rubisco was promoted by Rubisco activase and preceded

[1] Supported in part by grant No. 88–37234–3383 from the Competitive Research Grants Program of the U.S. Department of Agriculture.

[2] Present address: Department of Biology, The Johns Hopkins University, Baltimore, MD 21218. This work is a portion of the Ph.D. thesis of Z.Y.W.

[3] Abbreviations: RuBP, ribulose-1,5-bisphosphate; ATP-γ-S, adenosine-5′-*O*-(3-thiotriphosphate); K_{off}, dissociation rate constant.

Rubisco activation; and (c) the dissociation process was driven by the hydrolysis of ATP by Rubisco activase.

MATERIALS AND METHODS

Chemicals and Enzymes

RuBP and [1-^{3}H]RuBP were synthesized enzymically and purified as described by Jordan and Ogren (8) from ribose 5-phosphate and [2-^{3}H]glucose, respectively. Rubisco activase and Rubisco were purified from spinach (*Spinacia oleracea* L.) leaves as described previously (20). Rubisco was inactivated by gel filtration at 25°C through Sephadex[4] G-50 equilibrated with 20 mM Tricine, 0.2 mM EDTA, pH 8.0.

Dissociation (with Exchange) Kinetics of Rubisco-Bound RuBP

After preincubation with [1-^{3}H]RuBP and dilution with unlabeled RuBP as described in specific experiments, 160-μL samples were withdrawn, placed in an Ultrafree-MC device (300,000 nominal molecular weight limit), and centrifuged at 2070*g* for 20 s to obtain a sample containing no Rubisco protein. [1-^{3}H]RuBP in an aliquot of the eluent (20 μL) was determined by liquid scintillation counting.

Assay of Rubisco Activity

Rubisco activity was determined at 25°C by either a one- or two-step assay as indicated. In the one-step assay, RuBP-bound Rubisco was added to an assay medium as described in the figure legends and aliquots (50 μL) were withdrawn at various times, acidified, and dried to permit the determination of the incorporation of $^{14}CO_2$ into acid-stable products by liquid scintillation counting. The rate was calculated from the difference in incorporation between successive time intervals. In the two-step assay, aliquots of the reaction mixture were added to an assay medium (0.5 mL) containing 10 mM [^{14}C]$NaHCO_3$ (0.2 Ci mol^{-1}), 10 mM $MgCl_2$, 0.4 mM RuBP, and 50 mM Tricine (pH 8.0). After 30 s the reaction was stopped by addition of acid.

RESULTS

In the following experiments, dissociation was followed by preincubation of the enzyme with labeled RuBP and then dilution with excess unlabeled RuBP. Centrifugal ultrafiltration was used to allow the rapid isolation of a small sample that did not contain protein. In control experiments, 50-fold dilution of the specific radioactivity of the labeled RuBP prevented measurable binding to Rubisco, whereas no addition of unlabeled RuBP resulted in about 80% of the label being bound (data not shown). The dissociation and exchange of [1-^{3}H]RuBP from Rubisco determined with this method is shown in a semilogarithmic plot (Fig. 1), which is useful for

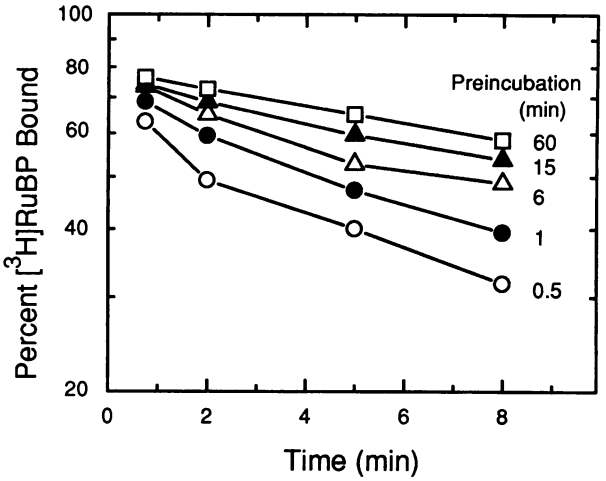

Figure 1. Effect of duration of Rubisco preincubation with [1-^{3}H]RuBP on the dissociation and exchange of RuBP bound to Rubisco. Inactive spinach Rubisco (2.3 mg mL^{-1}, 32-μM sites) and 18 μM of [1-^{3}H]RuBP (51.4 Ci mol^{-1}) were incubated for 0.5 to 60 min as indicated. Then unlabeled RuBP was added to the medium, diluting it to 0.5 mg mL^{-1} (7-μM sites) Rubisco and 200 μM RuBP. Dissociation was followed as described in "Materials and Methods."

determining rate constants for first-order kinetic processes. If all of the binding sites have the same intrinsic affinity for the RuBP, the plotted data are linear with a slope equal to the dissociation rate constant. However, the experimental results indicated that the dissociation was more complex, because the relationship between percent [1-^{3}H]RuBP bound and time is nonlinear and dependent on the time that Rubisco was preincubated with [1-^{3}H]RuBP.

After a short incubation (30 s), an initially rapid dissociation of the bound RuBP was followed by a much slower rate of dissociation, suggesting the existence of at least two types of sites. The evidence for the rapid dissociation rate gradually decreased with an increased time of preincubation with the [1-^{3}H]RuBP, such that after preincubation for 1 h at 25°C, the rate of dissociation was apparently constant and was the slowest observed. After this length of time the K_{off} of the bound RuBP was 4.8×10^{-4} s^{-1}, equal to a half-time of about 35 min. The initial dissociation rate observed after only 30 s of incubation was too fast to be determined by our method.

Further evidence for a time dependency in RuBP binding was also found in the activation kinetics of Rubisco in both the absence and presence of Rubisco activase. As shown in Figure 2A, an increase in activity was not detectable over the next 2 min if Rubisco was preincubated with RuBP for 1 h before the addition of CO_2 and Mg^{2+} to activate the enzyme. However, if the enzyme was exposed to the RuBP for only 30 s before addition of activating CO_2 and Mg^{2+}, a small but readily measurable increase in activity occurred. This result is consistent with the existence of a short period of weaker RuBP binding. In marked contrast to the spontaneous activation results, activation of Rubisco by Rubisco activase under otherwise identical conditions was slightly faster with the Rubisco that had been preincubated with RuBP for 1 h

[4] Mention of trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

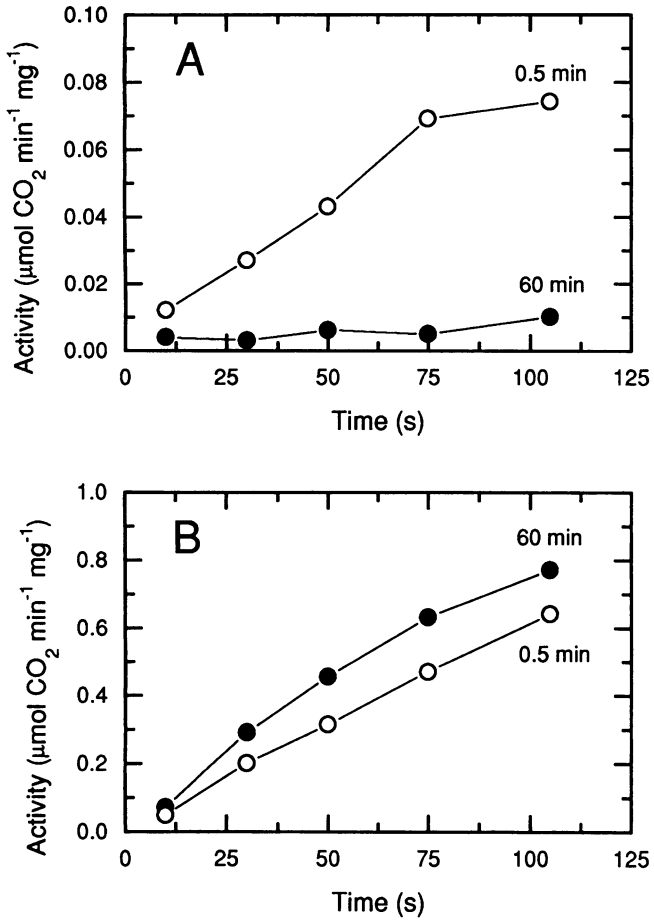

Figure 2. Effect of preincubation of Rubisco with RuBP on activation of Rubisco in the absence (A) or presence (B) of Rubisco activase. Inactive Rubisco (1.9 mg mL^{-1}, 26.6-μM sites) was incubated with 50 μM RuBP for either 0.5 min (○) or 1 h (●). The Rubisco was then added to a one-step assay mixture and assayed as described in "Materials and Methods." The final assay mixture contained 50 mM Tricine (pH 8.0), 10 mM $MgCl_2$, 5 mM [^{14}C]$NaHCO_3$ (0.3 Ci mol^{-1}), 2.0 mM RuBP, 2.0 mM ATP, 2.0 mM phosphocreatine, 30 U mL^{-1} phosphocreatine kinase, 0.32 mg mL^{-1} Rubisco, and (A) no or (B) 100 μg mL^{-1} Rubisco activase. The experiment was repeated with similar results.

(Fig. 2B). Thus, the slowly dissociating form of the enzyme appears to be a better substrate for Rubisco activase.

The dissociation constant of bound RuBP observed in the [1-^{3}H]RuBP-exchange experiment is less than one-third of the value reported by Cardon and Mott (K_{off} = 1.54 × 10^{-3} s^{-1}) (4). However, their measurements of the decrease in bound RuBP with time were done with spinach leaves in darkness where the RuBP concentration was less than the concentration of Rubisco binding sites. To estimate the dissociation rate of RuBP under conditions more similar to theirs, an indirect method that did not require [1-^{3}H]RuBP or the separation of the enzyme from RuBP was used. RuBP-bound Rubisco (with a slight excess of free RuBP) was added to a medium containing 4 mM $NaHCO_3$ and 4 mM Mg^{2+}, and both the decline in total RuBP (due to slow carboxylation) and the increase in Rubisco activity (with aliquots assayed at high RuBP) were followed with time.

The results (Fig. 3) indicated that a rapid increase in Rubisco activity was delayed until the level of RuBP dropped below the Rubisco catalytic site concentration (*i.e.* 8 mol/mol Rubisco), which occurred after 2 to 3 min in this experiment. The RuBP remaining after this time must be bound, because the measured Rubisco activity was more than sufficient to consume any free RuBP within seconds. A semilogarithmic plot of RuBP remaining *versus* time (not shown) indicated a K_{off} of the bound RuBP of about 3 × 10^{-3} s^{-1} at 25°C. This is about sixfold greater than the K_{off} measured in the previous experiments (Fig. 1), which were conducted in the absence of added CO_2 and with excess RuBP. These contrasting results clearly indicate that the dissociation rate of RuBP depends on the level of RuBP and perhaps also on the carbamylation state of the enzyme, which increases in the presence of CO_2 and Mg^{2+}.

The effect of pH on the dissociation of bound [1-^{3}H]RuBP was examined and is shown in Figure 4. There was not a significant change in the dissociation of bound RuBP between pH 7.0 and 8.0. However, a dramatic increase in the release of bound RuBP from Rubisco appeared at pH 8.5, implying that deprotonation of an amino acid(s) at the binding site might change the binding affinity of RuBP. This result is in agreement with previous observations by Mott and Berry (13) using 2-*p*-toluidinylnaphthylene-6-sulfonate fluorescence to monitor RuBP binding.

With the above experiments as a foundation, the effect of Rubisco activase on the dissociation of RuBP from inactive, noncarbamylated Rubisco was examined. When Rubisco ac-

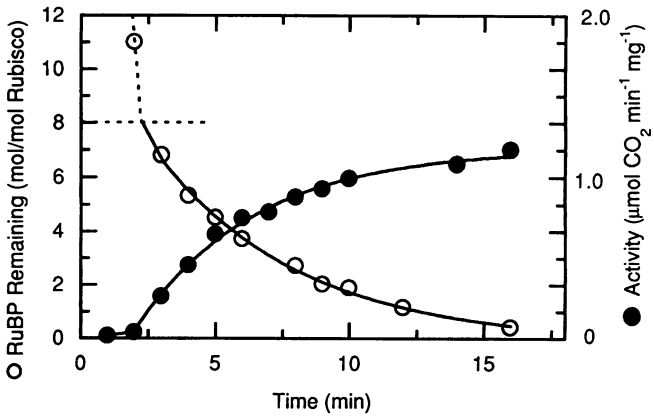

Figure 3. The dissociation of RuBP and activation of RuBP-bound Rubisco in the absence of "free" RuBP. Inactive Rubisco was incubated with RuBP for 1 h. Then a medium with either 0.17 Ci mol^{-1} $^{14}CO_2$ or without $^{14}CO_2$ was added to obtain final concentrations of 4 mM $MgCl_2$, 4 mM $NaHCO_3$, 50 mM Tricine (pH 8.0), 112 μM RuBP, and 3.0 mg mL^{-1} Rubisco. At the times indicated, 40 μL of the mixture with $^{14}CO_2$ was removed and added to 200 μL of 4 M formic acid and 1 M HCl to permit the incorporation of ^{14}C into acid-stable products to be determined and the amount of RuBP remaining (○) to be calculated. For the measurement of Rubisco activation (●), 40 μL of the mixture without $^{14}CO_2$ was assayed with the two-step assay described in "Materials and Methods."

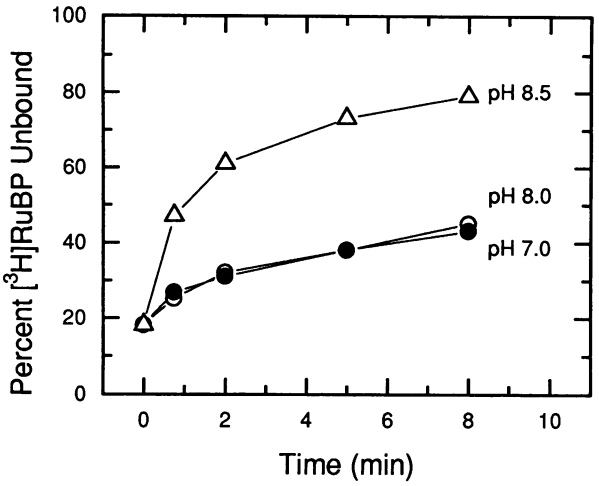

Figure 4. Effect of pH on the dissociation of [1-^{3}H]RuBP bound to Rubisco. Inactive Rubisco (3.0 mg mL^{-1}, 42-μM sites) was incubated with 24 μM [1-^{3}H]RuBP for 1 h. Then a medium containing unlabeled RuBP and 100 mM bis-Tris propane buffer at the different pH values was added to obtain a final concentration of 0.5 mg mL^{-1} Rubisco and 200 μM RuBP. Dissociation was followed as described in "Materials and Methods."

tivase, ATP, and an ATP-regenerating system was added along with the unlabeled RuBP to the Rubisco-RuBP complex, the dissociation of [1-^{3}H]RuBP was increased in proportion to the amount of Rubisco activase present (Fig. 5A). Thus, Rubisco activase is able to promote a rapid dissociation of bound RuBP in the absence of a high concentration of activating CO_2. Unfortunately, semilogarithmic plots of these data were nonlinear and the rate constant for the initially rapid dissociation of the [1-^{3}H]RuBP in the presence of Rubisco activase could not be determined accurately with the assay technique. This has precluded more detailed analysis of the data.

Previous research has indicated that ATP hydrolysis is specifically required for Rubisco activase-mediated Rubisco activation (19, 21). Whether this is also true for the enhanced RuBP dissociation was examined. Of the various nonhydrolyzable ATP analogs (ATP-γ-S, adenylyl-imidodiphosphate, and adenylyl-β-γ-methylenediphosphate) studied, only ATP-γ-S significantly inhibited both the Rubisco activation and ATPase activities of Rubisco activase (19; A.R. Portis, unpublished data). Therefore, ATP-γ-S appears to bind well to the same site as ATP. However, when we compared ATP-γ-S with ATP in an experiment in which [1-^{3}H]RuBP dissociation and exchange are followed, Rubisco activase and ATP-γ-S did not promote an enhanced dissociation of the bound RuBP (Fig. 5B). This result indicates that ATP binding alone is probably not sufficient and that hydrolysis of the ATP is required for the mobilization of the bound RuBP. It should be noted that the addition of Mg^{2+} alone (*i.e.* in the absence of ATP-γ-S or Rubisco activase) also caused some enhancement of the release of bound RuBP (Fig. 5B and data not shown). This effect could be due to the formation of the Mg^{2+}-RuBP complex or a difference in the affinity of Rubisco-Mg^{2+} for RuBP, but this issue was not explored further.

Finally, to determine if the release of bound RuBP by Rubisco activase precedes the activation of Rubisco and to determine the fate of the bound RuBP, the increase in Rubisco activity, [1-^{3}H]RuBP dissociation, and carboxylation of the labeled RuBP were followed in otherwise identical reaction mixtures. The results (Table I) clearly indicated that RuBP release was considerably faster than the increase in Rubisco activity. Furthermore, product analysis confirmed that even in the presence of CO_2 and Mg^{2+}, Rubisco activase promoted the release of the bound RuBP in an unchanged form and not through catalytic conversion to 3-phosphoglyceric acid.

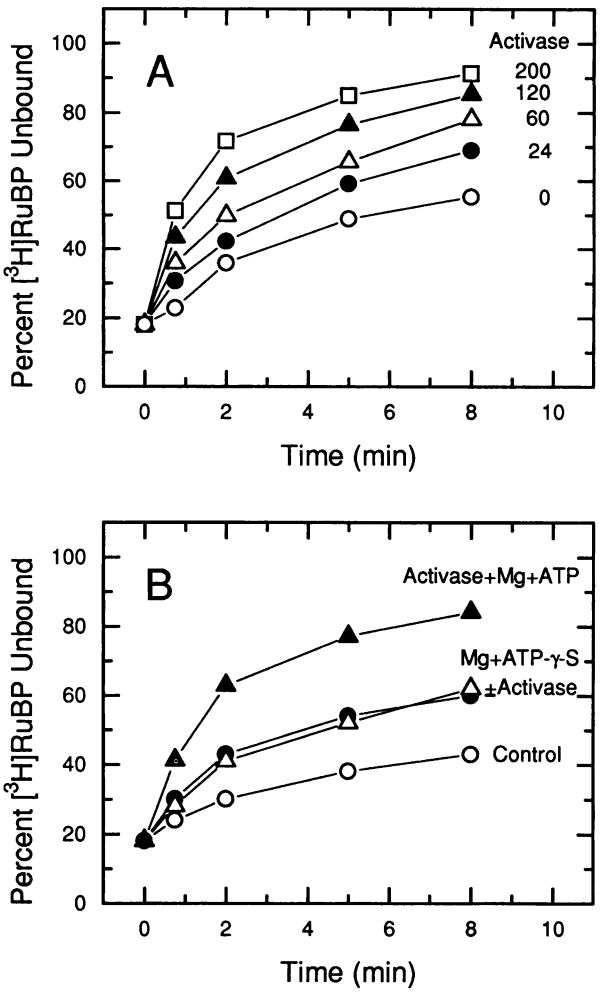

Figure 5. Effect of Rubisco activase and Mg^{2+}-ATP (A) *versus* the effect of Rubisco activase with Mg^{2+}-ATP-γ-S (B) on the dissociation of RuBP bound to Rubisco. Inactive Rubisco (2.0 mg mL^{-1}) was incubated with 16 μM [1-^{3}H]RuBP for 1 h. In A, the Rubisco was added to a medium to obtain 0.4 mg mL^{-1} Rubisco, 100 mM Tricine (pH 8.0), 4 mM $MgCl_2$, 1.5 mM ATP, 1.5 mM phosphocreatine, 70 U mL^{-1} creatine phosphokinase, 250 μM RuBP, and the indicated amount of Rubisco activase protein (in μg mL^{-1}). In B, the Rubisco was added to a medium to obtain 0.4 mg mL^{-1} Rubisco, 100 mM Tricine (pH 8.0), 250 μM RuBP (O), and one or more of the following: 4 mM $MgCl_2$ (Δ, $\blacktriangle$, $\bullet$,), 100 μM ATP-γ-S (Δ, $\bullet$), 150 μg mL^{-1} Rubisco activase (Δ, $\blacktriangle$), and 2 mM ATP with an ATP-regenerating system ($\blacktriangle$). Dissociation was assayed as described in "Materials and Methods."

Table I. *Rubisco Activation versus RuBP Dissociation and Carboxylation*

Rubisco (3.0 mg mL^{-1}) was preincubated with 15 μM [1-^{3}H]RuBP and then added to a reaction mixture with an ATP-regenerating system to obtain 0.6 mg mL^{-1} Rubisco, 100 μg mL^{-1} Rubisco activase, 4 mM $NaHCO_3$, 15 mM $MgCl_2$, 1 mM ATP, and 10 mM RuBP.

Time	Rubisco Activity[a]	[1-^{3}H]RuBP Released[b]	Percent as PGA[c]
s	μmol min^{-1} mg^{-1}	%	
30	0.08	65	4
60	0.26		
90	0.51		
150	0.79	98	12

[a] Rubisco activity was followed in an identical reaction mixture with unlabeled RuBP and [^{14}C]$NaHCO_3$ (0.4 Ci mol^{-1}). [b] RuBP release was assayed by using a Centricon-30 ultrafiltration device and centrifuging an aliquot at 700*g* for 1 min. Over 95% of the label was initially bound. [c] Samples from ([b]) were analyzed by ion-exchange chromatography (2). Metabolism of [1-^{3}H]RuBP added after preincubation and with the unlabeled RuBP, as measured in a parallel reaction mixture, was nearly identical to that shown. PGA, 3-phosphoglyceric acid.

DISCUSSION

The observed time dependency in the dissociation rate of Rubisco-bound RuBP along with previous evidence supports the idea that binding of RuBP to Rubisco is not a simple diffusion-controlled and rapid-equilibrium process. To explain the apparent slow association rate calculated from the dissociation rate constant and the equilibrium dissociation binding constant, Jordan *et al.* (6, 7) proposed a two-step binding mechanism in which a slow isomerization of the enzyme occurred after RuBP initially bound to the inactive form of the enzyme. In these experiments, we observed more direct evidence for an isomerization process and the existence of at least two forms of Rubisco-RuBP with distinct dissociation rate constants. A possibly related isomerization of spinach Rubisco (with a rate constant of $k = 0.04$ s^{-1}) was observed in the binding of the transition-state analog carboxyarabinitol bisphosphate (15). Based on the change in dissociation rate observed after various times of incubation and assuming first-order kinetics, we estimate that the isomerization rate constant of Rubisco-RuBP is also in the range of 10^{-2} s^{-1}, and thus, has a half-time of less than 0.5 min (Fig. 1). However, differences in the initial dissociation rate at times longer than 5 min suggest that even slower intermediate changes may be occurring.

Isomerization of the Rubisco-RuBP complex results in a slower dissociation rate of the bound RuBP and restricts the access of activator CO_2 and Mg^{2+} to the site of carbamylation (Fig. 2A). The time dependence of RuBP binding explains previously reported differences in the ability of RuBP to inhibit spontaneous activation (compare refs. 5, 9, and 16). Thus, a more rapid rate of activation and carbamylation is observed if inactive enzyme is added to RuBP and activating CO_2 and Mg^{2+} without preincubation with the RuBP. Edmondson *et al.* (5) observed that activity measurements did not accurately reflect the carbamylation level of the enzyme. Although it remains to be determined, we suspect that sufficient preincubation with RuBP severely inhibits carbamylation as well because almost no increase in activity occurred in our experiments as long as a considerable excess of RuBP was present.

It is interesting that isomerization results in an enzyme that is activated by Rubisco activase more rapidly (Fig. 2B). The effect was small because isomerization occurs at about the same rate as activation by Rubisco activase, but it was reproducible. This suggests that the conformational change induced by RuBP binding increases the affinity of Rubisco for Rubisco activase. Recently, it was found that Rubisco activase had a mixed specificity for some sugar phosphate-bound forms of Rubisco, but had low or no activity with others (11). The different specificities may be related to the nature or extent of the conformational change observed here.

There are a few previous measurements of the K_{off} of bound RuBP. The discrepancy between these values and ours may be attributed to different experimental conditions. The measurement by Cardon and Mott ($K_{off} = 1.54 \times 10^{-3}$ s^{-1} at 22°C), which is higher than that observed in our [1-^{3}H]RuBP-exchange experiment (4.8×10^{-4} s^{-1}), was made in spinach leaves under conditions where the RuBP concentration was less than the catalytic site concentration of Rubisco and the pH and Mg^{2+} concentration are low (4). We observed that Mg^{2+} appears to increase the dissociation of RuBP somewhat (Figs. 3 and 5B), but this effect and the different pH in the experiments (Fig. 4) may not account for the difference. More significantly, we found that in the presence of high CO_2 and Mg^{2+}, dissociation of the bound RuBP and the activation of Rubisco occurred quite rapidly once the RuBP level dropped below the catalytic site concentration of Rubisco (Fig. 3).

Therefore, the differences in observed values of K_{off} may result either from carbamylation of some catalytic sites and then an enhancement of RuBP release from the remaining sites or from an affect of the presence of excess RuBP on the dissociation rate of the bound RuBP due to allosteric interactions between the binding sites. The latter hypothesis is attractive because a tight binding of RuBP to the inactive form of the enzyme occurs at only about six of the eight available binding sites (6, 10). Additional experiments would be required to distinguish between these possibilities. However, because of RuBP consumption by catalysis when CO_2 and Mg^{2+} are present and because of the very tight binding of RuBP, direct measurements of the dissociation rate of RuBP with various concentrations of free RuBP are technically difficult and were not attempted here. Furthermore, the dissociation of RuBP in the presence of a high excess of RuBP was of primary interest because these are the conditions under which Rubisco activase enhances the activation of Rubisco.

In another case, Jordan and Chollet (6) reported that the K_{off} for RuBP from inactive enzyme was 4.9×10^{-5} s^{-1} at 2°C. Assuming a twofold increase in the rate with a 10°C increase in temperature, this would be equivalent to 2.4×10^{-4} s^{-1} at 25°C, which is closer to our value of 4.8×10^{-4} s^{-1}. However, in trying to repeat this observation, we could not observe evidence of the isomerization of the enzyme-RuBP complex

to a form with a slow dissociation rate if the enzyme was always kept at this low temperature (data not shown).

It has been demonstrated that some sugar phosphates preferentially stabilize the active form of Rubisco by inhibition of the exchange of activator CO_2 and Mg^{2+} (7), and x-ray structural studies indicate that access to the carbamate and Mg^{2+} site is blocked when the active site is occupied (1). Mobilization of the RuBP from the active site would seem to be a prerequisite for carbamylation. The data in Figure 5 clearly indicate that Rubisco activase increases the dissociation of RuBP from Rubisco in a process that appears to require the hydrolysis of ATP. It is difficult to envision how this might occur without postulating that Rubisco activase binds to Rubisco at some time during this process and induces a conformational change in the RuBP binding site. Assuming that the free energy of ATP hydrolysis is about 7 to 8 kcal mol^{-1} and that one ATP is required for each RuBP to be released, the rate of dissociation could be increased by up to 10^5. This is considerably greater than the effect of Rubisco activase observed here. Conditions under which ATP hydrolysis is strictly coupled to activation or RuBP release have not yet been found, and thus, the actual energetic relationships between these processes remain unclear.

The data in Table I indicate that during Rubisco activase-mediated activation the bound RuBP is rapidly released as RuBP and that this is followed by a much slower process in which activation of the enzyme occurs through carbamylation and Mg^{2+} binding. Whether Rubisco activase has additional effects on the rate of the latter process remains unclear, but this is certainly possible given that the RuBP binding site must be altered and Rubisco activase can remain bound until after carbamylation occurs. Nevertheless, we now have direct evidence for an enhanced dissociation process that was suggested by previous studies on the ability of Rubisco activase to reverse the inhibition of catalysis by carboxyarabinitol 1-phosphate (17) and the inhibition of Rubisco activation by other sugar phosphates (11, 18). The effect on RuBP dissociation is the earliest step established thus far in the process by which Rubisco activase promotes the activation of Rubisco.

In summary, the potent inhibition of Rubisco activation by preincubation with RuBP is due to an isomerization of the RuBP-inactive enzyme complex. There are several indications that allosteric interactions between the binding sites may be involved in the binding of several different sugar phosphates, but the characterization of such interactions remains largely obscure. Rubisco activase somehow binds to Rubisco and uses the energy of ATP hydrolysis to alter the active site and thus promote a more rapid dissociation of the bound RuBP and other inhibitory sugar phosphates. The release of the bound sugar phosphate allows access by CO_2 and Mg^{2+} for subsequent activation of the enzyme. Further progress in elucidation of the specific steps in the activation process will require the development of methods to study Rubisco-Rubisco activase binding and aspects of ATP hydrolysis that are specifically coupled to the RuBP release process.

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