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Rubisco Activase Mediates ATP-Dependent Activation of Ribulose Bisphosphate Carboxylase[1]

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ABSTRACT

The activation level of ribulosebisphosphate carboxylase following preincubation with ribulose 1,5-bisphosphate was increased by ATP and ribulosebisphosphate carboxylase activase in the absence of thylakoids or illumination. Maximal activation was obtained with 0.5 millimolar ATP in the presence of an ATP regenerating system (phosphoenolpyruvate and pyruvate kinase). Without the ATP regenerating system, activation was lower, linearly dependent on ATP concentration up to 1.0 millimolar, and was strongly inhibited by ADP.

Ribulosebisphosphate carboxylase/oxygenase (rubisco[2]), which catalyzes the first step in both the photosynthetic and photorespiratory carbon cycles, must be activated by the ordered addition of CO_2 and Mg^{2+} to be catalytically competent *in vitro* (12, 13). Activation of isolated rubisco, however, requires a CO_2 concentration 3-fold higher than atmospheric and is severely inhibited by physiological concentrations of RuBP (4, 8, 10). Recently, light dependent activation of rubisco at atmospheric CO_2 and in the presence of RuBP was achieved *in vitro* with a reconstituted system containing purified rubisco, washed thylakoid membranes, and a newly discovered enzyme, rubisco activase (16, 17). At the completion of this study, adenine nucleotides were discovered to be a contaminant of the RuBP used and ATP was found to be an additional requirement (16). We report here the first evidence that rubisco can be activated in the presence of RuBP by rubisco activase and ATP and that thylakoid membranes and light are not required in this system.

MATERIALS AND METHODS

RuBP was synthesized from ribose 5-P by the method of Jordan and Ogren (10) with minor modifications. Spinach was grown hydroponically (7) and chloroplasts were isolated following the method of Portis *et al.* (16). Rubisco and rubisco activase were obtained by fractionating stromal proteins on a Pharmacia[3] Mono Q (anion exchange) FPLC column following a modification of the method of Salvucci *et al.* (18).

The rubisco activation system contained 100 mM Tricine (pH 8.0), 4 mM $NaHCO_3$, and 5.8 mM free Mg^{2+}, maintained by adjusting the amount of $MgCl_2$ added along with changes in RuBP and ATP using an RuBP binding constant of 530 M^{-1}. RuBP, ATP, rubisco, and rubisco activase concentrations were as stated in the figure legends. The ATP regenerating system consisted of 0.5 mM PEP and 20 IU pyruvate kinase ml^{-1}. All incubations and assays were carried out at 25°C. Activation mixtures were preincubated for 6 min without rubisco activase to allow complete deactivation of rubisco. After assaying rubisco activity (zero time), activation was initiated by the addition of rubisco activase. Rubisco activation state was assayed by adding aliquots of the activation mixture to an assay solution containing 100 mM Tricine (pH 8.0), 10 mM $MgCl_2$, 0.4 mM RuBP, 10 mM $NaH^{14}CO_3$ (0.5 μCi/μmol). After 30 s, the assays were stopped by addition of 4 N formic acid in 1 N HCl. Samples were oven-dried at 65°C and acid-stable counts were determined by liquid scintillation spectroscopy.

RESULTS

In the presence of RuBP, $MgCl_2$, $NaHCO_3$, and an ATP regenerating system consisting of PEP and pyruvate kinase, a time dependent increase in rubisco activation was observed following the addition of rubisco activase (Fig. 1). The activation was relatively slow with an estimated half-time of 2 to 3 min of

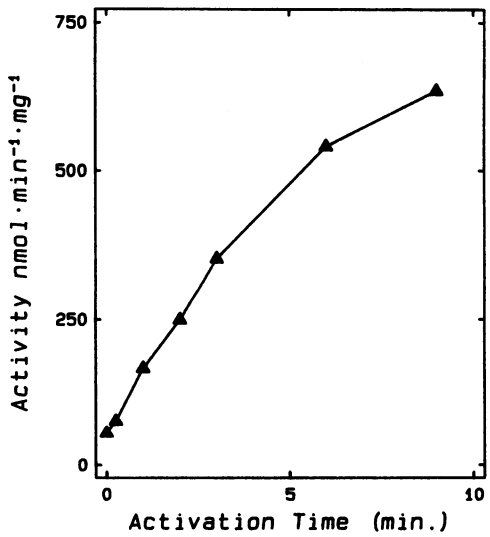

FIG. 1. Time course of activase-dependent rubisco activation in the presence of an ATP regenerating system. Reaction mixtures contained: 100 mM Tricine (pH 8.0); 5.8 mM free Mg^{2+}, 4 mM $NaHCO_3$; 1.0 mM ATP; 3.0 mM RuBP; activase, 0.12 mg ml^{-1}; rubisco, 0.12 mg ml^{-1}.

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[2] Abbreviations: Rubisco, ribulosebisphosphate carboxylase/oxygenase; RuBP, ribulosebisphosphate; PEP, phosphoenolpyruvate.

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incubation.

In the absence of an ATP regenerating system, the ATP dependence of rubisco activation was linear up to 1.0 mM ATP (Fig. 2, circles). In the presence of the ATP regenerating system, however, activity was enhanced by more than 2-fold and activation was more than 70% complete at 0.25 mM ATP. The system apparently saturates near 0.5 mM (Fig. 2, triangles).

Rubisco activation in the presence of RuBP and the regenerating system required the presence of rubisco activase (Fig. 3). RuBP consumption during the long incubation times and the high concentration of PEP in the activation mixture make RuBP concentrations lower than 0.5 mM RuBP difficult to assay. Maximal activity was attained at about 1 mM RuBP and remained at a high level with 6 mM RuBP (Fig. 3).

Because of the marked effect of the ATP regenerating system, the effect of ADP on activation in the absence of the ATP regenerating system was examined (Table I). In the presence of 1.0 mM ATP, 0.2 mM ADP decreased activation 50% and virtually no activation occurred in the presence of 1.0 mM ADP (Table I). These data indicate that activase and ATP alone would not appear to be sufficient to maintain the activation of rubisco *in vivo*, since high ATP/ADP ratios do not exist *in vivo* (11).

DISCUSSION

These results demonstrate that rubisco can be activated in the presence of physiological concentrations of RuBP by rubisco activase and ATP, in contrast to other proposed mechanisms of *in vivo* activation. Based on the *in vitro* model of rubisco activation by the ordered binding of CO_2 and magnesium (12, 13) it has been suggested that rubisco in the chloroplast was activated by light-induced changes in stromal pH and Mg^{2+} (5, 15, 23) and that perhaps some chloroplasts metabolites, such as NADPH and 6-phosphogluconate, stimulate activity *in vivo* (1, 3, 9). At physiological pH, however, activation of rubisco in the presence of RuBP does not occur, and the chloroplast metabolites which stimulate activity *in vitro* have no such effect in the presence of RuBP (4, 19).

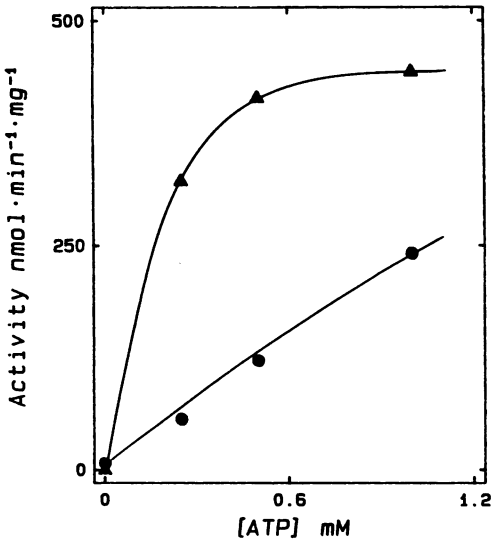

FIG. 2. ATP dependence of rubisco activation in the presence and absence of an ATP regenerating system. Activation was determined in the absence (●) and in the presence (▲) of the ATP regenerating system 9 min after the addition of activase. Concentrations in the reaction mixtures were: 100 mM Tricine (pH 8.0); 5.8 mM free Mg^{2+}; 4 mM $NaHCO_3$; 3.0 mM RuBP; rubisco, 0.12 mg ml^{-1}; activase, 0.13 mg ml^{-1}; ATP as shown. The regenerating system contained 0.5 mM PEP and 20 IU pyruvate kinase ml^{-1}. Zero time measurements after 6 min preincubation in the absence of activase were subtracted from the 9 min time points. Zero time control rates ranged from 107 to 159 nmol min^{-1} mg^{-1} except for the 0 mM ATP without the regenerating system, which was 32 nmol min^{-1} mg^{-1}.

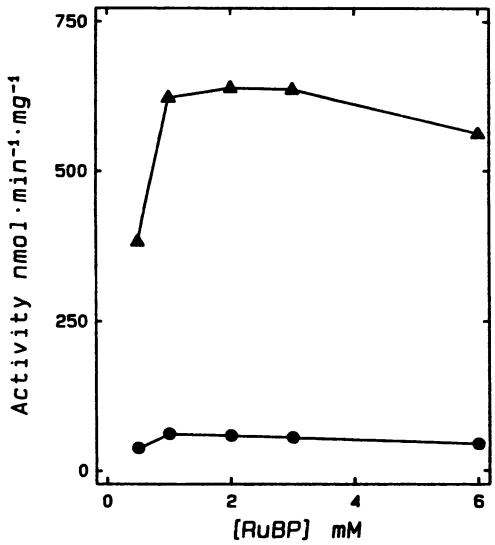

FIG. 3. RuBP dependence of rubisco activation in the presence of an ATP regenerating system. Rubisco activities are after 6 min preincubation in the absence of activase (●), followed by 6 min incubation in the presence of activase (▲). Concentrations in the incubation mixture were: 100 mM Tricine (pH 8.0); 5.8 mM free Mg^{2+}; 1.0 mM ATP; 4 mM $NaHCO_3$; RuBP as indicated in Figure; rubisco, 0.12 mg ml^{-1}; activase, 0.12 mg ml^{-1}. The regenerating system contained 0.5 mM PEP and 20 IU pyruvate kinase ml^{-1}.

Table I. *ADP Inhibition of ATP and Activase Dependent Rubisco Activation*

Concentrations were: RuBP, 3 mM; ATP, 1.0 mM; rubisco, 0.12 mg ml^{-1}; activase, 0.12 mg ml^{-1}. Rates shown are after 6 min incubation in the presence of activase. Rate after 6 min preincubation without activase (57 nmol min^{-1} mg^{-1}) was subtracted from all values. Control experiments indicated that ADP present in the assay did not affect activity measurements under the conditions used. No ATP regenerating system was present during activation.

Activation Treatment	Activity	Control
	nmol min^{-1} mg^{-1} protein	%
Control	422	100
+ 0.2 mM ADP	203	48
+ 1.0 mM ADP	9	2

Indications that a thylakoid membrane component plays a role in rubisco activation have come from several sources. Taylor and Terry (20) used iron deficiency to reduce the photosynthetic electron transport capacity in leaves and found a relationship between light activation of rubisco and photosynthetic electron transport. On this basis, they suggested that some product of photosynthetic electron transport has an allosteric effect on light activation of rubisco. Weis (21, 22) studied the effect of heat treating isolated chloroplasts and leaves on rubisco activation and found that, while activation in the dark was unaffected, light activation was inhibited. This inhibition occurred, however, without affecting light-induced stromal alkalization, linear electron transport and photophosphorylation, indicating again an indirect effect of the thylakoid membranes on rubisco light activation (22). A role for inorganic phosphate in the light regulation of rubisco activation has been suggested from phosphate deficiency studies with leaves (2) and isolated chloroplasts

(6). Mächler and Nösberger (14) measured light-dependent rubisco activation in isolated chloroplasts in the presence of inorganic phosphate and various metabolites. Their results indicate that rubisco activity declines in the presence of ATP consuming reactions (14), suggesting that ATP is necessary for rubisco activation in the light.

The data presented here may account for these observations and suggest a partial model for regulating rubisco activation *in vivo*. Rubisco activation by rubisco activase is dependent on ATP. Under *in vivo* conditions, ATP from photophosphorylation may act either as a substrate or regulatory metabolite for rubisco activase and lead to light-dependent rubisco activation at physiological levels of CO_2 and RuBP. ADP, however, appears to be such a strong inhibitor of the activase dependent process that maximal activation can only be achieved *in vitro* when an ATP regenerating system is used to keep the ADP concentration very low. Thus, it is necessary to account for the activation of rubisco by activase *in vivo* where ADP concentrations are known to be quite high (11). It is possible that some change occurs *in vivo* to rubisco activase which alters the inhibitory effects of ADP on the activase-catalyzed process. Energization of the thylakoid membranes may represent this change, as proposed (17) before the ATP requirement was known.

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