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The regulation of Rubisco activity

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The processes of photosynthesis and photorespiration are initiated by Rubisco, but the enzyme must be activated before it will catalyse either the carboxylation or oxygenation of ribulose bisphosphate. Rubisco is activated *in vitro* by CO_2 and Mg^{2+}. The dual roles of CO_2 as both an activator and a substrate led to anomalously high $K_m(CO_2)$ values until the activation requirement was recognized. During activation, CO_2 forms a carbamate at the ε-amino of a lysine residue on the large subunit of Rubisco. Under conditions thought to exist in the chloroplast during photosynthesis (10 μM CO_2, 5 mM Mg^{2+} and pH 8.0), Rubisco is only partially active since the $K_{act}(CO_2)$ is in the range 25–30 μM CO_2. Thus the mechanism of activation as deduced from *in vitro* studies is incomplete.

Higher activation levels can be obtained by preincubating Rubisco with phosphorylated metabolites, but these occupy ribulose bisphosphate binding sites and thus inhibit catalysis. Recently, a naturally occurring effector, which binds tightly to Rubisco and inhibits activity, has been found. This compound is synthesized in the dark and metabolized upon illumination, but its identity and physiological function are not yet known.

In leaves, Rubisco is nearly fully activated at high light intensities. By analysing an *Arabidopsis thaliana* mutant deficient in the ability to activate Rubisco, we have determined that a soluble protein is required for the *in vivo* activation process. This enzyme, designated Rubisco activase, reduces the high $K_{act}(CO_2)$, observed with the isolated enzyme, to physiological levels in an illuminated reconstituted assay system containing washed thylakoid membranes, Rubisco and ribulose bisphosphate.

Rubisco activation and the $K_m(CO_2)$

Rubisco activation by inorganic carbon and Mg^{2+} was first recognized by Pon *et al.* (1963), although more than a decade passed before the significance and details of the activation process began to be determined. Early research on activation was driven in large part by an anomaly in the kinetics of ribulose bisphosphate (RuBP) carboxylation: in chloroplasts and leaves of higher plants the $K_m(CO_2)$ was in the range of atmospheric CO_2 (about 10 μM), but the $K_m(CO_2)$ of isolated Rubisco was ten times higher (see review by Walker 1973). Bahr & Jensen (1974) were able to demonstrate a high-affinity form of the enzyme by lysing chloroplasts into hypotonic medium and rapidly assaying carboxylase activity. Further, the apparent $K_m(CO_2)$ of Rubisco could be decreased by preincubation in CO_2 and Mg^{2+}. Subsequently Laing *et al.* (1975) demonstrated that both RuBP carboxylase and RuBP oxygenase activities declined with time in low bicarbonate concentration and that the rate of this decline could be slowed by increasing the bicarbonate concentration. From these observations they deduced that there was a bicarbonate-dependent equilibrium between high- and low-V_{max} forms of Rubisco, with high bicarbonate concentration favouring the active form of the enzyme. At low bicarbonate concentration, active enzyme was deactivated. Thus the stimulatory effect of increasing

bicarbonate was on the V_{max} of the enzyme, not on the $K_m(CO_2)$. Andrews *et al.* (1975) similarly concluded that two forms of Rubisco existed, and that Mg^{2+} and bicarbonate (or CO_2) stabilized a low-K_m form. The recognition of this dual role of inorganic carbon acting as both substrate and activator solved the enigma of the high apparent $K_m(CO_2)$ values observed with Rubisco, relative to the $K_m(CO_2)$ for photosynthesis, reported prior to 1975.

The *in vitro* activation mechanism

In the initial report on Rubisco activation, Pon *et al.* (1963) found that maximum activation occurred when the enzyme was preincubated with CO_2 and Mg^{2+}, followed by initiation of the reaction with RuBP. Incubation of the enzyme with RuBP in the presence or absence of CO_2 and Mg^{2+} led to considerably reduced rates of RuBP carboxylation. From these experiments Pon *et al.* (1963) concluded that an enzyme–Mg^{2+}–inorganic carbon complex was required for full Rubisco activity, and that this complex formed just prior to the carboxylation of RuBP.

The nature of the enzyme–Mg^{2+}–CO_2 complex could not be determined from these experiments, but Pon *et al.* (1963) suggested the possibilities that either the activating bicarbonate also carboxylated RuBP or the activating and substrate bicarbonates were different molecules. From studies of 6-phosphogluconate on the activity of Rubisco, Chu & Bassham (1973) suggested that the activation bicarbonate was not the reacting species. This point was later established by Lorimer (1979), who showed that activating $^{14}CO_2$ remained bound to Rubisco after the enzyme had catalysed several carboxylations of RuBP in the presence of $^{12}CO_2$, and by Miziorko (1979), who trapped activator CO_2 on Rubisco with an analogue of the hypothetical six-carbon intermediate of the RuBP carboxylase reaction.

To determine whether the activation process was ordered, Lorimer *et al.* (1976) conducted kinetic analyses of Rubisco that had been inactivated by removal of inorganic carbon and Mg^{2+} by gel filtration. At short incubation times, in various CO_2 and Mg^{2+} concentrations, the activation rate was proportional to the CO_2 concentration but was independent of the Mg^{2+} concentration. From these observations Lorimer *et al.* (1976) concluded that Rubisco activation was an ordered reaction with the inorganic carbon adding first, followed by Mg^{2+}. The species of inorganic carbon involved in Rubisco activation was discovered to be CO_2 by following activation rates in the presence of CO_2, bicarbonate, and CO_2 plus carbonic anhydrase. The rate of activation was much greater with CO_2 than with bicarbonate. Carbonic anhydrase, which catalyses the interconversion between CO_2 and bicarbonate, reduced the activation rate when added with CO_2. Thus the active species in Rubisco activation is CO_2 and not bicarbonate, and the activation can be described by equation (1) (Lorimer *et al.* 1976), where E is Rubisco:

$$\underset{\text{(inactive)}}{E + CO_2} \rightleftharpoons E\text{–}CO_2 + Mg^{2+} \rightleftharpoons \underset{\text{(active)}}{E\text{–}CO_2\text{–}Mg^{2+}} \qquad (1)$$

The activation process is pH-dependent, being much more rapid and complete at alkaline pH. The increasing activation at high pH led Lorimer *et al.* (1976) to propose that the activating CO_2 reacted with an ε-lysyl amino group to form a carbamate. Such a reaction changes a positively charged or neutral amino group to a negatively charged carbonyl group, providing a site for interaction with the positively charged Mg^{2+} ion. Evidence to support this suggestion

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was obtained by Lorimer & Miziorko (1980), who methylated the carboxyl group after stabilizing the activating CO_2 on the enzyme with an analogue of the proposed intermediate of the RuBP carboxylation reaction. Thus the CO_2 reaction in activation can be written more specifically as:

$$E\text{–}Lys\text{–}NH_2 + CO_2 \rightleftharpoons H^+ + E\text{–}Lys\text{–}NH\text{–}CO_2^- + Mg^{2+} \rightleftharpoons E\text{–}Lys\text{–}NH\text{–}CO_2^- . Mg^{2+}. \quad (2)$$

The active lysine is amino acid residue number 201 in the spinach Rubisco large subunit (Lorimer 1981), and the neighbouring sequence has been highly conserved in all Rubisco sequences examined (Miziorko & Lorimer 1983).

Equations (1) and (2) indicate that the activation of Rubisco is promoted by CO_2, Mg^{2+} and alkaline pH. Although the CO_2 concentration in the chloroplast probably changes little between light and dark, illumination increases the Mg^{2+} concentration and pH in the stroma (Werdan & Heldt 1972). Rubisco activation by CO_2 and Mg^{2+}, as summarized in (1) and (2), has formed the basis of suggested mechanisms of light activation *in vivo* (Werdan & Heldt 1972; Walker 1973; Bahr & Jensen 1974; Chu & Bassham 1975; Laing *et al.* 1975; Andrews *et al.* 1975; Lorimer *et al.* 1976). In this scheme, light-induced increases in Mg^{2+} and pH promote the carbamylation of the lysine ε-amino group. The reactions would be reversed upon the cessation of illumination. However, the $K_{act}(CO_2)$ for Rubisco is in the range 25–30 μM CO_2 (Lorimer *et al.* 1976; Jordan & Ogren 1981), while nearly full activation of Rubisco was observed at high light intensity in air, which provides about 10 μM CO_2 in the chloroplast (Perchorowicz & Jensen 1983; Salvucci *et al.* 1986). Thus the mechanism established for Rubisco activation *in vitro*, as described in equations (1) and (2), does not fully account for activation as it occurs in the leaf.

Effectors

In addition to activation by CO_2 and Mg^{2+}, Rubisco is activated by preincubation with many phosphorylated metabolites. Chu & Bassham (1973) nearly doubled the activity of Rubisco by adding 50 μM 6-phosphogluconate to a preincubation mixture containing 1 mM $NaHCO_3$ and 10 mM $MgCl_2$. They later obtained a similar stimulation with NADPH, and lesser amounts of stimulation with fructose 1,6-bisphosphate and 3-phosphoglycerate (Chu & Bassham 1974). Stimulation was observed with these compounds only at low (1 mM or less) concentration. At higher concentrations these compounds inhibited RuBP carboxylation. Chu & Bassham (1975) concluded that 6-phosphogluconate and NADPH regulated Rubisco activity through interaction at allosteric sites on the enzyme.

Buchanan & Schürmann (1973) found that several metabolites of photosynthesis, including fructose 6-phosphate and 6-phosphogluconate, reduced the $K_m(CO_2)$ of Rubisco, and postulated that the interaction of these compounds with Rubisco in the chloroplast could explain the anomalously high $K_m(CO_2)$ of isolated Rubisco compared with the $K_m(CO_2)$ of leaf and chloroplast photosynthesis. In this regulatory scheme, illumination of chloroplasts activates fructose bisphosphatase, leading to an increase in the fructose 6-phosphate level. Fructose 6-phosphate, in the presence of CO_2 and Mg^{2+}, promotes the activation of Rubisco.

The physiological significance of phosphate effectors, in terms of increasing the affinity of Rubisco for CO_2 *in vivo*, was disputed by Badger & Lorimer (1981). From kinetic studies of the influence of NADPH and 6-phosphogluconate on the stability and activity of the

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Rubisco–CO_2–Mg^{2+} complex, they concluded that effectors bind to the RuBP site, not to an allosteric site. In the presence of an effector, they argued, the effector bound to Rubisco at the RuBP site, forming a Rubisco–CO_2–Mg^{2+}–effector complex. Upon dilution into a reaction mixture for assay of activity, the effector either came off rapidly leaving the active Rubisco–CO_2–Mg^{2+} complex (positive effector), or came off slowly. In the latter case, since the RuBP binding site was occupied, the effector inhibited carboxylation rate. Thus the question of whether a specific metabolite stimulated or inhibited Rubisco activation depended upon its affinity for the Rubisco RuBP site, and the activation promoted by effectors was an artefact of the standard assay procedures used. *In vivo*, since the effector occupies the RuBP binding site, the Rubisco molecule may be in the active state with regard to CO_2 and Mg^{2+} but is catalytically incompetent because the effector prevents the binding of RuBP (Badger & Lorimer 1981). Thus metabolic effectors, even though they increase the activation state of Rubisco and decrease the apparent $K_m(CO_2)$ *in vitro*, cannot explain the discrepancy between the low activation level reached by the isolated enzyme under physiological conditions and the high photosynthesis rates catalysed by leaves and chloroplasts.

A naturally occurring inhibitor of Rubisco

McDermitt *et al.* (1983) reported that total extractable RuBP carboxylase activity in soybean leaves was lower in the morning than at midday. This light response was distinct from the light activation phenomenon discussed above. Increasing light intensity, in addition to converting a greater amount of Rubisco from the inactive to the active state, led to an increase in the total amount of Rubisco that could be activated at high Mg^{2+} and saturating CO_2 conditions (McDermitt *et al.* 1983). Vu *et al.* (1983) quantitated these effects and found that total carboxylase activity was approximately doubled by increasing the light intensity from 0 to 600 $\mu mol\ m^{-2}\ s^{-1}$, which saturated photosynthesis. Over this intensity range, the percentage activation of extractable enzyme activity increased from about 25% to 80%. Similar responses in total RuBP carboxylase activity were observed in leaves of tomato grown in a controlled environment chamber, with activity doubling after about 2.5 h in 80 $W\ m^{-2}$ of photosynthetically active irradiation (Besford 1984). Total activity declined to the preillumination level during a subsequent 12 h dark period. In a survey of several species, Vu *et al.* (1984) showed that dark inactivation of Rubisco was a common but not universal phenomenon in C_3 plants (five out of six species examined showed a marked increase in total RuBP carboxylase activity in the light), but less common in C_4 plants (the response was seen in only one of five species examined). The increase in Rubisco activity was most dramatic in two CAM species examined, with a 27-fold increase in *Bromelia pinguin* and a 33-fold increase in *Ananas comosus* (pineapple). In C_3/C_4 intermediate photosynthesis species, light increased activatable Rubisco by 50% in *Panicum milioides*, but had no effect on Rubisco in *Moricandia arvensis*.

These observations on the light-dependent increase in total extractable Rubisco activity led Vu *et al.* (1984) to conclude that light had two effects: to convert Rubisco from the inactive to active state, as discussed earlier, and to convert an inhibited or inactivated enzyme to an activatable form. Evidence that an endogenous inhibitor caused the low Rubisco activity in the dark was developed by Servaites (1985), who purified *Nicotiana tabacum* Rubisco from predawn and midday leaves. The enzyme from predawn leaves remained in an inhibited state after polyethylene glycol precipitation, gel filtration, heat treatment and extensive dialysis. After

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precipitation in 50% ammonium sulphate, the specific activity doubled and reached the level found in midday leaves. Subsequent purification of predawn and midday enzyme by gel filtration, and analysis of protein and phosphate, showed that the predawn enzyme contained about 0.55 mol inorganic phosphate per mol enzyme, while negligible amounts of phosphate coeluted with the midday enzyme. Thus Servaites (1985) concluded that the predawn enzyme was inhibited by a tightly binding phosphate compound in a manner analogous to the known Rubisco inhibitor carboxyarabinitol bisphosphate (Miziorko 1979). On illumination, the inhibitor was metabolized or released from the enzyme and full activity was restored.

The metabolic function of the phosphate inhibitor is not known. While Rubisco inhibition is gradually reduced in the light and the amount of extractable enzyme activity is a function of the light intensity, the inhibition of total extractable activity in the dark takes an hour or more (Servaites *et al.* 1984). Thus the inhibitor cannot be involved in the short-term Rubisco deactivation observed in the dark in leaves, since the return of activation to the dark level occurs within several minutes after illumination ceases or is reduced (Perchorowicz *et al.* 1981; Salvucci *et al.* 1986).

Light activation in leaves

Historically, the light and CO_2 response of photosynthetic CO_2 fixation has been considered to be caused by a direct energy limitation at low light or high CO_2 concentration and by biochemical limitation at high light intensity or low CO_2 concentration. One recent model (Farquhar *et al.* 1980) expresses CO_2 assimilation as being limited by Rubisco activity at low CO_2 concentration and by RuBP regeneration capacity at high CO_2 concentration. However, Mächler & Nösberger (1980) demonstrated, by rapid extraction and assay of Rubisco activity, that activation in wheat leaves was increased by increasing light intensity. Perchorowicz *et al.* (1981) also found that extractable activity was a function of light intensity, and that RuBP concentration was saturating at irradiance levels greater than 300 $\mu mol\ m^{-2}\ s^{-1}$. They also showed that photosynthesis, when varied by light intensity, was correlated with Rubisco activation level. When the light intensity was reduced, [RuBP] dropped below saturating concentrations because Rubisco activity declined more slowly than did the capacity of the plant to regenerate ribulose bisphosphate (Mott *et al.* 1984). Thus, under these transient conditions, the rate of RuBP utilization by carboxylation and oxygenation exceeded the rate of regeneration. After several minutes, the Rubisco activation level reached a new steady state and [RuBP] had increased to above saturation.

These observations clearly indicate that there is an additional means of photosynthetic regulation by Rubisco. Not only does the enzyme regulate photosynthetic CO_2 fixation because of a low turnover number and by partitioning carbon between photosynthesis and photorespiration, but activity also responds directly to the light intensity sensed by the leaf. The correlation between Rubisco activity and photosynthetic rate as a function of light intensity has also been observed in leaves of wild-type *Arabidopsis* and of a mutant incapable of activating Rubisco (Salvucci *et al.* 1986).

Rubisco activase

Rubisco activation *in vitro* is promoted by CO_2, Mg^{2+}, pH and certain effectors as discussed above. By analogy with activation of the purified enzyme, Rubisco activation *in vivo* has been universally considered to be regulated by some combination of these factors. On illumination,

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H^+ ions are taken up by the thylakoid membrane and, to maintain electrical neutrality, Mg^{2+} ions are released into the stroma (Werdan & Heldt 1972). Thus both the stromal pH and Mg^{2+} concentration are increased in the light. Further, the concentrations of sugar phosphates, the primary effector compounds, also change and could collectively promote Rubisco activation. Nonetheless, the mechanism proposed for Rubisco activation *in vitro*, i.e. spontaneous carbamate formation and Mg^{2+} addition (Lorimer *et al.* 1976), cannot account for Rubisco activation *in vivo*. Rubisco in leaves is almost completely activated by saturating light at CO_2 concentrations less than those found in air (Perchorowicz & Jensen 1983; Salvucci *et al.* 1986), yet the $K_{act}(CO_2)$ for the purified enzyme is 25–30 μM CO_2 (Lorimer *et al.* 1976; Jordan & Ogren 1981). Also, activation of the isolated enzyme by physiological concentrations of CO_2 and Mg^{2+} is strongly inhibited by RuBP concentrations of 10 μM and less (Jordan & Chollet 1983). In contrast, full activation of the enzyme proceeds *in vivo* in the presence of 4–12 mM RuBP (Perchorowicz *et al.* 1981; Perchorowicz & Jensen 1983). Further, the light intensity needed to saturate stromal Mg^{2+} influx is much less than that needed to saturate Rubisco activation (Heber *et al.* 1982). Finally, Somerville *et al.* (1982) isolated a mutant *Arabidopsis thaliana* strain that was not able to activate Rubisco in the light. These observations collectively suggested that an additional component was needed for light activation of Rubisco *in vivo*.

The nature of this unidentified component was first revealed by two-dimensional gel electrophoresis of chloroplast stromal proteins of wild-type and the activation-deficient mutant of *Arabidopsis* (Salvucci *et al.* 1985). Two polypeptides of about 47 and 50 kDa were missing in the mutant extract. Genetic analysis of an F_2 population containing the activation lesion, by one-dimensional gel electrophoresis, demonstrated that the absence of the 50 kDa polypeptide cosegregated with the physiological phenotype (the 47 kDa polypeptide was masked by other proteins). Thus it was concluded that the missing activation factor was a soluble chloroplast protein that participated in the activation process.

Additional evidence for the involvement of a protein in Rubisco activation was obtained from the development of a reconstituted chloroplast assay system which included washed chloroplast thylakoids, Rubisco, RuBP, and a partially purified extract of chloroplast proteins (Salvucci *et al.* 1985). In this system, chloroplast extracts from spinach and wild-type *Arabidopsis* stimulated Rubisco activity in the light, but the extract of mutant chloroplasts did not (table 1). Stimulation of activity was also observed in lysed spinach and *Arabidopsis* wild-type chloroplasts, but not in lysed chloroplasts from the mutant (table 1).

It was later determined that the lesion did not affect the activation of fructose bisphosphatase, an enzyme that is light-activated by electrons from the photosynthetic electron transport chain via thioredoxin and ferredoxin (Salvucci *et al.* 1986). Also, pretreatment of leaves with methyl viologen, which diverts electrons from the thioredoxin–ferredoxin system, did not abolish Rubisco activation although fructose bisphosphatase activation was inhibited (Salvucci *et al.* 1986). Finally, the $K_{act}(CO_2)$ for Rubisco activation was 4 μM CO_2 in the reconstituted system, well within the physiological range of CO_2 concentrations (unpublished data). Thus the mechanism of Rubisco activation is distinct from the mechanism of activation of other photosynthetic enzymes. The soluble enzyme catalyzing this reaction has been designated Rubisco activase.

The interdependence and coregulation of the light and dark reactions of photosynthesis has been the subject of considerable comment and speculation. Generally, it is thought that low light limits the availability of ATP and NADPH and thus the rate of RuBP regeneration.

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TABLE 1. LIGHT ACTIVATION OF RUBISCO IN A RECONSTITUTED CHLOROPLAST SYSTEM AND IN LYSED CHLOROPLASTS (DATA FROM SALVUCCI *ET AL.* (1985))

			Rubisco activity (1) nmol CO_2 mg Rubisco protein^{-1} min^{-1}; (2) nmol CO_2 min^{-1}		
experiment	source of chloroplasts	source of extract	dark	light	light–dark
(1)	spinach	none	32	36	4
	spinach	spinach	40	523	483
	spinach	*A. thaliana* (w.t.)	36	119	83
	spinach	*A. thaliana* (mutant)	34	37	3
(2)	spinach	(lysed)	24	84	60
	A. thaliana (w.t.)	(lysed)	13	33	20
	A. thaliana (mutant)	(lysed)	16	18	2

In the reconstituted light activation system (experiment 1), chloroplast stromal extracts were obtained by 10-fold dilution of intact spinach and *Arabidopsis* chloroplasts (2–3 mg chlorophyll ml^{-1}) into Buffer A (50 mM Tricine–NaOH, pH 8.0 and 4 mM β-mercaptoethanol) for 5 min at 0 °C, followed by centrifugation at 2000 *g* for 4 min. The pelleted thylakoid membranes were resuspended at 2–3 mg chlorophyll ml^{-1} in a small volume of buffer A containing 5 mM $MgCl_2$. To obtain a concentrated extract free of Rubisco, the stromal extract (3.0 ml) was fractionated by sucrose density centrifugation (Covey & Taylor 1980) in buffer A. Proteins less than 17S, in the upper fractions of the gradient, were freed of low molecular weight compounds (< 30 kDa) by ultrafiltration on an Amicon YM–30 membrane, and the remaining proteins were concentrated sixfold. Rubisco, recovered from the lower fractions of the sucrose gradient, was used as a source of purified enzyme following the removal of sucrose by ultrafiltration. The reconstituted activation assay was formed by adding, in the order given: 200 µl concentrated stromal extract, spinach thylakoid membranes (30 µg chlorophyll), 10 µl spinach Rubisco (4.5–4.9 mg ml^{-1}), 4 mM ribulose 1,5-bisphosphate (RuBP), 8 mM $MgCl_2$, 3000 units catalase, 40 µM pyocyanine, and 3.1 mM $NaHCO_3$ in a final volume of 300 µl. Following 6 min preincubation in the dark at 25 °C, the assay mixtures were kept in the dark or illuminated for 6 min at 800 µmol photon m^{-2} s^{-1}. 50 µl aliquots were then removed for assay of Rubisco activity by addition to 450 µl of a solution containing 100 mM Tricine-NaOH, pH 8.0, 0.4 mM RuBP, 5 mM $MgCl_2$, 0.2% Triton X-100, and 1.67 mM $NaH^{14}CO_3$ (2 µCi µmol^{-1}) at 25 °C and stopped after 30 s by the addition of HCOOH. Acid-stable incorporation of ^{14}C was determined by liquid scintillation spectroscopy. In the assay of light activation in lysed chloroplasts (experiment 2), intact chloroplasts from *Arabidopsis* (150 µg chlorophyll ml^{-1}) and spinach (250 µg chlorophyll ml^{-1}) were lysed by dilution into buffer A for 5 min at 25 °C. The *in vitro* activation assay mixture was prepared as described above starting with the addition of RuBP to the lysed chloroplasts. After a 2 min dark preincubation period at 25 °C, reaction mixtures were maintained in the dark or illuminated for 6 min at 800 µmol photons m^{-2} s^{-1}. Rubisco assays on the dark and illuminated samples were performed as described above.

However, the RuBP concentration in leaves was found to be saturating over an extended range of light intensities (Perchorowicz *et al.* 1981; Perchorowicz & Jensen 1983) and RuBP became transiently limiting only when the light intensity was quickly reduced (Mott *et al.* 1984). After a reduction of light intensity, the rate of RuBP carboxylation and oxygenation exceeds the rate of RuBP synthesis because the Rubisco activation level does not reach a new steady state immediately, but takes several minutes. Once a new steady-state activation level is achieved, however, [RuBP] has again increased to a saturating level. Thus during steady state photosynthesis, RuBP concentration is always saturating (Perchorowicz *et al.* 1981; Perchorowicz & Jensen 1983; Mott *et al.* 1984). The steady-state saturated level of RuBP may be one aspect of the control of the Rubisco activation system *in vivo*. In the reconstituted assay system, Rubisco activation proceeds in the presence of RuBP (Salvucci *et al.* 1985), while with purified Rubisco, RuBP forms a stable complex and leads to deactivation of the enzyme (Pon *et al.* 1963; Laing *et al.* 1975; Chu & Bassham 1973; Jordan & Chollet 1983).

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Conclusions

Our recent identification of a soluble protein required for Rubisco activation *in vivo* has resolved the enigmatic observation that the Rubisco activation level in illuminated leaves is much greater than can be accounted for by the spontaneous activation of the isolated enzyme at physiological concentrations of CO_2, Mg^{2+} and pH. This system also provides the framework of a regulatory mechanism for integrating the rate of CO_2 fixation with the rate of photosynthetic energy transduction. In illuminated leaves, the steady-state RuBP concentration is saturating for Rubisco, regardless of the CO_2 concentration or light intensity. RuBP forms a complex with inactivated Rubisco and only very slowly activates in the presence of CO_2 and Mg^{2+}, so the default state of Rubisco *in vivo* is the inactive one. Rubisco activase uses Rubisco–RuBP as a substrate, and since Rubisco activase is driven by illuminated thylakoids, the amount of active Rubisco is directly determined by the light intensity received by the chloroplast. This mechanism ensures that the RuBP concentration is always saturating and thus sufficient to maintain the maximum possible rates whatever the ambient light intensity, CO_2 concentration and other environmental parameters. A constant, saturating RuBP concentration also ensures that the photosynthetic carbon reduction cycle maintains homeostasis in photosynthetic metabolite concentrations and thus in CO_2 fixation rates. In contrast to previous models regarding light and CO_2 limitations of photosynthesis, which considered that energy supply directly regulated RuBP synthesis and utilization, our interpretation of the Rubisco activase system is that the activation level of Rubisco and the rate of RuBP synthesis are coordinately regulated by processes in the thylakoid membrane to maintain a saturating level of RuBP and thus maximum rates of photosynthesis.

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Discussion

R. C. Leegood (*Research Institute for Photosynthesis, Department of Botany, University of Sheffield, Sheffield, S. Yorks. S10 2TN, U.K.*) Professor Ogren stated that he believed that RuBP never limited the rate of carboxylation during steady-state photosynthesis, whether leaves were illuminated in high light or in low light. Certainly Mott *et al.* (1984) claim that the pool of RuBP remains constant in the steady state at different light intensities and that the pool is maintained in low light by deactivation of Rubisco. However, the low light intensities they employed were

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relatively high (600 μmol PAR m^{-2} s^{-1}). Clearly there will be considerable variation between different leaves and different species, but our own results show both that the pool of RuBP in spinach leaves in air is always lower in low light and that in the steady state in low light amounts of RuBP are often well below the binding-site concentration. There are also a number of other reports in which RuBP is decreased in low light in air compared with high light and in the steady state in low light RuBP may be below the binding-site concentration (see, for example, Badger *et al.* 1984; Dietz & Heber 1984).

W. L. Ogren. The context of the comment by Dr. Leegood is embodied in the question 'What limits photosynthesis rate at low light intensity, RuBP regeneration rate or Rubisco activation state?' Based on the RuBP response to changes in light intensity observed by Mott *et al.* (1984) in *Xanthium strumarium* and the observations that photosynthesis rate closely paralleled Rubisco activation state in wheat (Perchorowicz *et al.* 1981) and in *Arabidopsis thaliana* wild type and activation mutant (Salvucci *et al.* 1986), we concluded that Rubisco activation state, not RuBP regeneration rate, is the primary site of regulation. The generality of this conclusion remains to be determined.

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