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Phil. Trans. R. Soc. Lond. B 1989 **323**, 435-448
doi: 10.1098/rstb.1989.0022

Phil. Trans. R. Soc. Lond. B **323**, 435–448 (1989)
Printed in Great Britain

Evaluating the role of Rubisco regulation in photosynthesis of C_3 plants

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The enzyme ribulose 1,5-bisphosphate carboxylase (Rubisco) catalyses the entry of carbon dioxide into photosynthetic metabolism, provides acceptor molecules that consume the products of the light reactions of photosynthesis, and regulates the pool sizes of important photosynthetic intermediates. Studies of the regulation of Rubisco *in vivo* have required the development and adaptation of biochemical techniques to physiological questions. For example, the analogue of the six-carbon intermediate 2-carboxyarabinitol bisphosphate is now used in several ways to assess regulation of Rubisco. The advances in understanding Rubisco regulation allow a re-evaluation of the concepts of regulation and limitation of photosynthesis. The Blackman view of limiting factors in photosynthesis is supported by measurements and can be explained by the regulation of Rubisco. This regulation of Rubisco can result in the efficient use of resources. The Blackman view remains a useful framework for discovering patterns in the complex regulation of Rubisco.

Introduction

The enzyme ribulose 1,5-bisphosphate carboxylase (Rubisco) catalyses the entry of carbon dioxide into photosynthetic metabolism, provides acceptor molecules to use the products of the light reactions of photosynthesis, and regulates the pool sizes of important photosynthetic intermediates by changes in its activity. The role of Rubisco in regulating photosynthesis was reviewed recently by Ogren *et al.* (1986), Andrews & Lorimer (1987), and Woodrow & Berry (1988). Recent studies have examined effectors such as Rubisco activase and carboxyarabinitol 1-phosphate (CA1P) that were unknown only five years ago. These studies have enhanced our understanding of the role of Rubisco regulation in photosynthesis.

The capacity to predict outcomes is one of the few measures of scientific truth available to us. The process of making predictions, testing the predictions, then revising initial viewpoints is readily apparent in the study of Rubisco regulation. Within the last ten years, quantitative predictions were made about the pool size of RuBP, measurement techniques were developed, measurements were made, conditions which led to large disagreement between the prediction and measurement were investigated, and an updated view of the role of Rubisco regulation in photosynthesis has emerged. Here I shall discuss the role of Rubisco regulation in establishing and maintaining the instantaneous rate of photosynthesis.

I shall distinguish throughout between regulation and limitation of the instantaneous rate of photosynthesis. I shall use the term 'regulation' to mean adjustment for proper functioning or according to a rule, whereas 'limitation' is what establishes the maximum. I hope to show how these dictionary definitions of regulation and limitation can be applied to regulation and limitation of photosynthesis by Rubisco. Two senses of regulation will be discussed: regulation of photosynthesis by Rubisco and regulation of Rubisco during photosynthesis.

The role of Rubisco in the CO_2 response of photosynthesis

Leaf photosynthesis depends on the concentration of CO_2 in solution because CO_2 is a substrate of Rubisco. The concentration of CO_2 in solution in turn depends on the partial pressure of CO_2 in the gas phase in equilibrium with a solution (Henry's Law). This substrate effect of p_{CO_2} is most plainly seen when the other substrate, ribulose 1,5-bisphosphate (RuBP), is freely available.

When this is so, a small hypothetical increase in the amount of Rubisco protein would result in an increase in the rate of photosynthesis. Because the amount of Rubisco is determining the maximum rate, it is limiting the rate of photosynthesis according to the definition of limitation given above. When Rubisco is setting the upper bound to the rate of photosynthesis the substrate-level effect of CO_2 is evident.

A second effect of CO_2

When the photon flux density (PFD) is low, the ability of the photosynthetic carbon reduction (PCR) cycle to regenerate RuBP is often much lower than the ability of Rubisco to convert RuBP to products. In C_3 plants, photosynthesis still responds to p_{CO_2} for the second reason that increases in p_{CO_2} suppress photorespiration. In this case the properties of Rubisco are regulating photosynthesis by determining how much RuBP will go to carboxylation and how much to oxygenation. A small hypothetical increase in the amount of Rubisco protein would not increase the rate of photosynthesis and so Rubisco does not limit the rate of photosynthesis.

The distinction between regulation and limitation is clearly demonstrated by this second effect of CO_2 on photosynthesis. The quantum yield in C_3 plants is CO_2 dependent when O_2 is present (Ehleringer & Björkman 1977). This is because the properties of Rubisco regulate how much RuBP is used for carboxylation and how much is used for oxygenation. Rubisco adjusts photosynthesis in accordance with a rule, the specificity factor for CO_2 and O_2. The quantum yield is independent of the amount of Rubisco, therefore photosynthesis in the quantum yield region of a PFD–response curve is not limited by Rubisco even though it is CO_2 sensitive. This can also happen at higher rates of photosynthesis: photosynthesis can be CO_2 sensitive but not limited by Rubisco.

The role of Rubisco in the light response of photosynthesis

Electron transport provides the energy needed to regenerate RuBP for Rubisco, but given the cyclical nature of photosynthetic carbon reduction it is also legitimate to say that Rubisco activity provides the acceptor molecules (primarily 3-phosphoglycerate (PGA)) necessary to hydrolyse ATP and oxidize NADPH to allow electron transport to continue. The response of photosynthesis to light can be either regulated or limited by Rubisco. As discussed above, Rubisco regulates photosynthesis in the quantum yield region of a light response curve. Under other conditions, the rate at which Rubisco can supply PGA will determine the rate of electron transport and so determine the maximum rate of photosynthesis. In such conditions, Rubisco can limit photosynthesis: plants with more Rubisco have higher rates of light-saturated photosynthesis (Björkman 1981).

Rubisco limitation of photosynthesis

When photosynthesis in leaves of C_3 plants is limited predominantly by Rubisco, photosynthesis in leaves takes on characteristics similar to those of purified Rubisco supplied with saturating amounts of RuBP. Among these characteristics are a very strong response to p_{CO_2} caused by both the substrate level effect and the suppression of photorespiration, and a weak or absent response to temperature and light (von Caemmerer & Farquhar 1981). The rate of photosynthesis measured under these conditions is well correlated with the amount of Rubisco present in leaves (Seemann *et al.* 1981; von Caemmerer & Farquhar 1981). When the instantaneous rate of photosynthesis is limited this way, we can reasonably surmise that a hypothetical increase in the amount of Rubisco would increase the rate of photosynthesis.

A strong response to p_{CO_2} at very low p_{CO_2} is a necessary but not sufficient character to postulate that photosynthesis is limited by Rubisco activity. The suppression of photorespiration by p_{CO_2} in leaves at low PFD also results in a steep increase in photosynthesis at low p_{CO_2}. Because CO_2 sensitivity arises by two different mechanisms, analyses of CO_2 responses of intact leaves without calculating rates of RuBP regeneration (Farquhar *et al.* 1980) or measuring rates of electron transport (Sharkey *et al.* 1989) cannot be interpreted in biochemical terms (Dietz 1986; Dietz & Heber 1986).

If Rubisco limits photosynthesis in a particular situation, then reducing the efficiency of Rubisco by reducing the p_{CO_2} should cause feedback on the rate of electron transport. This can be detected as a change in fluorescence from the leaf. On the other hand, if Rubisco only regulates photosynthesis, by determining the fate of a constant supply of RuBP for either carboxylation or oxygenation, then no feedback is expected as p_{CO_2} is changed. Both of these cases are illustrated in figure 1. At moderate PFD, the rate of electron transport calculated from fluorescence parameters (Weis & Berry 1987) increases at low p_{CO_2} indicating feedback from carbon reactions on electron transport and that Rubisco limits photosynthesis. However, at low PFD, there is no change in the calculated rate of electron transport with CO_2, indicating that there is no feedback from carbon metabolism on electron transport and that Rubisco regulates but does not limit photosynthesis.

When Rubisco activity limits the rate of photosynthesis, the nocturnal inhibitor carboxyarabinitol 1-phosphate (CA1P, described below) is not present and Rubisco is usually fully activated (Seemann & Sharkey 1986; von Caemmerer & Edmondson 1986). Exceptions to this generalization are the work of Brooks (1986), Perchorowicz & Jensen (1983) and R. F. Sage (personal communication). Brooks found that Rubisco from plants starved for inorganic phosphate never reached full activation. Perchorowicz and Jensen (1983) found that Rubisco in 6–8 day old wheat seedlings never achieved full activation and Sage found that plants growing in elevated CO_2 lost the ability for full activation at low CO_2 levels. These effects may reflect the metabolic costs of the regulatory machinery (discussed by Woodrow & Berry (1988)).

The pool of RuBP is generally high and is probably saturating when Rubisco limits photosynthesis (Badger *et al.* 1984; Dietz & Heber 1986; Seemann & Sharkey 1986; von Caemmerer & Edmondson 1986). The question of how much RuBP is required to saturate Rubisco has sparked some debate.

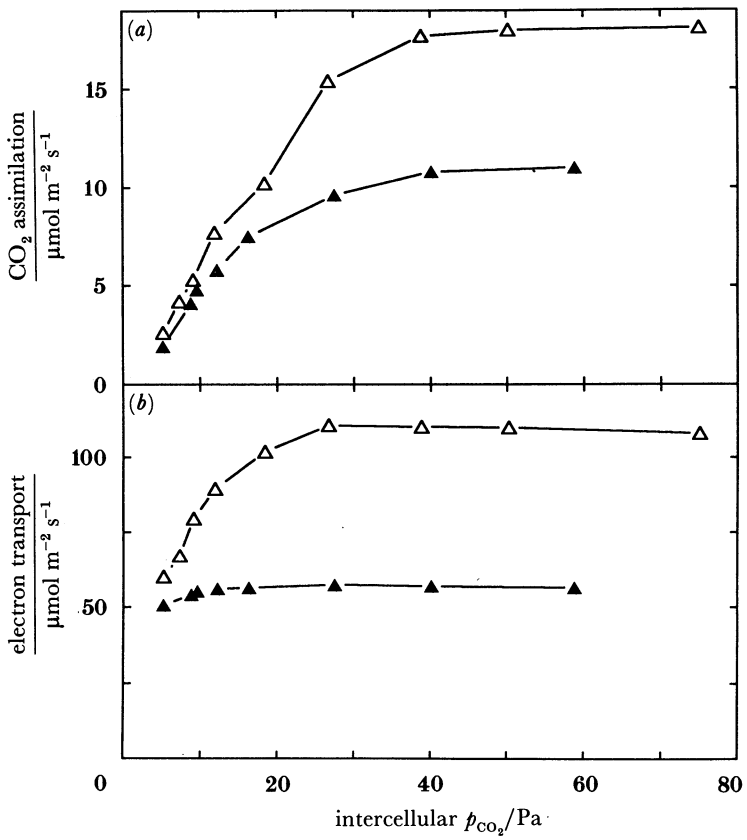

FIGURE 1. Rate of photosynthetic CO_2 assimilation (*a*) and electron transport (*b*) as functions of intercellular CO_2 partial pressure for a leaf of *Phaseolus vulgaris*. The open symbols were determined with a PFD of 380 μmol quanta m^{-2} s^{-1}, the closed symbols with 175 μmol quanta m^{-2} s^{-1}. Leaf temperature was 25 °C. Electron transport was determined from fluorescence parameters as described by Weis & Berry (1987). These results illustrate that CO_2 can increase the rate of photosynthesis by suppressing photorespiration (at low PFD) or by making Rubisco more efficient (at moderate PFD) (data from Sharkey *et al.* 1989).

How much RuBP *is required to saturate Rubisco in vivo?*

Farquhar *et al.* (1980) originally proposed that if there were more than one RuBP for every catalytic site of Rubisco, then the activity of Rubisco would be saturated with RuBP. Perchorowicz *et al.* (1981) and Perchorowicz & Jensen (1983) measured RuBP pools in six-to eight-day old wheat seedlings under a variety of conditions. They found that the pools of RuBP were always very high. (It is possible that this was because they used young plants which were, in all likelihood, still receiving carbohydrate from the endosperm. We now know that when there are insufficient sinks for photosynthate, as can occur in low p_{O_2}, the pools of RuBP are often quite high (Badger *et al.* 1984; Schnyder *et al.* 1986). In addition, the RuBP levels reported by Perchorowicz & Jensen may have been high because they plunged leaves into liquid N_2. Although liquid N_2 is colder than most other commonly used cryogens, it stops metabolism more slowly because it is a boiling liquid that forms an insulating layer of gaseous N_2 around leaf material. As this gas layer is pure N_2, any RuBP made during the slow freezing would accumulate in the leaves.)

Badger *et al.* (1984) measured RuBP pools in leaves of *Phaseolus vulgaris* plants three to six weeks old placed under a range of conditions. They used a specially constructed freeze-clamp

to stop metabolism rapidly. When photosynthesis appeared Rubisco limited (strong response to CO_2 at high PFD), the RuBP pool was two to four times greater than the pool of Rubisco binding sites. When the gas exchange behavior of the leaves indicated that Rubisco was no longer saturated with RuBP, the concentration of RuBP was still greater than 1.5 times the concentration of binding sites. Subsequent studies with improved techniques have confirmed that the pool of RuBP rarely falls below 1.5 per binding site (Seemann & Sharkey, 1986; von Caemmerer & Edmondson 1986) except at very low PFD.

An important improvement in technique has been the measurement of the amounts of both RuBP and Rubisco in opposite halves of the same leaf disc killed in a freeze clamp. The concentration of Rubisco is measured by incubating a plant extract with radioactive 2-carboxyarabinitol 1,5-bisphosphate (CABP). CABP is very similar to the intermediate formed by the addition of CO_2 to RuBP before cleavage into two molecules of 3-PGA. The binding of CABP to Rubisco is extremely tight and specific. Rubisco bound with CABP is precipitated from solution by anti-Rubisco antibodies (Collatz *et al.* 1978) or by polyethylene glycol (McCurry *et al.* 1981). The importance of expressing RuBP concentrations per binding site is illustrated by the data in table 1.

TABLE 1. POOL SIZE OF RUBP IN *ALOCASIA MACRORRHIZA* AND *PHASEOLUS VULGARIS* GROWN IN SHADE

(RuBP concentration is expressed on a chlorophyll basis or per CABP binding site. Data from Sharkey *et al.* (1986*b*) given as mean ± s.e.)

pool	*Alocasia*	*Phaseolus*
chlorophyll/ng mol^{-1}	17 ± 1	112 ± 5
CABP sites/mol mol^{-1}	1.5 ± 0.2	3.4 ± 0.3

The shade plant *Alocasia* has a very high concentration of chlorophyll and a low concentration of Rubisco per unit leaf area relative to *Phaseolus*. The concentration of RuBP per chlorophyll is in *Alocasia* one sixth of that in *Phaseolus*, but per binding site the concentration of RuBP in *Alocasia* is one half that in *Phaseolus*.

The amount of RuBP present is often expressed as a volume concentration (discussed by Walker *et al.* 1986). However, there is no indication that concentration is the relevant parameter and it involves estimating the chloroplast volume. Because the concentration of sites is so much higher than the binding affinity of Rubisco for RuBP and because expressing RuBP relative to binding sites does not involve estimates of volume from chlorophyll measurements, this seems a better way to express amounts of RuBP in leaves.

It is now established that a concentration of 1.5 mol RuBP mol^{-1} CABP binding sites occurs when gas exchange behaviour predicted for RuBP regeneration-limited photosynthesis is observed. Why is the value 1.5, and not one as originally postulated (Farquhar *et al.* 1980)? von Caemmerer & Farquhar (1985) (their analysis can also be found in an appendix to von Caemmerer & Edmondson (1986)) developed a theoretical treatment of ionization state and Mg^{2+} binding of RuBP. In their analysis, 1.5–2 RuBPs per site may be required to saturate Rubisco because some of the RuBP is in the wrong ionic form or complexed with Mg^{2+} and so unavailable for binding to Rubisco.

There may also be more RuBP than expected when Rubisco does not limit photosynthesis

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because Rubisco activity is highly regulated. Woodrow & Berry (1988) point out that RuBP is a unique metabolite in photosynthesis because it is produced and consumed by irreversible reactions. It also contains more phosphate per carbon than any other metabolite in the photosynthetic carbon reduction cycle. Thus changes in the RuBP pool will be reflected by twofold changes of the opposite sign in the P_i pool. Regulation of P_i pool sizes may be a more important factor in determining the steady-state pool size of RuBP than is the need to saturate Rubisco.

In summary, when Rubisco limits photosynthesis, photosynthesis takes on the characteristics of Rubisco, Rubisco is not regulated, and more than 1.5 RuBP molecules per active site of carboxylase are present. The CO_2 responsiveness of photosynthesis occurs because CO_2 is a substrate for the reaction limiting photosynthesis.

Rubisco regulation

In the above I have used the term 'regulation' to mean the determination by Rubisco of the fate of products of electron transport, specifically RuBP. In addition, the activity of Rubisco is adjusted for proper functioning of photosynthesis. These changes in activity are another aspect of regulation, this time a regulation of Rubisco rather than a regulation by Rubisco. Substantial progress has been made in understanding two of the methods by which Rubisco activity is regulated.

Methods of regulation

The response of Rubisco to changes in the substrate CO_2 is a very useful probe of photosynthesis in the laboratory, but in nature the partial pressure of CO_2 does not usually change dramatically over short times. The other substrate, RuBP, also changes very little as photosynthesis changes over a wide range except at very low or very high PFD (figure 2) (Woodrow & Berry 1988). Under natural conditions, Rubisco is primarily regulated by changes in the amount of activator CO_2 bound to it (carbamylation) (Miziorko & Lorimer 1983) and by the presence of CA1P (Seemann *et al.* 1985; Servaites 1985; Gutteridge *et al.*

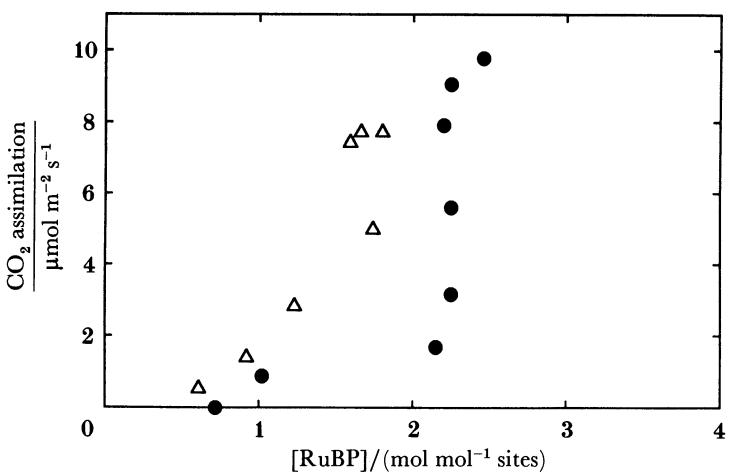

Figure 2. Rate of photosynthesis as a function of ribulose 1,5-bisphosphate for intact leaves of *Phaseolus vulgaris* (△) and *Beta vulgaris* (●). The date are replotted from Kobza & Seemann (1988).

1986; Berry *et al.* 1987). Regulation of Rubisco by factors such as pH and Mg^{2+}, other than through their effects on carbamylation, will not be considered, because at present there are no techniques to assess the physiological importance of these effects.

The carboxylase and oxygenase functions of Rubisco require CO_2 and Mg^{2+} for activation. The CO_2 is bound to a lysine residue to form a carbamate in a slow, pH-dependent step (Miziorko & Lorimer 1983). This activation will occur spontaneously at elevated CO_2 levels, but at air levels of CO_2 this activation is catalysed by the enzyme Rubisco activase (Salvucci *et al.* 1986). Rubisco activase depends on ATP (Streusand & Portis 1987; Robinson & Portis 1988) and so provides the link between the ATP status of photosynthetic tissue and Rubisco activation that had been postulated on physiological grounds (Sharkey *et al.* 1986*a*; Seemann & Sharkey 1987).

New techniques for assessing Rubisco regulation

The changes in activation state under physiological conditions have been measured as activity observable immediately upon extraction of the leaf material (initial activity) relative to the activity measured after incubating the extract with CO_2 and Mg^{2+} (total activity). Upon extraction from the leaf, Rubisco activation state is remarkably stable for up to 5 min providing the extraction buffer contains 5 mM Mg^{2+} and the temperature is kept at 0 °C. The requirement for low temperature may be related to the tight binding of RuBP to inactive Rubisco at low temperature (see below).

There is an independent method for measuring the carbamylation state of Rubisco. CABP binds essentially irreversibly to carbamylated Rubisco, but less tightly to Rubisco lacking the carbamate (Hall *et al.* 1981). Incubation of Rubisco with radioactive CABP causes all the binding sites of Rubisco to become labelled. By presenting a 500–1000-fold excess of unlabelled CABP, the radioactive CABP bound to uncarbamylated sites will be displaced. By comparing the amount of label retained by Rubisco immediately upon extraction with the amount retained by Rubisco incubated with CO_2 and Mg^{2+}, a carbamylation ratio can be determined. Butz & Sharkey (1989) have compared carbamylation ratios with activation states and found them to be similar when deactivation occurs in low O_2 (table 2).

Table 2. Carbamylation, activation and catalytic constant of Rubisco from *Phaseolus vulgaris*

(Data of Butz & Sharkey (1989), expressed as mean ± s.e.)

	carbamylation ratio (%)	activity ratio (%)	k_{cat}/s^{-1}
normal air	80 ± 5	80 ± 4	20 ± 1
low O_2 and high CO_2	56 ± 3	54 ± 1	18 ± 1
low light	69 ± 2	59 ± 6	11 ± 1

This technique for measuring the degree of carbamylation of Rubisco is very robust. After adding the radiolabelled CABP, unlabelled CABP can be added immediately or up to 30 min later. The protein can be precipitated immediately or several hours later (Butz & Sharkey 1989).

The inhibitor CA1P is detected by a reduction in the catalytic constant of Rubisco (k_{cat}). The k_{cat} is V_{max} (total activity as measured above) divided by the amount of enzyme present. The amount of enzyme present is measured by binding radioactive CABP to Rubisco incubated with CO_2 and Mg^{2+}. The presence of CA1P reduces k_{cat} because it blocks the

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binding of RuBP, and so prevents activity of the enzyme, but does not affect the binding of CABP. It is also possible to extract CA1P from plant tissue and measure its presence by its effect on the activity of purified Rubisco (Kobza & Seemann 1988).

Enzyme sites that have CA1P bound to them will not affect activation state determinations, but they will affect carbamylation ratio measurements. This is evident in the data in table 2 collected at low light. The low k_{cat} indicates the presence of CA1P. The activation state is lower than the carbamylation state, indicating that Rubisco bound to CA1P is more active than Rubisco not bound to CA1P. Measurements of activation will be valid only for enzyme not bound with CA1P and CA1P absence and activation state are multiplicative. For example, if 45% of the Rubisco sites have CA1P bound to them (55% unbound) and the activation state is 59%, then $0.55 \times 0.59 = 0.32$, and so 32% of the sites are functional.

The effect of Rubisco regulation on metabolite pools

An important role for Rubisco in photosynthetic metabolism is the regulation of pool sizes (Sage *et al.* 1988). The precursor, RuBP, and the product, glycerate 3-phosphate (PGA), of the Rubisco reaction are, in almost all cases, the metabolites that change most in concentration over a very wide range of environmental conditions (Badger *et al.* 1984; Dietz & Heber 1986; Prinsley *et al.* 1986; Sharkey *et al.* 1986*b*, *c*).

The changes in RuBP pool size as photosynthesis responds to light (figure 2) have great significance for understanding the regulation of photosynthesis. When photosynthesis is not limited by Rubisco, Rubisco can be regulated by either starvation for RuBP or by direct regulation of its activity by other mechanisms. The starvation for RuBP is only seen at extremely low light (Perchorowicz & Jensen 1983; Badger *et al.* 1984; Dietz & Heber 1986; Kobza & Seemann 1988) or during transients (Mott *et al.* 1984; Prinsley *et al.* 1986; Sharkey *et al.* 1986*c*). In most circumstances, the activity of Rubisco is regulated by changes in carbamylation and CA1P so that RuBP starvation does not occur. A plot of photosynthetic rate against RuBP is sigmoidal, when leaves are allowed to equilibrate at each PFD. Steep increases in photosynthetic rate occur when the RuBP concentration is between 1.5 and 2.5 per site (figure 2) (see also Woodrow & Berry 1988). This is in contrast to the straight-line relation between substrate and enzyme activity which is observed in transient experiments (Mott *et al.* 1984) and had been predicted (Farquhar *et al.* 1980).

Why does RuBP *rarely fall below 1.5 per site?*

It is believed that the purpose served by keeping the RuBP pool high is regulation of the level of inorganic phosphate inside the stroma (Sage *et al.* 1988; Woodrow & Berry 1988). Accordingly, the pool of RuBP that is found when Rubisco regulates photosynthesis is determined by the requirements for sequestering P_i and need not reflect the concentration of RuBP needed to saturate Rubisco.

A second effect of keeping the pool of RuBP high is to prevent Rubisco from binding other photosynthetic metabolites in the stroma. Rubisco will bind a number of photosynthetic metabolites, presumably at the active site of the enzyme (Badger & Lorimer 1981). This observation has given rise to the idea that Rubisco can act as a metabolite buffer (Ashton 1982; Furbank *et al.* 1987). However, calculations used to support the metabolite buffer theory (Ashton 1982) were made assuming RuBP levels too low by a factor of five. Other phosphate esters could affect Rubisco during transients, as hypothesized for PGA (Prinsley *et al.* 1986;

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Foyer *et al.* 1987). In the steady state these effects are unlikely because all sites, active or inactive, are probably bound with RuBP.

Brooks & Portis (1988) have found that deactivated Rubisco has RuBP bound to it under steady-state conditions. At low temperature, RuBP is bound more tightly to deactivated than to activated Rubisco (Jordan & Chollet 1983). If this also occurred at normal temperatures, photosynthesis would soon stop as RuBP bound to inactive sites and prevented them from becoming active (von Caemmerer & Farquhar 1985). Three hypotheses have been advanced to explain how this problem is overcome. Portis *et al.* (1986) and Salvucci *et al.* (1986) have shown that Rubisco activase can keep Rubisco active despite the tight binding of RuBP to deactivated Rubisco. Mott & Berry (1986) have shown that at high pH RuBP does not bind tightly to Rubisco. Seemann & Kobza (1988) suggest that the binding of RuBP to inactive sites is extremely temperature dependent, and so the problem is not as severe at normal temperatures. These solutions to this problem of RuBP binding to inactive Rubisco are not mutually exclusive.

Ideal regulation of Rubisco

An ideal regulation of Rubisco would impose no cost of regulation on the metabolism of photosynthesis. The rate of Rubisco activity would increase as required, for example as the supply of RuBP increased, until the enzyme was 100% active. Once this was attained, any increase in availability of RuBP would have no effect. There would be an abrupt transition from the regulatory role to the limiting role of Rubisco. Kobza & Seemann (1988) found that in *Beta vulgaris* the rate of photosynthesis increased over fivefold with no increase in RuBP (figure 2). In this case Rubisco activity increased, by activation and loss of CA1P, as the rate of supply of RuBP increased and so the pool size of RuBP was independent of the flux rate.

If Rubisco regulation only occurs when Rubisco activity is greater than the capacity for RuBP regeneration, then Rubisco activation should fall with increasing CO_2 even though CO_2 is required for activation. This behaviour was observed by von Caemmerer & Edmondson (1986) (see also figure 3). R. F. Sage (personal communication) has modelled this behaviour and shown that the changes in activation state can be predicted by the following guidelines. (1) Rubisco activation is set so that the maximum rate of use of RuBP by Rubisco is equal to

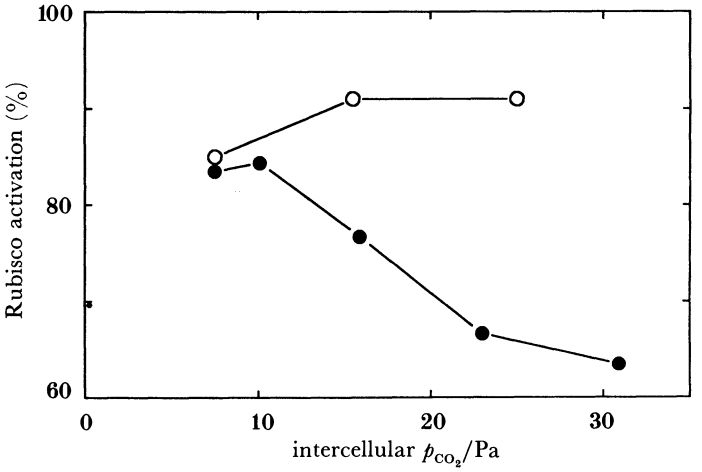

FIGURE 3. Activation of Rubisco as a function of intercellular p_{CO_2} for a leaf of *Chenopodium album*. The open symbols were determined with 1800 μmol quanta m^{-2} s^{-1}, the closed symbols with 500 μmol quanta m^{-2} s^{-1}. Leaf temperature was 25 °C, p_{O_2} was 18 kPa (unpublished data of R. F. Sage).

the capacity for RuBP production. (2) When more than 100% activation is called for, the activation state is 100% and Rubisco limits the rate of photosynthesis. (3) Rubisco cannot deactivate to less than 30%. These simple rules account for many of the complex relations between activation state, CO_2 and PFD.

If Rubisco is ideally regulated, then photosynthesis should undergo an abrupt transition from Rubisco limited to Rubisco regulated as the p_{CO_2} around a leaf is increased. This behaviour has been observed many times in intact leaves (figure 4) (von Caemmerer & Farquhar 1981, 1984; Seemann & Sharkey 1986). There are many non-biochemical reasons why abrupt transitions might not be observed in intact leaves, including variations in photosynthetic capacity across the leaf and light gradients through the leaf. These physical factors obscuring the abrupt transitions in intact leaves should be ruled out before it is concluded that the biochemistry of photosynthesis does not undergo abrupt transitions. That abrupt transitions can be observed in intact leaves indicates a remarkable homogeneity of the photosynthetic apparatus over the leaf and that the underlying biochemistry has the capacity to undergo abrupt transitions.

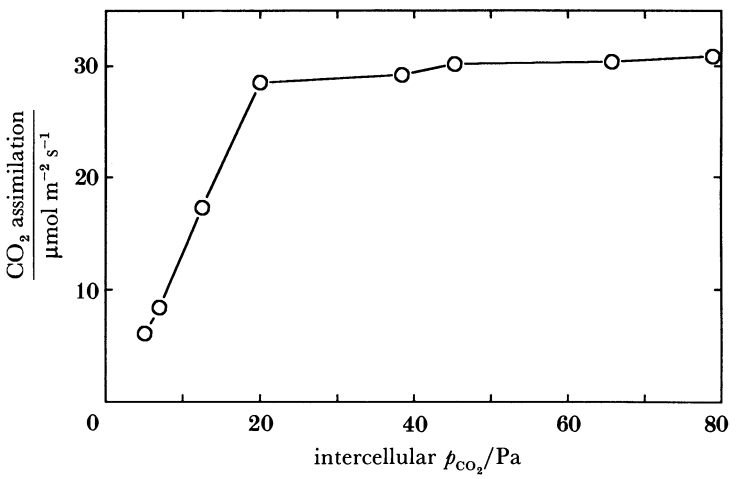

FIGURE 4. The rate of photosynthetic CO_2 assimilation as a function of intercellular pCO_2 for a leaf of *Phaseolus vulgaris*. The p_{O_2} was 2 kPa, leaf temperature was 25 °C, PFD was 1000 μmol quanta m^{-2} s^{-1}. These data illustrate that abrupt transitions occur in photosynthesis of C_3 plants when experiments are done in reduced O_2 atmosphere to eliminate the second effect of CO_2 on photosynthesis (T. D. Sharkey, unpublished data).

In many experiments with intact, thin leaves, abrupt transitions are observed. It was originally thought that this resulted from a high concentration of enzyme relative to the affinity of Rubisco for RuBP (Farquhar *et al.* 1980; Sharkey 1985). However, I now believe that the abrupt transitions result from regulation of the irreversible steps in photosynthesis.

The Blackman view of photosynthesis

The view that intact leaf photosynthesis is limited by a single factor at any given instant was first put forward by Blackman (1905). This idea has been criticized because it implies inefficient use of resources in non-limiting reactions. This intuitive criticism has merit when long timescales are considered (Sharkey 1985). However, there is no reason to impose such logic on a timescale over which the plant cannot respond. As shorter and shorter timescales are considered, rapid formation and degradation of sufficient amounts of proteins becomes

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energetically unfavourable when compared with the increased photosynthesis made possible. Over short time periods the enzyme capacity is fixed and ideal regulation will keep a process from limiting photosynthesis until an absolute maximum capacity is needed. Then, abruptly, this process will change from its role in regulation to its role in limitation of photosynthesis. Allowing only one factor to limit makes more efficient use of resources compared with colimited photosynthesis. The modern Blackman view is that the rate of photosynthesis in leaves approaches the theoretical limit allowed by electron transport and Rubisco capacity. The metabolic cost of regulatory processes (as discussed by Woodrow & Berry (1988)) is presumed to be low and so the rate of photosynthesis that can be measured approaches the maximum possible activity of extant electron transport components and Rubisco, whichever is less.

If photosynthesis is limited solely by Rubisco in the middle hours of the day, and by electron transport capacity at the beginning and end of the day, this may be the best use of scarce resources. The fact that long-term plant growth responds to increases in PFD and CO_2 does not preclude photosynthesis from being limited by Rubisco alone for some minutes and by PFD alone for others. Through the day a range of factors will limit the rate of photosynthesis but at any given instant, photosynthesis will be limited by only one or a few reactions.

The alternative to the Blackman view is that everything or several things always limit to some degree (Taylor & Terry 1984, 1986). Often called co-limitation, it is believed that control of the photosynthetic rate is distributed among the various reactions in photosynthesis and that an increase in capacity of any one of these reactions will increase the overall rate of photosynthesis. This view is implicitly based on Michaelis–Menten (or similar) kinetics: an increase in pool size will result in a greater rate of the reaction. However, this view should be re-examined in the light of the finding that mass action ratios for some of the important reactions in photosynthesis fall as the rates of those reactions increase (Dietz & Heber 1986).

Evidence in favour of the co-limitation view of photosynthesis is the observation that photosynthesis is often sensitive to both CO_2 and PFD at the same time. However, it has already been demonstrated that photosynthesis can be CO_2 sensitive without being Rubisco limited because CO_2 suppresses photorespiration. Under non-photorespiratory conditions, the slow saturation of photosynthesis with CO_2 is not observed (figure 4). Thus photosynthesis may be co-limited by PFD and CO_2, but this observation does not provide a useful framework for interpreting biochemical data.

The distinction between the co-limitation view and the Blackman view is important. If the deactivation of Rubisco is regulatory and does not limit the overall rate of photosynthesis then it is a desirable trait to have in engineered plants. If, on the other hand, deactivation of Rubisco significantly limits the overall rate of photosynthesis, then engineering a new, permanently activated, Rubisco would result in greater rates of photosynthesis. The difference in viewpoint of limitations results in opposite recommendations from the physiologist to the plant breeder or genetic engineer.

The difference in viewpoint is also important for interpreting photosynthetic data. Changes in such things as gas composition of the atmosphere will have dramatically different effects depending on whether or not a transition between limitations is crossed. If plant growth is colimited by PFD and CO_2 then photosynthesis should undergo transition from Rubisco regulation to Rubisco limitation several times in a day and these transitions will occur around

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the operating condition of the plant. Therefore studies of Rubisco regulation should be conducted under nearly natural conditions as much as possible.

In summary, the Blackman view is more useful than the co-limitation view for interpreting biochemical data, can be explained by the regulation of key photosynthetic enzymes and it is supported by available data (figures 2 and 4). I continue to use the Blackman view because it provides a useful framework for discovering patterns in the complex regulation of Rubisco.

I thank Dr Susanne von Caemmerer, Dr Graham Farquhar, and Professor Barry Osmond for their helpful comments on this manuscript.

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