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Interactions between ribulose-1,5-bisphosphate carboxylase and stromal metabolites. I. Modulation of enzyme activity by Benson-Calvin cycle intermediates

Christine H. Foyer, Robert T. Furbank and David A. Walker

Research Institute for Photosynthesis, University of Sheffield, Sheffield (U.K.)

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Changes in the concentrations of glycerate 3-phosphate, ribulose 1,5-bisphosphate, fructose 1,6-bisphosphate and dihydroxyacetone phosphate were measured in leaf discs under various conditions of light intensity and CO_2 concentration. The range of measured metabolite concentrations was then used to study metabolite regulation of ribulose-1,5-bisphosphate carboxylase. The effects of physiological concentrations of glycerate 3-phosphate, fructose 1,6-bisphosphate and dihydroxyacetone phosphate on ribulose-1,5-bisphosphate carboxylase activity of isolated stromal protein at saturating and limiting ribulose 1,5-bisphosphate were measured by incorporation of $^{14}CO_2$. A system containing ribulose 5-phosphate and an ATP-generating mechanism is described which was used to produce a constant low level of ribulose 1,5-bisphosphate in the reaction mixture. Of the metabolites measured glycerate 3-phosphate was the only one which was found to be present at a sufficiently high concentration relative to ribulose 1,5-bisphosphate in the light to be an effective inhibitor of ribulose-1,5-bisphosphate carboxylase activity in situ. Physiological concentrations of glycerate 3-phosphate were found to inhibit carboxylation severely, particularly at rate-limiting ribulose 1,5-bisphosphate concentrations. The rate of $^{14}CO_2$ assimilation was decreased 85% (with 0.2 mM ribulose 1,5-bisphosphate) by the presence of 20 mM glycerate 3-phosphate and the K_m (ribulose 1,5-bisphosphate) of ribulose-1,5-bisphosphate carboxylase was increased by over 10-fold. The activity of the enzyme was inhibited substantially by a mixture of metabolites chosen to mimic in vivo conditions (20 mM glycerate 3-phosphate, 0.5 mM fructose 1,6-bisphosphate and 1.5 mM dihydroxyacetone phosphate). With this mixture of metabolites the K_m (ribulose 1,5-bisphosphate) of ribulose-1,5-bisphosphate carboxylase was increased 10-fold above the control. A pulse of 10 mM glycerate 3-phosphate completely inhibited CO_2 uptake when the activity of the Benson-Calvin cycle as a whole was assayed in chloroplast extracts. Using these data we have examined the physiological significance of glycerate 3-phosphate modulation of ribulose-1,5-bisphosphate carboxylase at different metabolite levels.

Introduction

At saturating light intensities and air levels of CO_2, turnover of the Benson-Calvin cycle is primarily limited by the the rate of production of

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Correspondence: C.H. Foyer, Research Institute for Photosynthesis, University of Sheffield, Sheffield, S10 2TN, U.K.

glycerate 3-phosphate that can be supported by ribulose-1,5-bisphosphate carboxylase [1,2]. The activity of this enzyme is only barely sufficient to support photosynthesis measured under these conditions in vivo [3] and factors affecting the activity of the enzyme may substantially affect the rate of photosynthesis [4]. Under sub-saturating illumination and low CO_2 the regeneration of ribulose 1,5-bisphosphate limits carboxylation [5] and under these conditions it is not clear how modulation of ribulose-1,5-bisphosphate carboxylase contributes to the regulation of photosynthesis.

The catalytic activity of ribulose-1,5-bisphosphate carboxylase may be modulated either by modification of the activation state of the enzyme [6] or by alteration of the rate of catalysis by the fully active enzyme (e.g., competitive inhibition by stromal metabolites or other potential inhibitors of catalysis [7,8]). Modification of the activation state of ribulose-1,5-bisphosphate carboxylase has been extensively studied [6,9–11] and there is considerable evidence that the activation state changes with light intensity and possibly with the composition of the gas phase. However, despite observations of a close correlation between extracted catalytic activity and the rate of photosynthesis in vivo, there is little information available on regulation of catalysis rather than activation state.

Recent measurements indicate that leaf levels of metabolites such as glycerate 3-phosphate may be as high as 800 nmol $\cdot$ mg^{-1} chlorophyll [12,13]. In the case of glycerate 3-phosphate and fructose 1,6-bisphosphate between 70 and 80% of the leaf metabolite pool is present in the chloroplast under a wide range of conditions [14,15], while ribulose 1,5-bisphosphate is exclusively located in the chloroplast stroma. Therefore, the levels of ribulose 1,5-bisphosphate, glycerate 3-phosphate and fructose 1,6-bisphosphate measured in leaf extracts approximate chloroplast levels. Assuming a chloroplast stromal volume of 25 μl $\cdot$ mg^{-1} chlorophyll and that 70% of the metabolite level measured in the leaf is present in the chloroplast, the glycerate 3-phosphate concentration in the stroma can reach over 25 mM. Thus, although this metabolite is a relatively poor inhibitor of purified ribulose-1,5-bisphosphate carboxylase [7], this may be offset in vivo by the high concentrations present. Conversely, fructose 1,6-bisphosphate, known to be a potent inhibitor of ribulose-1,5-bisphosphate carboxylase [7], is present only at very low concentrations in the chloroplast stroma in vivo [15].

The significance of metabolite modulation of ribulose-1,5-bisphosphate carboxylase in vivo can be assessed by identifying potential regulatory metabolites, determining concentrations of these metabolites in vivo and how they change with photosynthetic flux and, most importantly, comparing these changes in metabolite levels with the K_d values for binding of these metabolites to ribulose-1,5-bisphosphate carboxylase. In addressing the latter point, the mechanism of inhibition of catalysis by metabolites (including the possibility of synergistic and allosteric effects), the relative concentrations of ribulose 1,5-bisphosphate and metabolite effectors and the enzyme concentration must all be considered. To include all these factors in a theoretical frame-work would be immensely complicated, particularly since there appears to be disagreement in the literature over the nature of inhibition by sugar phosphates and the binding sites involved (compare Refs. 7 and 16). In this paper a chloroplast extract system is used to examine the effect of metabolite regulation of ribulose-1,5-bisphosphate carboxylase on $^{14}CO_2$ fixation by assaying this in the presence of physiological concentrations of metabolites known to inhibit the purified enzyme. This system permits the simulation of changes in metabolite levels seen in vivo during rapid transitions in photosynthetic flux and separation of the effects on ribulose-1,5-bisphosphate carboxylase from those on ATP and NADPH production. The effects of metabolite regulators on ribulose 1,5-bisphosphate at high stromal protein concentrations were also examined.

Materials and Methods

Plant material and intact chloroplast preparation

Spinacia oleracea L. cv virtuosa was grown in hydrophonic culture in a glasshouse as described by Edwards and Walker [17]. Leaf metabolites were extracted and assayed as described previously [13]. Intact spinach chloroplasts were prepared as described by Walker [18].

Preparation of stromal proteins

Stromal proteins were prepared from intact chloroplasts as follows. Intact chloroplast pellets were lysed in 2 ml of a medium containing 10 mM Hepes/KOH (pH 7.6), 1 mM $MgCl_2$, 1 mM EDTA and 5 mM dithiothreitol and centrifuged at $6000 \times g$ for 2 min. The supernatant (chloroplast stromal extract) was retained and passed through a PD 10 gel filtration column (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with a medium containing 2 mM EDTA, 2 mM $MgCl_2$, 4 mM ascorbate, 1 mM dithiothreitol, 10 mM KCl and 50 mM Hepes/KOH buffer (pH 7.9) and stored on ice. The protein concentration was estimated by the dye-binding protein assay (Bio-Rad laboratories, München, F.R.G.).

Ribulose-1,5-bisphosphate carboxylase activity

Ribulose-1,5-bisphosphate carboxylase in stromal protein extracts was activated prior to use by incubation for 10 min at 40 °C in a medium containing 20 mM $MgCl_2$, 10 mM $NaHCO_3$, 2 mM EDTA, 10 mM KCl, 50 mM Hepes/KOH buffer (pH 7.9), 4 mM ascorbate, 1 mM dithiothreitol and stromal protein at 5 or 10 mg/ml. Ribulose-1,5-bisphosphate carboxylase activity (with ribulose 1,5-bisphosphate supplied as substrate) was measured at 25 °C over a 30 s period via the incorporation of $^{14}CO_2$ in 0.1 ml reaction mixtures containing either 100 μg or 2 mg of stromal protein and 20 mM $MgCl_2$, 10 mM $NaH^{14}CO_3$ (0.4 mCi · mmol^{-1}), 0.33 M sorbitol, 2 mM EDTA, 10 mM KCl, 50 mM Hepes/KOH buffer (pH 7.9), 4 mM ascorbate, 1 mM dithiothreitol and various concentrations of ribulose 1,5-bisphosphate. Where ribulose 1,5-bisphosphate was generated in the reaction medium 5 mM ribose 5-phosphate plus 10 mM creatine phosphate and creatine phosphate kinase (0.1 units · ml^{-1}) were added to the above reaction mixture and $^{14}CO_2$ incorporation was measured over a 1 min timecourse. Ribulose 1,5-bisphosphate was estimated either spectrophotometrically at 340 nm using pure ribulose-1,5-bisphosphate carboxylase coupled to the reduction of glycerate 3-phosphate, or by $^{14}CO_2$ incorporation [13].

Glycerate 3-phosphate, fructose 1,6-bisphosphate and dihydroxyacetone phosphate were added to this reaction mixture (immediately before ribulose 1,5-bisphosphate addition) at the concentrations given in the figure legends.

Other assays

Chlorophyll was estimated by the method of Arnon [19]. Carbon fixation in stromal extracts was assayed with 2 mM triose phosphate as substrate in the standard reaction mixture, except that 5 mM dithiothreitol, creatine phosphate kinase (2.2 units · ml^{-1}), 5 mM creatine phosphate and 4 mM ATP were included. $^{14}CO_2$ (0.8 Ci/mol) was included and 100-μl aliquots were removed and injected into formic acid. These samples were dried under a stream of air and acid-stable ^{14}C incorporation measured by scintillation counting. ATP was assayed as previously described [20].

Results

Large changes in the concentrations of leaf metabolites have been found to occur following transitions in light intensity or CO_2 concentration [12,13]. Table I shows the changes in the concentrations of ribulose 1,5-bisphosphate, glycerate 3-phosphate, fructose 1,6-bisphosphate and triose phosphate which have been measured in this study and in other studies in our laboratory in steady-state conditions and also immediately following transitions from darkness to light, high to low light and air levels of CO_2 to air supplemented with 5% CO_2. These values provide a good indication of the range of variation in the pool sizes of these metabolites. It can be seen the concentration of glycerate 3-phosphate is always significantly higher than the ribulose 1,5-bisphosphate concentration, being double the ribulose 1,5-bisphosphate concentration in air and high illumination and over 10-fold higher following a transition in light intensity or CO_2 concentration.

Using the data obtained from such measurements, we studied the effect of physiological concentrations of metabolites, particularly glycerate 3-phosphate, on the activity of ribulose-1,5-bisphosphate carboxylase over a physiological range of ribulose 1,5-bisphosphate levels. It is experimentally difficult to examine metabolite regulation of ribulose-1,5-bisphosphate carboxylase activity at sub-saturating ribulose 1,5-bisphosphate

TABLE I

CHANGES IN THE LEVELS OF CALVIN CYCLE METABOLITES IN SPINACH LEAF DISCS IN STEADY-STATE CONDITIONS AND IMMEDIATELY FOLLOWING TRANSITIONS IN LIGHT AND CO_2

Metabolite data under transient conditions correspond to the point where glycerate 3-phosphate was at a maximum following the transition. In the measurement of metabolite concentration, it is assumed that the metabolites are exclusively in the chloroplasts and that the stromal volume is 25 $\mu l \cdot mg^{-1}$ chlorophyll.

Experimental conditions	Concentration of metabolite (mM)			
	ribulose 1,5-bis-phosphate	glycerate 3-phosphate	fructose 1,6-bis-phosphate	dihydroxyacetone phosphate
Air, dark	0.4	7.6	0.8	1.4
Transition, dark to 200 $W \cdot m^{-2}$	2.2	10.8	3.8	3.4
Air, 200 $W \cdot m^{-2}$	1.0	4.8	0.6	1.6
Transition [13], air to air + 5% CO_2	0.2	11.6	1.0	2.8
Air, 150 $W \cdot m^{-2}$	7.2	14.0	–	–
Transition [12], 150 to 15 $W \cdot m^{-2}$	2.4	31.2	–	–

and physiological protein concentrations as the substrate is consumed during the timecourse of the experiment. This was overcome in Figs. 1 and 2 by maintaining the ribulose 1,5-bisphosphate concentration in the assay mixture at a constant low level using an ribulose 1,5-bisphosphate-generating system consisting of 5 mM ribose 5-phosphate, 10 mM creatine phosphate and creatine phosphate kinase (0.1 unit per ml). Relatively high

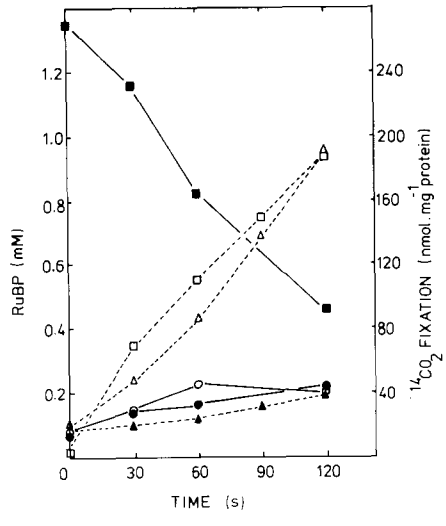

Fig. 1. The use of creatine phosphate and creatine phosphate kinase to control the ribulose 1,5-bisphosphate (RuBP) level during the assay of ribulose-1,5-bisphosphate carboxylase. CO_2 fixed (□) and ribulose 1,5-bisphosphate level (■) during assay in the presence of 1.4 mM ribulose 1,5-bisphosphate (initial concentration) were compared with CO_2 fixed (△) and ribulose 1,5-bisphosphate level (▲) using the creatine phosphate/kinase system to generate ribulose 1,5-bisphosphate from ribose 5-phosphate. The effect of 20 mM glycerate 3-phosphate on CO_2 fixed (○) and ribulose 1,5-bisphosphate level (●) using this system is also shown. The stromal protein concentration was 2.0 $mg \cdot ml^{-1}$.

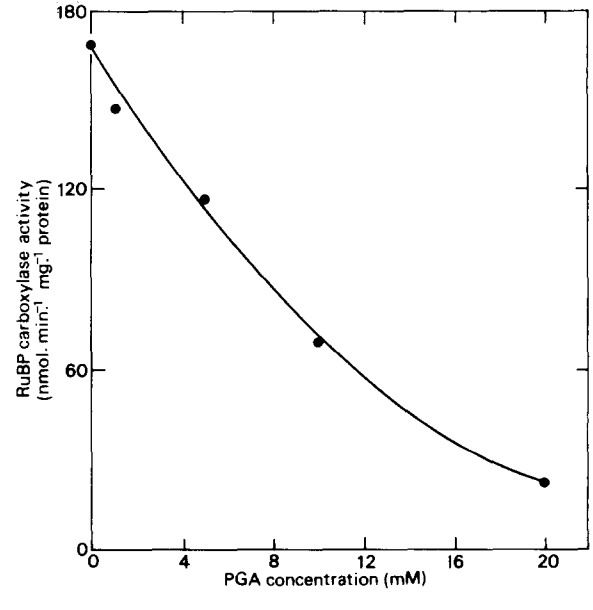

Fig. 2. The effect of glycerate 3-phosphate (PGA) concentration on ribulose-1,5-bisphosphate (RuBP) carboxylase activity at ribulose 1,5-bisphosphate concentrations of approx. 200 μM, generated from ribose 5-phosphate. The stromal protein concentration was 2.0 $mg \cdot ml^{-1}$.

enzyme protein concentrations (2 mg · ml^{-1}) were used in these experiments since the enzyme is present in the stroma at extremely high concentrations [2] and this is of considerable significance in considerations of the mechanisms involved in the regulation of activity. Fig. 1 shows the ribulose 1,5-bisphosphate level maintained in the reaction mixtures during CO_2 fixation and the timecourse of CO_2 fixation obtained using this system. Between 60 and 120 s after the addition of creatine kinase to start the assay, CO_2 uptake was linear and ribulose 1,5-bisphosphate levels were between 180 and 200 μM. When the enzyme was assayed after the addition of a much higher concentration of ribulose 1,5-bisphosphate (1.4 mM) in the absence of the ribulose 1,5-bisphosphate-generating system, the level of ribulose 1,5-bisphosphate showed a rapid progressive decline, as expected, being reduced to one-third the original value within 2 min.

Under conditions where ribulose 1,5-bisphosphate was maintained at 200 μM in the presence of 20 mM glycerate 3-phosphate, severe inhibition of CO_2 fixation was observed (Figs. 1 and 2). The effect of glycerate 3-phosphate concentration on ribulose-1,5-bisphosphate carboxylase using the creatine phosphate/creatine phosphate kinase system is shown in Fig. 2. The inhibition of carboxylation showed broadly hyperbolic kinetics with respect to glycerate 3-phosphate concentration (Fig. 2), and at higher concentrations (10–20 mM) of glycerate 3-phosphate (which may be encountered in vivo) inhibition was severe, with approx. 85% inhibition of carboxylation at 20 mM glycerate 3-phosphate. When the level of added stromal protein was varied with saturating substrates, $^{14}CO_2$ incorporation was constant (on a protein basis) with increasing protein concentration.

The effect of glycerate 3-phosphate concentration on the affinity of ribulose-1,5-bisphosphate carboxylase for ribulose 1,5-bisphosphate is shown in Table II. The presence of 20 mM glycerate 3-phosphate considerably reduced the rate of CO_2 incorporation at low ribulose 1,5-bisphosphate levels, confirming the data of Figs. 1 and 2. The K_m values shown were obtained using direct linear plots. The apparent K_m for ribulose 1,5-bisphosphate increased by approx. 10-fold in the presence of 20 mM glycerate 3-phosphate. Physiological concentrations of triose phosphate and fructose 1,6-bisphosphate (1.5 and 0.5 mM, respectively) also caused some inhibition of ribulose-1,5-bisphosphate carboxylase activity, but only marginally affected the K_m for ribulose 1,5-bisphosphate. In order to simulate the conditions which might be encountered in vivo, these experiments were repeated, varying the concentration of glycerate 3-phosphate from 2 to 20 mM in the presence of 0.5mM fructose 1,6-bisphosphate and 1.5 mM triose phosphate. Changes in glycerate 3-phosphate concentration in excess of this range occur during transitions in light intensity and gas phase composition in intact leaves (Table I). Despite the presence of triose phosphate and fructose 1,6-bisphosphate (both of which must compete with glycerate 3-phosphate for the ribulose 1,5-bisphosphate-binding site) a 5-fold increase in the apparent K_m for ribulose 1,5-bisphosphate was observed (Table II).

Fig. 3(a) shows the effect of adding a 'pulse' of glycerate 3-phosphate (10 mM) on $^{14}CO_2$ incorporation in a chloroplast extract supplied with 1.2 mM ribulose 1,5-bisphosphate. Despite the presence of nominally saturating ribulose 1,5-bis-

TABLE II

THE EFFECT OF pH AND BENSON-CALVIN CYCLE METABOLITES ON THE APPARENT K_m OR RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE FOR RIBULOSE 1,5-BISPHOSPHATE

Conditions	Apparent K_m for ribulose 1,5-bisphosphate
pH 7.0	0.25
pH 7.9	0.25
pH 7.0, 20 mM glycerate 3-phosphate	2.50
pH 7.9, 20 mM glycerate 3-phosphate	2.50
pH 7.9, 0.5 mM fructose 1,6-bisphosphate	0.28
pH 7.9, 1.5 mM dihydroxyacetone phosphate	0.22
pH 7.9, 2 mM glycerate 3-phosphate, 0.5 mM fructose 1,6-bisphosphate, and 1.5 mM triose phosphate	0.60
pH 7.9, 20 mM glycerate 3-phosphate, 0.5 mM fructose 1,6-bisphosphate, and 1.5 mM triose phosphate	3.00

phosphate, glycerate 3-phosphate addition caused a large and immediate decrease in the rate of CO_2 uptake, as would be predicted from Fig. 3. A more physiological approach was used in Fig. 3(b),

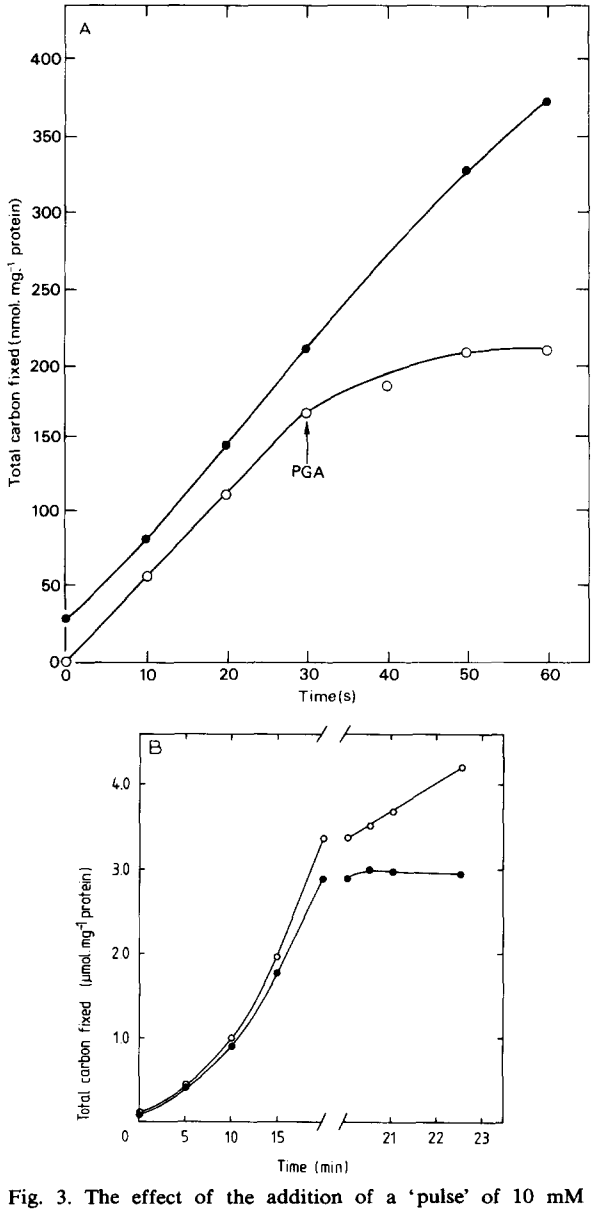

Fig. 3. The effect of the addition of a 'pulse' of 10 mM glycerate 3-phosphate (PGA) on $^{14}CO_2$ by stromal protein (2 $mg \cdot ml^{-1}$) supplied with either 1.2 mM ribulose 1,5-bisphosphate (A) or 0.5 mM triose phosphate to generate a steady-state ribulose 1,5-bisphosphate pool (B). Note the change in time scale in B, used to show the kinetics of the inhibitory effect where glycerate 3-phosphate was added at 20 min.

where a steady-state ribulose 1,5-bisphosphate concentration was maintained in a stromal extract at high protein concentration (2 $mg \cdot ml^{-1}$) using triose phosphate as substrate. ATP in these assays was maintained at high levels by the inclusion of creatine kinase and creatine phosphate. The addition of glycerate 3-phosphate reduced the rate of CO_2 uptake almost to zero, although the ATP level was relatively constant and the ATP/ADP ratio never fell below 4. This suggests that the inhibition observed did not result from reduced conversion of ribulose 5-phosphate to ribulose 1,5-bisphosphate (due to a lower ATP/ADP ratio). Consequently, it appears that the rate of carboxylation of ribulose 1,5-bisphosphate could be significantly affected, in vivo, by changes in stromal glycerate 3-phosphate concentration.

Discussion

In this study of metabolite regulation of ribulose-1,5-bisphosphate carboxylase activity we have attempted to mimic conditions which exist in the chloroplast stroma as closely as possible and considering also the inhibition constants in the literature [7–9]. It is only in the light of the actual physiological concentrations of metabolites relative to ribulose 1,5-bisphosphate that the in vivo relevance of the inhibition caused by a given metabolite can be assessed. The data presented here suggest that glycerate 3-phosphate will be an effective competitive inhibitor of ribulose-1,5-bisphosphate carboxylation in vivo, particularly when the level of ribulose 1,5-bisphosphate is low. Although this metabolite has been shown to be a weaker inhibitor of purified ribulose-1,5-bisphosphate carboxylase than fructose 1,6-bisphosphate or 6-phosphogluconate [7], it is present in vivo at such high concentrations that it is a most potent inhibitor. This inhibition be particularly important when considering the effects of transitions in light intensity on carboxylation rate. When irradiance is decreased the activation state of the enzyme declined relatively slowly compared to changes in the rate of photosynthesis [12]. In this situation the glycerate 3-phosphate level was found to increase to 15-fold that of ribulose 1,5-bisphosphate and direct inhibition of carboxylation would occur through product inhibition of ribulose-1,5-

bisphosphate carboxylase activity. The inhibition may also be exacerbated when other conditions in the stromal environment are sub-optimal for catalysis. In addition, Von Caemmerer and Farquhar [3] have suggested that the stromal Mg^{2+} concentration may limit the proportion of free ribulose 1,5-bisphosphate available for catalysis. They also discuss the possibility of only partial activation of ribulose-1,5-bisphosphate carboxylase and stress the importance of the ratio of the K_d of ribulose 1,5-bisphosphate for the active enzyme compared to that for the inactive enzyme. If this value is high then catalysis would be severely impeded. If it is proposed that the presence of modulators such as glycerate 3-phosphate may influence this ratio, in vivo, then these changes in glycerate 3-phosphate concentration may be of even greater significance in modulating catalytic activity.

An important parameter in a prediction of the physiological significance of these observations is the effective K_m for ribulose 1,5-bisphosphate in vivo. In the crude extracts used here, K_m (ribulose 1,5-bisphosphate) values were relatively high [21], but compare with those reported for purified enzyme preparations from sunflower and soybean leaves [22]. Laisk et al. [23] found that there was an apparent non-saturation of ribulose 1,5-bisphosphate carboxylation even at high ribulose 1,5-bisphosphate concentrations which occur in intact leaves and which are far above the K_m ribulose 1,5-bisphosphate [24] and the ribulose 1,5-bisphosphate-binding site concentration. They suggest that the non-saturation phenomenon may be related to inhibition of ribulose-1,5-bisphosphate carboxylase activity by glycerate 3-phosphate (and also by P_i). The results presented here show that glycerate 3-phosphate can effectively compete with ribulose 1,5-bisphosphate for binding sites on the carboxylase. The resulting large increase in K_m (ribulose 1,5-bisphosphate) caused by the binding of glycerate 3-phosphate is clearly significant and this effect is not prevented by the presence of tightly binding metabolites such as fructose 1,6-bisphosphate or dihydroxyacetone phosphate [7].

The mode of action of glycerate 3-phosphate on catalytic activity of ribulose-1,5-bisphosphate carboxylase is complex. Badger and Lorimer [7], using purified ribulose-1,5-bisphosphate carboxylase, have shown that glycerate 3-phosphate acts as a competitive inhibitor with a K_i of approx. 1 mM. However, mixed inhibition has also been observed [16,25] and multiple binding sites for glycerate 3-phosphate have been proposed [16]. From the kinetics of inhibition shown in Fig. 2, it would appear that the system is more complex than single component competitive inhibition. High glycerate 3-phosphate produced less than a 20% decrease in V_{max} (calculated by the direct linear method), suggesting competitive inhibition, although it does not preclude the possibility of allosteric modulation at a second binding site.

We may examine the implications and importance of the data presented here in relation to the theoretical maximum catalytic activity of ribulose-1,5-bisphosphate carboxylase, in the presence of varying metabolite levels, calculated from the equations of Farquhar, Von Caemmerer and coworkers [5,26,27], assuming conditions of $[CO_2]$ of 340 μbar ($K_C = 460$ μbar), $[O_2]$ of 250 μbar (K_O of 330 μbar):

$$\frac{V_C}{V_{C\,max}} = \frac{C}{C + K_C(1 + O/K_O)} \cdot \frac{R}{R + K_r} \qquad (1)$$

where V_C = carboxylation rate, $V_{C\,max} = V_{max}$ of ribulose-1,5-bisphosphate carboxylase at saturating ribulose 1,5-bisphosphate and CO_2, $C = CO_2$ concentration, $O = O_2$ concentration, $K_O = K_mO_2$, $K_C = K_mCO_2$, R = ribulose 1,5-bisphosphate concentration, K_r = effective K_m for ribulose 1,5-bisphosphate (= 250 μM or 2 mM).

If ribulose 1,5-bisphosphate is high, for example 10 mM (as would occur with high light and air levels of CO_2), a glycerate 3-phosphate concentration of 2 mM (an extremely low concentration, in vivo) would cause only a 5% inhibition of the maximum carboxylation rate in air (according to Eqn. 1). If the glycerate 3-phosphate concentration was high, for example 20 mM, in these circumstances then the rate would be inhibited by 13%. However, should the ribulose 1,5-bisphosphate level fall to 1.5 mM with an accompanying rise in glycerate 3-phosphate to 20 mM (as happens after a transition from high to low light in air [12]) then a considerably greater effect would be predicted, resulting in 43% of the maximum

carboxylation rate. The value of K_r is of key importance in this calculation. If the K_m values for ribulose 1,5-bisphosphate (20–50 μM) commonly found in the literature for the purified enzyme is used the effects of glycerate 3-phosphate would be much less significant [28]. However, the results presented here and those of Laisk et al. [23] would suggest that glycerate 3-phosphate will decrease the affinity of the enzyme for ribulose 1,5-bisphosphate and is therefore of significance in the regulation of ribulose-1,5-bisphosphate carboxylase activity.

This effect may also be important during oscillatory behaviour in photosynthetic metabolism [29] induced by a transition from air to 5% CO_2 [13]. Under these conditions, ribulose 1,5-bisphosphate fell to less than 1 mM while glycerate 3-phosphate rose to above 10 mM (Table I), a situation which could potentially reduce catalytic activity of ribulose-1,5-bisphosphate carboxylase by up to 70%. In addition, such calculations do not include the possibility of partial activation of ribulose-1,5-bisphosphate carboxylase or changes in pH or magnesium concentration, factors which may further decrease catalytic activity. It is clear from the effects of glycerate 3-phosphate on CO_2 fixation that a rigid control of ribulose-1,5-bisphosphate carboxylase (via product inhibition) will occur.

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