Biochimica et Biophysica Acta 849 (1986) 254–263
Elsevier

BBA 41986

Regulation of photosynthetic carbon assimilation in spinach leaves after a decrease in irradiance

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(Received November 12th, 1985)

Key words: Photosynthesis; Carbon assimilation; Benson-Calvin cycle; Ribulose-1,5-bisphosphate carboxylase; (Spinach leaf)

The aim of this work was to investigate the factors which regulate photosynthetic carbon assimilation in leaves following a large step decrease in irradiance. Metabolites were measured in intact leaves and in chloroplasts isolated by non-aqueous means from leaves. The following observations were made. (i) After a decrease in irradiance, the [ATP]/[ADP] and [NADPH]/[$NADP^+$] ratios decreased immediately due to a rapid decrease in the rate of electron transport. The assimilatory power, ATP · NADPH/ADP · P_i · $NADP^+$, decreased almost to dark levels during the transition from high to low irradiance. It then increased slowly to the new steady state. (ii) The decline in the ratios [ATP]/[ADP] and [NADPH]/[$NADP^+$] resulted in the accumulation of a large pool of glycerate-3-P. (iii) The amount of Rbu-1,5-P_2 declined as it was carboxylated to glycerate-3-P and its regeneration was limited by the supply of ATP and NADPH. (iv) The activation state of Rbu-1,5-P_2 carboxylase changed little during the transient, but its activity was strongly inhibited by stromal concentrations of glycerate-3-P. (v) The rate of carbon drainage into starch adjusted slowly to the decrease in irradiance as indicated by a displacement of the reaction between Fru-6-P and Glc-6-P. This delay in adjustment to a lower rate of carbon assimilation may contribute to the initial decrease in sugar phosphates observed after the irradiance was lowered. (vi) Eventually the pools of metabolites assumed their correct proportions and the concentration of Rbu-1,5-P_2 was high enough for the resumption of steady-state photosynthesis at the lower irradiance.

Introduction

Rapid fluctuations in irradiance are a common occurrence in the field, but we know little about the regulation of photosynthesis during such fluctuations. When illuminated leaves are subjected to a sudden decrease in irradiance, a pronounced lag is evident in the rate of photosynthetic carbon assimilation before it proceeds at a new lower steady-state rate. Two phenomena are associated with this lag which follows a sudden decrease in irradiance. The first is a post-lower-irradiance CO_2 burst [1,2] which results from the continued turnover of photorespiratory intermediates. The second is a true lag in the rate of photosynthetic carbon assimilation [3]. Since an efficient response to a step decrease in irradiance would be a step decrease in the steady-state rate of carbon assimilation, a lag indicates a period of 'lost' carbon assimilation and must result from a regulatory imbalance within the system.

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Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Rbu-1,5-P_2, ribulose-1,5-bisphosphate; P_i, inorganic phosphate.

A sudden reduction in irradiance would be expected to lead to an immediate decrease in the rate of electron transport and therefore a rather rapid limitation on the supply of ATP and NADPH. On the other hand, the reactions of carbon assimilation would be supported at a high irradiance both by high levels of some intermediates (both inside and outside the chloroplast) and by high activities of light-modulated enzymes which may be inappropriate to steady-state carbon assimilation at the lower irradiance. For example, in leaves from wheat seedlings, Perchorowicz et al. [4] found that the activation state of Rbu-1,5-P_2 carboxylase responded much more slowly than the rate of Rbu-1,5-P_2 regeneration to a lowering of the irradiance. They have argued that Rbu-1,5-P_2 consumption by the carboxylase is greater than Rbu-1,5-P_2 regeneration, and have shown that Rbu-1,5-P_2 levels drop below the concentration of Rbu-1,5-P_2 binding sites on Rbu-1,5-P_2 carboxylase. As a result, Rbu-1,5-P_2 regeneration temporarily limits carbon assimilation following a decrease in irradiance [5].

However, the responses of electron transport and carbon assimilation to a step decrease in irradiance are unlikely to be merely chaotic. A relatively well-controlled adjustment would be expected to occur upon decreasing the irradiance, minimising an imbalance between the reactions of electron transport and those of carbon assimilation and hence the loss of carbon assimilation. In practice, CO_2 assimilation in air is poised between limitation by CO_2, which is mediated by Rbu-1,5-P_2 carboxylase and limitation by light which powers the reactions of Rbu-1,5-P_2 regeneration [5]. On transition to a different irradiance, control of the activities of both the carboxylase and the reactions of Rbu-1,5-P_2 regeneration must undergo relatively rapid adjustment to the new light environment. In this study we have investigated how carbon assimilation is regulated following a decrease in irradiance, through measurements both of metabolites and of the activation state of Rbu-1,5-P_2 carboxylase.

Materials and Methods

Materials. Spinacia oleracea L. (Yates hybrid 102) was grown in hydroponic culture in a greenhouse with supplemental lighting with an 11 h light/13 h dark period. For each experiment, leaf discs from a single, large leaf were used to obtain reproducible metabolite measurements. Leaves were harvested at the end of a light cycle and discs were cut from leaves under water with a sharp cork-borer. The discs were then left overnight floating on water in the dark. Three discs of 2.45 cm^2 were used for each time point. Rbu-1,5-P_2 was obtained from Sigma Chemical Company (Poole, U.K.). Rbu-1,5-P_2 carboxylase was isolated as in Ref. 6. All other biochemicals were from Boehringer (Mannheim, F.R.G.)

Treatment and extraction of leaf discs. Leaf discs were placed in a cylindrical chamber (5 cm high and 10 cm in diameter) which was sealed at the top and the bottom with cling-film held in place by rubber bands. Gas was humidified and passed through the chamber. Side-illumination was provided by two quartz halide projector lamps fitted with cylindrical light guides which were mounted at 45° to the leaf surface. Changes in irradiance were effected by the introduction of neutral density filters. Similar behaviour of gas exchange and metabolite levels was observed for the different transitions in irradiance reported here. At appropriate times, leaf discs were freeze-clamped at the temperature of liquid N_2 and transferred to a mortar and pestle pre-cooled with liquid N_2. The leaf material was ground to a powder together with a pellet of 500 μl of frozen 1 M $HClO_4$ [7]. When the mixture had thawed, the pestle and mortar were washed with 3×150 μl aliquots of 0.1 M $HClO_4$, and the mixture centrifuged for 2 min at $2000 \times g$. The supernatants were then neutralized (to pH 7) either with 5 M K_2CO_3 or with 5 M KOH (if used for Rbu-1,5-P_2 determination). 100 μl charcoal suspension (100 $mg \cdot ml^{-1}$) was added (except where adenylates were to be measured) and the neutralized samples were centrifuged at $2000 \times g$ for 2 min. All metabolites except Rbu-1,5-P_2 were measured spectrophotometrically as described by Lowry and Passonneau [8]. Recoveries of metabolites added prior to killing and extraction were within 10% of the expected values.

Measurement of Rbu-1,5-P_2. Rbu-1,5-P_2 was determined by $^{14}CO_2$ incorporation into acid-stable compounds in the presence of Rbu-1,5-P_2

carboxylase. The assay contained 50 mM Tris-HCl/20 mM $MgCl_2$/2 mM EDTA/3 mM dithiothreitol. Assays were run in 300 µl total volume, including 12 mM $NaH^{14}CO_3$ (2 Ci · mol^{-1}), 0.2 units of Rbu-1,5-P_2 carboxylase and 100 µl sample. The reaction was terminated after 30 min by adding 100 µl 5 M HCl.

Non-aqueous fractionation of leaves. Leaves were fractionated by a non-aqueous technique as described by Dietz and Heber [6]. Cytosolic contamination of the chloroplastic fraction was estimated by measurement of the activities of pyruvate kinase and by comparison of the distribution of chlorophyll, Rbu-1,5-P_2 and UDP-glucose [6].

Assay of Rbu-1,5-P_2 carboxylase. Two or three leaf discs of 2.45 cm^2 were freeze-clamped. The frozen discs were ground in a mortar and pestle with a little liquid N_2 and then 500 µl 100 mM ice-cold, CO_2-free Hepes (pH 8.1)/20 mM KCl/30 mM $MgCl_2$ was added. Immediately the homogenate had thawed, 20 µl aliquots were stirred into the assay mixture, incubated for 30 s, and the reaction stopped with 100 µl 5 M HCl. The standard assay mixture for the determination of initial activity contained 100 mM Hepes (pH 8.1)/30 mM $MgCl_2$/1 mM dithiothreitol/2 mM KH_2PO_4/20 mM KCl/12 mM $NaH^{14}CO_3$ (1 Ci · mol^{-1})/0.6 mM Rbu-1,5-P_2 at 25°C [9]. Rbu-1,5-P_2 carboxylase activity was also determined after full activation by adding 20 µl crude extract to the assay medium (minus Rbu-1,5-P_2). After 10 min at 25°C, 20 µl of 15 mM Rbu-1,5-P_2 was added to start the reaction. The reaction was stopped after 30 s by addition of 100 µl 5 M HCl.

Gas exchange. CO_2 uptake by leaf discs was measured as in Ref. 3.

Chlorophyll. Chlorophyll was measured according to the method of Arnon [10].

Results and Discussion

The nature of the response of carbon assimilation to a lowered irradiance

Fig. 1 shows the typical pattern of CO_2 uptake observed when spinach leaf discs which had reached a steady-state rate of carbon assimilation at a high irradiance were subjected to a sudden decrease in irradiance. The rate of carbon assimilation displayed a pronounced lag, only part of which can be attributed to a post-lower-illumination CO_2 burst [1–3].

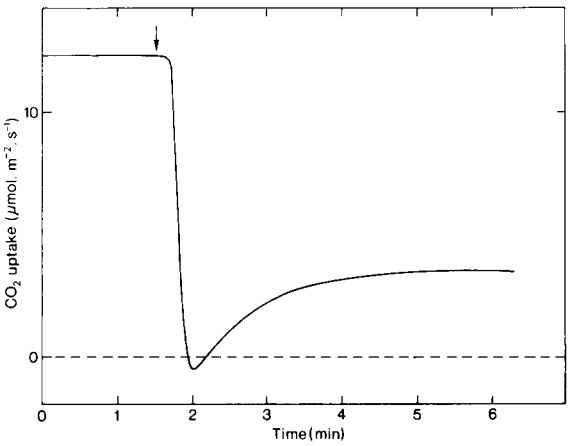
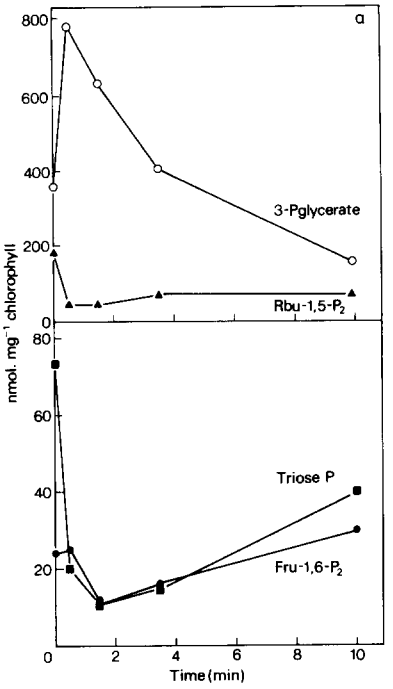


Fig. 1. The effect of a decrease in irradiance on the rate of CO_2 assimilation of a spinach leaf disc. After the rate of photosynthesis had been at steady state for 15 min at 250 W · m^{-2}, the irradiance was decreased to 25 W · m^{-2}. The arrow indicates the time at which irradiance was decreased.

Changes in metabolites following a decrease in irradiance

Metabolites in intact leaves. Metabolite pools in leaf discs were measured after a transition from 150 to 15 W · m^{-2} in air (Fig. 2). There were three main features of these measurements. First, 30 s after the irradiance was decreased, there was a 2–3-fold increase in the pool size of glycerate-3-P. This was matched by a decrease in the pool size of Rbu-1,5-P_2. A large decrease in most other metabolites occurred during this time, although the concentration of Fru-1,6-P_2 usually increased slightly during the first 30 s. Second, during the next few minutes, the size of the pool of glycerate-3-P decreased and the amount of Rbu-1,5-P_2 usually increased to a new steady-state level. The amounts of triose-P and Fru-1,6-P_2 continued to decrease until 90 s after the irradiance was lowered, and then increased over the next few minutes. Third, the [ATP]/[ADP] ratio in intact leaves decreased during the first 30 s. It then increased over the next few minutes (see Fig. 2).

Fig. 3 shows the change in metabolite levels during the first 40 s after a decrease in irradiance. The important feature of these measurements was that the amounts of Rbu-1,5-P_2 and ATP decreased



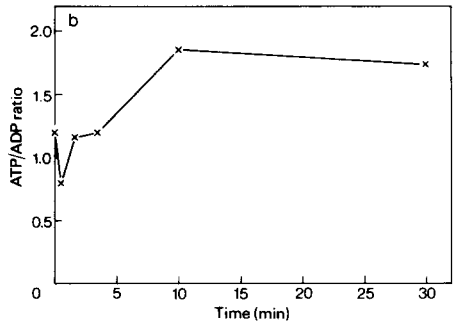


Fig. 2. (a) Changes in the levels of photosynthetic metabolites in spinach leaf discs on a change in irradiance from 150 to 15 $W \cdot m^{-2}$ in air. (b) Changes in the ratio of ATP to ADP in spinach leaf discs on a change in irradiance from 400 to 30 $W \cdot m^{-2}$ in air.

immediately, whereas the level of glycerate-3-P increased relatively slowly. This slow build-up of glycerate-3-P therefore reflects the relatively gradual impact of unfavourable ATP/ADP and NADPH/$NADP^+$ ratios on the reduction of glycerate-3-P [11], while a flux through the Calvin cycle continues, rather than the immediate appearance of glycerate-3-P as a product of the carboxylation of Rbu-1,5-P_2.

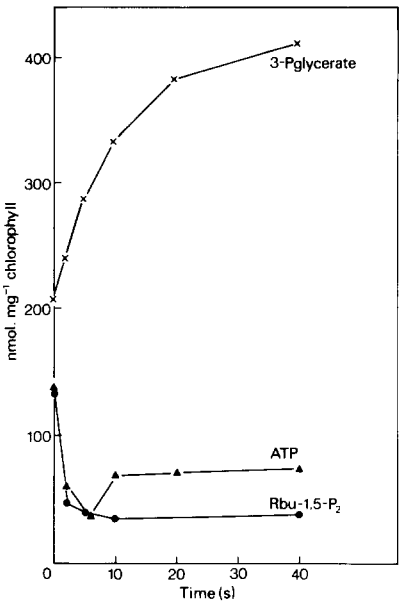


Fig. 3. Changes in the levels of photosynthetic metabolites in spinach leaf discs on a change in irradiance from 150 to 15 $W \cdot m^{-2}$ in air for the first 40 s after the irradiance was decreased.

Metabolites in chloroplasts isolated by non-aqueous fractionation of leaves. As in intact leaves the ATP content in the chloroplasts decreased initially and then rose to a new steady-state level (Fig. 4). The NADPH/$NADP^+$ ratio also decreased to values typical of darkened chloroplasts [27] and then rose again. The amount of triose-P decreased markedly in the chloroplasts, from 60 nmol per mg chlorophyll to 30 nmol per mg chlorophyll during the first 90 s, and then increased to about 45 nmol per mg chlorophyll. The changes in Fru-1,6-P_2 and glycerate-3-P were similar both in chloroplasts and in intact leaves (data not shown). This is to be expected as the major portion of these metabolites are compartmented within the chloroplast [12].

The reduction of 3-P-glycerate during the transient. The glycerate-3-P pool underwent a 3-fold increase whereas the amount of triose-P showed a large decrease (Fig. 2), reflecting the decline in [ATP]/[ADP] and NADPH/$NADP^+$ ratios. The peak of glycerate-3-P was coincident with the maximum depression of the rate of CO_2 fixation below the final steady-state value. It is therefore likely that at least part of the regulation of the

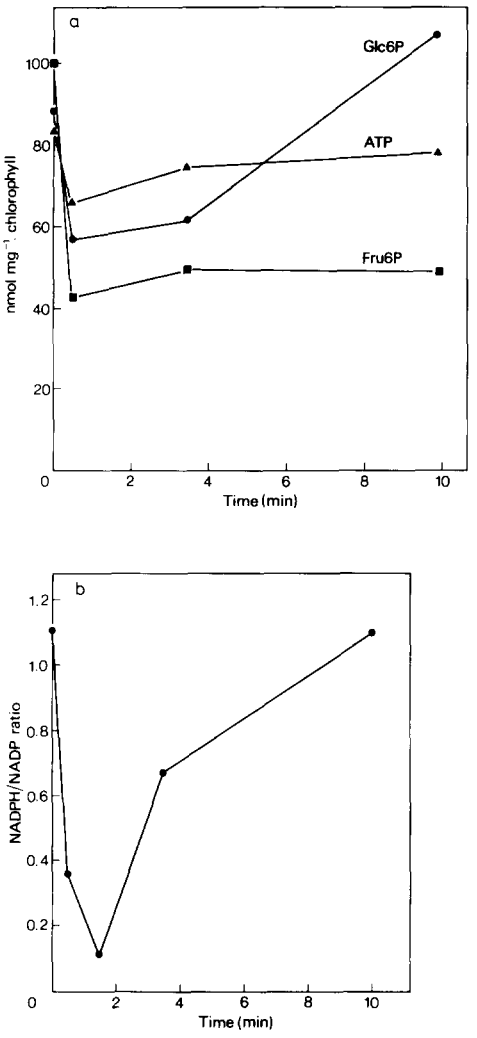


Fig. 4. (a) Changes in thel evels of metabolites in chloroplasts isolated by non-aqueous means from spinach leaf discs undergoing a transition from 250 to 25 $W \cdot m^{-2}$ in air. (b) Changes in the NADPH/$NADP^+$ ratio in chloroplasts isolated by non-aqueous means from spinach leaves undergoind a transition form 250 to 25 $W \cdot m^{-2}$ in 5000 μl per l CO_2, 20% O_2.

flux through the Calvin cycle when the irradiance is decreased lies within the reactions involving glycerate-3-P reduction. The decrease in the ATP/ADP ratio shown in Table I is probably responsible for the build-up of glycerate-3-P levels and for the decline in the rate of triose phosphate formation, because the glycerate-3-P kinase reaction is particularly sensitive to the [ATP]/[ADP] ratio [11]. Although it is known that the capacity of the enzymic system in leaves responsible for glycerate-3-P reduction can cope with various fluxes in the steady-state [6], and that the reactions remain near equilibrium under these conditions, it is possible that this is not so during transients, since the activity of glyceraldehyde-3-P dehydrogenase, for example, is known to be modulated by light [13].

The mass-action ratios were calculated for the reactions involved in the reduction of glycerate-3-P to triose-P to determine whether these reactions moved from equilibrium when the irradiance was lowered. Table I shows mass-action ratios and calculated P_i levels in chloroplasts isolated non-aqueously from leaves undergoing a transition in irradiance from 250 to 25 $W \cdot m^{-2}$. The values of the mass-action ratio shown in Table I are all close to the value of the equilibrium constant. The evidence indicates that the reactions do not deviate appreciably from equilibrium throughout the transient. Therefore the enzyme catalysing these reactions do not directly regulate the flux during the decrease in irradiance.

Changes in assimilatory power. Photosynthetic carbon assimilation is driven by ATP and NADPH. An estimate of the driving force of photosynthesis may be obtained by calculating P_A, the assimilatory power, defined as the product of the chloroplast phosphorylation potential and the NADPH/$NADP^+$ ratio, from the ratio triose-P/glycerate-3-P [27]. In Table I values of P_A have been calculated during the transition in irradiance in air. In the steady state in high light, P_A was 52 M^{-1}. It decreased to 8 M^{-1} when the irradiance was decreased, and then increased slowly to the new steady-state value of 20 M^{-1}. The supply of assimilatory power was therefore reduced below that subsequently reached in the steady state in low light. The mechanism responsible for this undershoot in assimilatory power is unclear, but it could clearly contribute to the observed lag in CO_2 assimilation.

Factors affecting regeneration of Rbu-1,5-P_2 from triose P. A fall in the amount of triose-P will immediately influence the rate of Rbu-1,5-P_2 regeneration through a simple reduction in the availability of substrate. However, the activities of enzymes controlling the rate of Rbu-1,5-P_2 regeneration may also require modification. At a high

TABLE I

CHANGES IN ASSIMILATORY POWER AND MASS-ACTION RATIOS FOR THE CHLOROPLASTIC REACTIONS INVOLVED IN THE REDUCTION OF GLYCERATE-3-P

Chloroplasts were isolated by non-aqueous means from leaves undergoing a decrease in irradiance from 250 to 25 $W \cdot m^{-2}$ in air. It is assumed for the purpose of calculation that the stromal pH remains constant, although it may decline during the transition (see Results and Discussion). The P_i concentration was calculated by assuming that at steady state in air in saturating light, the P_i concentration was 730 nmol per mg chlorophyll [6] in the chloroplast. At various time intervals, the P_i concentration was calculated by subtracting the organic phosphate from this value for total phosphate. This was possible because the amounts of the majority of the phosphorylated intermediates were measured. Lesser values for the stromal phosphate concentration would result in a proportionally greater decline in P_A than calculated here. The mass-action ratio glycerate-3-P·ATP·NADPH·H^+/DHAP·ADP·$NADP^+ \cdot P_i = 5.4 \cdot 10^{-6}$ at equilibrium. The assimilatory power, P_A = ATP·NADPH/ADP·P_i·$NADP^+$ [27].

Time at low irradiance	[glycerate-3-P] / [DHAP]	[ATP] / [ADP]	[NADPH] / [$NADP^+$]	[P_i] (nmol per mg Chl)	[H^+] (nmol per mg Chl)	Mass/action ratio	P_A (M^{-1})
0 min	2.9	2.7	0.46	730	$3 \cdot 10^4$	$1.48 \cdot 10^{-6}$	52
0.5 min	13.7	1.5	0.18	1000	$3 \cdot 10^4$	$1.1 \cdot 10^{-6}$	8
3.5 min	12.6	2.3	0.32	1300	$3 \cdot 10^4$	$2.1 \cdot 10^{-6}$	17
10 min	5.1	2.25	0.34	1160	$3 \cdot 10^4$	$1.01 \cdot 10^{-6}$	20

irradiance, fluxes through individual enzymes catalysing the regeneration of Rbu-1,5-P_2 will be higher than at a low irradiance. If any of these enzymes contribute to control of flux at the lower irradiance then their activities must be reduced accordingly.

It was observed that the level of Fru-1,6-P_2 increased for 30 s after a decrease in irradiance, and only after 30 s in low light did Fru-1,6-P_2 levels decrease. Fru-1,6-P_2 levels then increased again to a level higher than that at a high irradiance (Fig. 2). Taken at their face value, these data indicate that Fru-1,6-P_2-ase activity was immediately modulated so as to regulate the flux when the irradiance was decreased. It is, however, questionable whether the levels of Fru-1,6-P_2 reflect corresponding amounts of the thermodynamically-active metabolite, because some of the Fru-1,6-P_2 would be bound to the Rbu-1,5-P_2 carboxylase when the concentration of Rbu-1,5-P_2 fell below the concentration of substrate binding sites on Rbu-1,5-P_2 carboxylase [14,15]. Thirty seconds after a decrease in light intensity, there was a fall in the amount of Rbu-1,5-P_2 below the estimated binding site concentration on Rbu-1,5-P_2 carboxylase (approx. 4 mM, [4]). After 90 s at the low irradiance it appears that Fru-1,6-P_2ase activity may have been in excess because Fru-1,6-P_2 levels continued to fall even when the Rbu-1,5-P_2 concentration remained below that of the binding sites. Fru-1,6-P_2 content eventually rose at a time when the rate of CO_2 assimilation would have

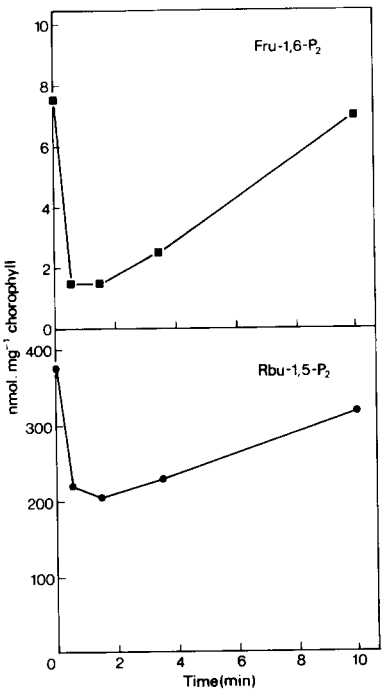


Fig. 5. The effect of 2% O_2 and 120 μl per l CO_2 on the amounts of Rbu-1,5-P_2 and Fru-1,6-P_2 in spinach leaves after a decrease in irradiance from 250 to 15 $W \cdot m^{-2}$.

been constant or rising [3] which could have resulted from the downward modulation of Fru-1,6-P_2-ase activity.

In order to avoid substantial binding of Fru-1,6-P_2 to Rbu-1,5-P_2 carboxylase during the transient, the Rbu-1,5-P_2 pool was maintained above the concentration of binding sites during the transient by employing a gas phase comprising 120 ppm CO_2 and 2% O_2. The low CO_2 and oxygen concentrations restricted consumption of Rbu-1,5-P_2 (Fig. 5). Under these conditions, Fru-1,6-P_2 levels decreased immediately when the irradiance was lowered (Fig. 5) and rose again as the pool of Rbu-1,5-P_2 increased. However, the comparison of measurements of Fru-1,6-P_2 in 20% O_2 and 2% O_2 must be approached with caution. This is because fructose-1,6-bisphosphatase does not redox-inactivate when the irradiance is decreased in 2% O_2 [16], and may therefore have remained more active in 2% O_2 than it would have in air. However, the data do indicate that the lag in carbon assimilation was not a result of slow modulation of fructose-1,6-bisphosphatase activity.

Changes in stromal metabolite concentrations will influence reactions of Rbu-1,5-P_2 regeneration apart from the reactions of glycerate-3-P reduction. For example, Gardemann et al. [17] have studied the effects of various stromal metabolites on the light-activated form of Rbu-5-P kinase from spinach chloroplasts. Perhaps the most significant of these effects is inhibition by glycerate-3-P. A three-fold increase in the concentration of glycerate-3-P from 2.5 mM to 7.5 mM in the assay medium inhibited the activity of Rbu-5-P kinase by 45% at pH 7.9 [17]. A decrease in assay pH exacerbated the inhibition by glycerate-3-P, which would be important if a decrease in stromal pH occurs upon decreasing the irradiance. Increased concentrations of ADP also inhibit this enzyme [17]. Thus the high levels of stromal ADP and glycerate-3-P (the changes shown in Fig. 4 would be equivalent to a transient increase in the latter from 8 mM to 24 mM) which occurred when the irradiance was lowered in these experiments would be likely to inhibit the activity of Rbu-5-P-kinase substantially. This would be important in balancing the activities of Rbu-5-P kinase and glycerate-3-P kinase, because, if both these enzymes were freely competing for ATP, Rbu-5-P-kinase would compete for ATP more effectively than the glycerate-3-P kinase reaction [11].

One further aspect which has to be considered when the irradiance is decreased is that the rates of starch and sucrose synthesis must be adjusted to an appropriate level in order to limit depletion of Calvin cycle intermediates. In Fig. 4 the transient changes in chloroplastic Glc-6-P and Fru-6-P levels are shown which occurred after a decrease in irradiance. At a high irradiance the ratio of Glc-6-P to Fru-6-P was very low (0.9). This strong displacement of hexose-P isomerisation from thermodynamic equilibrium (Glc-6-P/Fru-6-P = 3.4 at equilibrium at 30°C) indicates effective drainage of carbon into the pathway of starch synthesis [18]. When the irradiance was lowered, levels of hexose-P fell approximately in parallel, thus the ratio of the hexose monophosphates did not change much during the first 3.5 min after the decrease (Glc-6-P/Fru-6-P = 1.33 after 30 s, Glc-6-P/Fru-6-P = 1.25 after 3.5 min). This indicates continued consumption of hexose-P in starch synthesis. Only after 10 min did the ratio increase (Glc-6-P/Fru-6-P = 2.2), which indicates adjustment of the rate of starch synthesis to the conditions in low light. It is known that starch synthesis is controlled at the level of ADP-glucose pyrophosphorylase by orthophosphate, which is a negative modulator and by glycerate-3-P, which is a positive modulator [19]. The results in Figs. 2 and 3 show that the amount of glycerate-3-P increased dramatically before falling to the new steady-state level. This may explain why it takes several minutes for the rate of starch synthesis to adjust to the rate of the Calvin cycle turnover, because 3-phosphoglycerate, as a positive modulator, would keep ADP-glucose pyrophosphorylase in an activated state.

There is also good evidence for overshoots in the rate of sucrose synthesis when the concentration of CO_2 or the irradiance is lowered [3,20]. A slow adjustment in the rates of starch and sucrose synthesis may therefore contribute to the decrease in the levels of several sugar phosphates observed after the change in light intensity (Figs. 2–5).

Changes in the activity of Rbu-1,5-P_2 carboxylase following a decrease in irradiance

Factors affecting the consumption of Rbu-1,5-P_2.

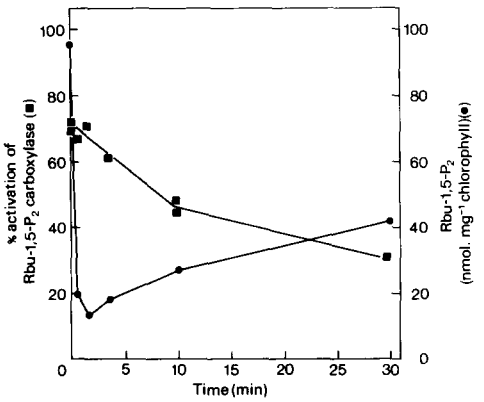


Fig. 6. The effect of a decrease in irradiance from 150 to 15 $W \cdot m^{-2}$ on the activation state of Rbu-1,5-P_2 carboxylase (■) isolated from spinach leaves and the effect of a decrease in irradiance from 300 to 30 $W \cdot m^{-2}$ on levels of Rbu-1,5-P_2 (●) in spinach leaf discs.

A study of the activation state of Rbu-1,5-P_2 carboxylase was made during the transition from high light to low light in spinach leaf discs. Fig. 6 shows that the activation state of Rbu-1,5-P_2 carboxylase changed very slowly for 30 min after the irradiance was lowered from 150 to 15 $W \cdot m^{-2}$. The activation state (expressed as a percentage of full activation) decreased from about 70% to about 30% during this time. However, during the first 90 s of the induction period (the period during which the rate of CO_2 uptake changed most dramatically), the degree of activation remained constant, while by the end of the induction period, after 3.5 min at the low irradiance, the degree of activation had fallen by only 10%. These results therefore agree with the findings of Perchorowicz et al. for wheat seedlings [4]. Although caution should be exercised when considering these data in absolute terms (i.e., percentage activation) because of the possibility of artefactual changes in the activation state of the carboxylase during extraction and assay, these data nevertheless represent valid comparisons of changes in the activation state of the enzyme throughout the transient.

If the activity of Rbu-1,5-P_2 carboxylase immediately after the irradiance is decreased was determined merely by its activation state, it might be predicted that the rate of consumption of Rbu-1,5-P_2 by a highly active carboxylase would so much outweigh the rate of regeneration of Rbu-1,5-P_2 that Rbu-1,5-P_2 would virtually disappear, as it does when leaves are darkened (e.g., Ref. 4). One important factor is that Rbu-1,5-P_2 carboxylase activity will itself be reduced by the lowered concentration of Rbu-1,5-P_2 [21], but there is also the possibility that modulation of Rbu-1,5-P_2 carboxylase activity by changing stromal metabolites will minimise the decrease in the pool of Rbu-1,5-P_2.

Upon lowering the irradiance, there were large changes in both the amounts of glycerate-3-P (Fig. 4) and of P_i (Table I). Both these metabolites are competitive inhibitors of Rbu-1,5-P_2 carboxylase. Glycerate-3-P has a K_i value of 840 μM and P_i has a K_i value of 900 μM, compared to a K_d for Rbu-1,5-P_2 by Rbu-1,5-P_2 carboxylase of 0.6 μM [14]. As shown in Fig. 4, the level of glycerate-3-P increased 3-fold (equivalent to a concentration increase from 8 to 24 mM) when the irradiance was decreased, coinciding with a large decrease in the pool size of Rbu-1,5-P_2 from 4 to 1 mM. The effect of changes in glycerate-3-P concentration on the activity of Rbu-1,5-P_2 carboxylase during this transient was measured (it should be stressed that there was no difference in activation state from the results shown in Fig. 6). At each point during the time-course, leaf discs were extracted and the ac-

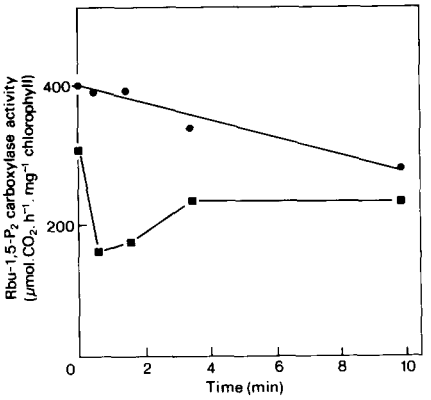


Fig. 7. The effect of changes in glycerate-3-P concentration in the assay medium on the activity of Rbu-1,5-P_2 carboxylase extracted from spinach leaves after a decrease in irradiance from 300 to 15 $W \cdot m^{-2}$. At each time point, leaves were rapidly extracted and the activity of Rbu-1,5-P_2 carboxylase was measured in the presence of glylcerate-3-P. The concentration of glycerate-3-P in the assay was 8 mM at 0 and 10 min, 24 mM at 30 s, 21 mM at 90 s, 14 mM at 3.5 min. Activity in the absence of glycerate-3-P (●), activity in the presence of glycerate-3-P (■).

tivity of the carboxylase was measured in an assay medium which contained a concentration of glycerate-3-P equivalent to that obtaining in the stroma (assuming a stromal volume of 25 $\mu l \cdot mg^{-1}$ chlorophyll). In the experiment shown in Fig. 7, Rbu-1,5-P_2 concentration was maintained at 1 mM throughout. The increase in glycerate-3-P concentration from 8 to 24 mM led to a 42% inhibition of the activity of the enzyme compared to the control. For the rest of the time-course the concentration of glycerate-3-P in the assay was decreased, so that after 10 min the concentration was again 8 mM. When Rbu-1,5-P_2 concentration was increased to 4 mM (the stromal concentration in high light) for the first point in the time-course, the activity of Rbu-1,5-P_2 carboxylase increased from 308 to 500 μmol CO_2/h per mg chlorophyll. In this case the activity of the carboxylase 30 s after decreasing the irradiance would have been reduced by 64% in the presence of 24 mM glycerate-3-P when compared with the control. The extent to which the stromal pH decreases in leaves with a decrease in irradiance is not known. Light scattering is the only available probe of ΔpH in leaves [22] and in high-light-adapted spinach leaves it was found to decrease gradually after decreasing the irradiance [23] (results not shown), which implies that the stromal pH will also change. We investigated the effect of changing the pH in the assay of the carboxylase during the transient. When the concentration of CO_2 in the assay was held constant at 220 μM, changes in the assay pH from pH 8.1 to 7.6 had little effect on the activity of the carboxylase (see, e.g., Ref. 24). However, a decrease in pH exacerbated inhibition by glycerate-3-P. The activity of the carroxylase at constant CO_2 in the presence of glycerate-3-P was decreased by a further 25% at pH 7.9 and by a further 50% at pH 7.6 (both compared to pH 8.1), suggesting that glycerate-3-P^{2-} may possibly inhibit the activity of the carboxylase to a greater extent than glycerate-3-P^{3-}.

It is therefore apparent that factors beside the activation state of the carboxylase will influence its activity profoundly following a decrease in irradiance. Clearly the concentration of glycerate-3-P will not be the only other factor involved, but the accumulation of glycerate-3-P (and of P_i (Table I), which has a similar K_i value and the decrease in Rbu-1,5-P_2 will result in rapid inhibition of the activity of the carboxylase and hence in a tendency to minimise the decrease in the pool of Rbu-1,5-P_2. This may mean that the system achieves something closer to a step change in the concentration of Rbu-1,5-P_2 than would otherwise be achieved and hence minimises any induction loss.

The relationship between the content of Rbu-1,5-P_2, the activity of Rbu-1,5-P_2 carboxylase and the photosynthetic rate. Perchorowicz et al. [4] and Mott et al. [25] have measured Rbu-1,5-P_2 concentration, the activation state of the carboxylase and the rate of CO_2 uptake in leaves of wheat and *Xanthium strumarium* following a reduction in irradiance. Rbu-1,5-P_2 levels dropped below the binding site concentration of the carboxylase and CO_2 fixation decreased rapidly, both within 90 s. Following this, both the rate of CO_2 fixation and Rbu-1,5-P_2 concentration increased, but after about 5 min the rate of CO_2 uptake remained constant while the concentration of Rbu-1,5-P_2 continued to rise [4]. Mott et al. [25] have proposed that a slow decrease in the activation state of the carboxylase occurs following a decrease in irradiance, resulting in a slow rise in the concentration of Rbu-1,5-P_2, while the capacity to regenerate Rbu-1,5-P_2 remains constant. The result is that in the steady-state the content of Rbu-1,5-P_2 is constant at different light intensities. It is clear that for the results of this study this proposal should be viewed with caution. Modulation of the activity of the carboxylase by effectors seems inescapable when the concentration of Rbu-1,5-P_2 falls and the concentration of effectors rises dramatically. The activity of the carboxylase is not determined by its activation state alone and it cannot remain constant, even for a short period, during the transient. Indeed, if Rbu-1,5-P_2, glycerate-3-P, P_i and pH changes were the sole effectors involved, then the activity of the carboxylase (as opposed to its activation state) would tend to fall and rise in synchrony with the fall and rise in the concentration of Rbu-1,5-P_2 (see, for example, Fig. 7) for the first few minutes of the transient. This is difficult to reconcile with the proposal of Mott et al. [25], unless other effectors are involved. Indeed, the results pre-

sented here are more amenable to the notion that control is not wholly invested in the reactions of Rbu-1,5-P_2 regeneration upon lowering the irradiance and that the rate of carbon assimilation may initially be controlled both by the activity of Rbu-1,5-P_2 carboxylase and by the rate of Rbu-1,5-P_2 regeneration. In the long term (over 30–60 min) the decline in activation state of the carboxylase may allow the pool size of Rbu-1,5-P_2 to increase gradually as Mott et al. [25] suggest. It should be noted that the pool of Rbu-1,5-P_2 is not necessarily constant at different light intensities, especially at those light intensities lower than employed by Mott et al. (i.e., lower than 600 $\mu E \cdot m^{-2} \cdot s^{-1}$ [25]). In this study the pool of Rbu-1,5-P_2 only rarely returned to its value in high light when the irradiance was lowered. In the example in Fig. 6, Rbu-1,5-P_2 remained at a concentration (40 nmol per mg chlorophyll, equivalent to approx. 1 mM in the stroma), well below the binding site concentration, for as long as 30 min at the lower irradiance. Steady-state contents of Rbu-1,5-P_2 in air have also been found to decrease in low light compared with high light by others [4,6,26].

Mott et al. [25] also propose a linear relationship between Rbu-1,5-P_2 concentration and the transient rate of carbon assimilation when the irradiance is decreased. This too must be viewed with caution because it is assumed both (i) that the rate of carbon assimilation drops immediately to a new low value which is constant during the first 3 min after the irradiance is lowered and, again, (ii) that the activity of the carboxylase does not change. From results obtained by Perchorowicz et al. [4] and from these and other results [3] it is clear that the photosynthetic rate changes dramatically during this time, and although there does appear to be a close relationship between the concentration of Rbu-1,5-P_2 and the rate of carbon assimilation (compare Fig. 1 with Fig. 6), this cannot be well-defined because of the difficulties involved in mesuring the latter, which is complicated by the photorespiratory CO_2 burst.

Acknowledgements

This work was supported by the Agricultural and Food Research Council, U.K., by the Science and Engineering Research Council, U.K. and by the Deutsche Forschungsgemeinschaft. We thank David Walker and Ulrich Heber for their advice, criticism and encouragement, Claudia Zink and Georg Kaiser for their help and Spidola Neimanis for measuring $NADP^+$ and NADPH.

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