Plant Physiol. (1986) 81, 1123–1129
0032-0889/86/81/1123/07/$01.00/0

Limitation of Photosynthesis by Carbon Metabolism[1]

II. O_2-INSENSITIVE CO_2 UPTAKE RESULTS FROM LIMITATION OF TRIOSE PHOSPHATE UTILIZATION

Received for publication January 14, 1986 and in revised form April 3, 1986

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ABSTRACT

The occurrence of O_2-insensitive photosynthesis at high quantum flux and moderate temperature in *Spinacia oleracea* was characterized by analytical gas exchange measurements on intact leaves. In addition photosynthetic metabolite pools were measured in leaves which had been rapidly frozen under defined gas conditions. Upon switching to low O_2 in O_2-insensitive conditions the ATP/ADP ratio fell dramatically within one minute. The P-glycerate pool increased over the same time. Ribulose bisphosphate initially declined, then increased and exceeded the pool size measured in air. The pools of hexose monophosphates and UDPglucose were higher at a partial pressure of O_2 of 21 millibars than at 210 millibars. These results are consistent with the hypothesis that the rate of sucrose synthesis limited the overall rate of assimilation under O_2-insensitive conditions.

In this article we ask whether manipulating the ambient O_2 concentration provides a way of recognizing a situation in which sucrose or starch synthesis limit the rate of photosynthesis. During photosynthesis, CO_2 and Pi are converted to triose-P in the chloroplast. The triose-P is converted to sucrose in the cytosol, or starch in the chloroplast, liberating Pi for use in subsequent CO_2 fixation. The production of triose-P in the chloroplast requires electron transport, regeneration of RuBP[3] and activity of Rubisco. As all these processes are also needed for photorespiration, there is normally a stimulation of photosynthesis when the ambient O_2 concentration is lowered to suppress photorespiration. However, photorespiration does not compete for the reactions by which triose-P is converted into end products such as sucrose and starch. This means that the rate of photosynthesis would not be reduced by photorespiration when photosynthesis is limited by the utilization of photosynthesis products provided there is adequate electron transport and carbon reduction cycle activity to cope with the additional fluxes required when photorespiration occurs. The rate of photosynthesis would then be independent of the ambient O_2 level.

It has been observed that decreasing the O_2 concentration from 210 to 21 mbar does not always lead to an increase of photosynthesis, especially when the light intensity is high and the temperature is below 20°C (3, 12, 14, 24). It has been proposed that this O_2 insensitivity of photosynthesis is due to a limitation of photosynthate utilization, in particular triose-P utilization for sucrose and starch synthesis (17, 18). When O_2 sensitivity is lost photosynthesis also fails to respond to CO_2 (18) indicating that the phenomenon is not simply an effect of O_2 on pseudo-cyclic photophosphorylation.

A limitation of the utilization of phosphorylated intermediates would lead to a fall of the stromal Pi. Many studies have shown that the rate of CO_2 fixation in isolated chloroplasts depends on the stromal Pi level. Upon lowerng the stromal Pi concentration from 7 to 2 mM, CO_2 fixation was decreased by 40% (11) and this inhibition of CO_2 fixation was accompanied by lower ATP/ADP ratios, an accumulation of P-glycerate, an increase of RuBP and a decrease in the activation state of Rubisco (11). There are indications that O_2 insensitivity of photosynthesis in leaves is associated with a limitation of phosphate. Thus, depletion of the cytosolic Pi by feeding mannose to leaf discs leads to O_2 insensitivity (10). In leaves which were photosynthesizing rapidly and were presumed to be limited by use of photosynthate, high pools of P-glycerate and RuBP have been measured (1) as expected by analogy with isolated chloroplasts in low Pi. However, there is still no direct evidence that triose-P utilization limits CO_2 assimilation during O_2-insensitive photosynthesis in whole leaves, and that the phosphate level could be involved in this limitation.

To elucidate the matter we have defined what conditions are needed to reproducibly obtain O_2-insensitive photosynthesis in spinach leaves and then used a high-speed freeze clamp gas exchange apparatus (19) to quench leaf material for metabolite analysis. Photosynthesis in low O_2 caused an accumulation of hexosephosphates, RuBP and P-glycerate, and a decline in ATP/ADP ratio, supporting the notion that O_2 insensitivity of photosynthesis is associated with a limitation in triose phosphate utilization.

MATERIALS AND METHODS

Spinach (*Spinacia oleracea* var. Mazurka) was grown in a growth chamber in hydroponic culture with a 9 h light/15 h dark cycle, a quantum flux of 340 μmol m^{-2} s^{-1}, partial pressure of CO_2 in the light phase at 380 μbar, and temperatures of 22°C in the light and 16°C in the dark. Spinach was grown in similar conditions in a glasshouse, the temperature was 24 to 28°C in the light.

Gas Exchange Measurements. CO_2 assimilation was assessed by measuring the depletion of CO_2 in an air stream that had

[1] Supported by the Deutsche Forschungsgemeinschaft, National Science Foundation grants INT-8411180 and PCM 8304775, and Department of Energy grant DE-FG08-84ER13234.

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[3] Abbreviations: RuBP, ribulose 1,5-bisphosphate; Rubisco, ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39).

passed over a leaf. The CO_2 content of the gas stream was adjusted by bleeding 5% CO_2 in air into either CO_2 free air (210 mbar O_2 treatment) or 21 mbar O_2 in N_2 which was mixed in a Wösthoff mixing pump. This mixed air passed over 9 cm^2 of leaf material in an aluminum chamber. The aluminum chamber was temperature regulated by water. The windows of the chamber were made of Frapan S (similar to Saran). Light was provided by a quartz halogen lamp through light guides. Leaf temperature was measured with a fine wire thermocouple threaded through the leaf. The air streams that had passed over the upper and lower surface were combined and circulated by means of an air pump back over the leaf to reduce the boundary layer over the leaf. Humidification and CO_2 depletion of the air flowing out of the loop was measured with an IR gas analyzer (Binos, Leybold Heraeus, Hanau, W. Germany).

When desired conditions had been established and measurements had been made, the metabolism of the leaf was stopped by bringing two liquid N_2 cooled copper heads together at the plane of the leaf inside the chamber. The Frapan windows kept the leaf material from sticking to the copper heads. Between the time that the light beam was interrupted and the time the leaf was below 0°C, 0.25 s had elapsed. Samples were collected and stored in liquid N_2 until extracted for metabolite assay.

Photosynthetic CO_2 assimilation was calculated from the depletion of CO_2 in the gas stream. Intercellular CO_2 concentration was calculated as recommended by von Caemmerer and Farquhar (25). The rate of use of RuBP was calculated as described in (5) and briefly described here. The CO_2 assimilation rate, A, depends on the velocity of carboxylation, V_c, the ratio of oxygenation to carboxylation ϕ, and the rate of mitochondrial respiration, R_d which is occurring (taken to be 1 μmol m^{-2} s^{-1}).

$$A = (1 - 0.5\phi)V_c - R_d. \quad (1)$$

The rate of carboxylation will depend on the rate of RuBP regeneration, R.

$$V_c = \frac{R}{(1 + \phi)}. \quad (2)$$

By substitution and rearrangement R can be calculated as

$$R = \frac{(1 + \phi)(A + R_d)}{(1 - 0.5\phi)}. \quad (3)$$

ϕ was calculated from equations given by Farquhar and von Caemmerer (5) and was 0.128 in 210 mbar and 0.012 in 21 mbar O_2. The ATP use rate and P-glycerate reduction rate was calculated as described in (5).

Metabolite Assays. Metabolites were measured as described in (22).

Compartmentation Analysis. The subcellular distribution of P-glycerate was determined by nonaqueous density gradient centrifugation (8). Chl, NADP dependent glyceraldehyde-P dehydrogenase, and RuBP were used as chloroplast markers, P-enolpyruvate carboxylase was used as the cytosol marker and α-mannosidase was used as the vacuole marker. The distribution of the three chloroplast markers was the same indicating that the thylakoids, the stromal protein, and the stromal metabolites moved through the gradient together.

RESULTS

Characterization of O_2 Insensitivity of Spinach by Gas Exchange Measurements. When CO_2 assimilation was limited by light, switching from air to N_2 to suppress photorespiration led to a rapid, smooth increase in the CO_2 assimilation rate until a new, steady state rate of assimilation was reached (Fig. 1). However, at higher quantum fluxes there was a large initial inhibition of assimilation after switching from air to N_2, with CO_2 maintained at 550 μbar (Fig. 1). In some experiments this initial inhibition was more than 50% of the rate of CO_2 assimilation. Following the initial inhibition, CO_2 assimilation recovered again over 5 to 20 min, but the rate of assimilation in N_2 was not much higher than that in air even after 20 min (Fig. 1). In these and all subsequent experiments, the partial pressure of CO_2 in the air was maintained at 550 μbar because this level was high enough to reproducibly cause O_2 insensitivity at high quantum flux, but still provided a clear indication of O_2 sensitivity at lower quantum flux. A postillumination burst of CO_2 release was found at 550 μbar CO_2, 880 μmol photons m^{-2} s^{-1}, and 210 mbar O_2.

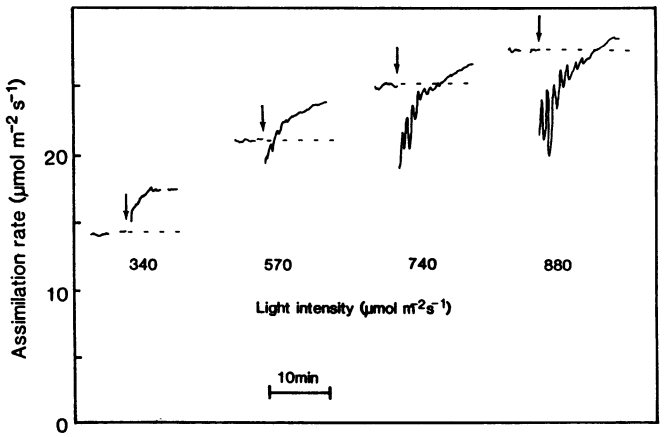

FIG. 1. Response of assimilation rate to N_2 at four light intensities. The gas phase was changed from air to N_2 at the arrow. CO_2 was maintained at 550 μbar throughout. Leaf temperature was 15°C.

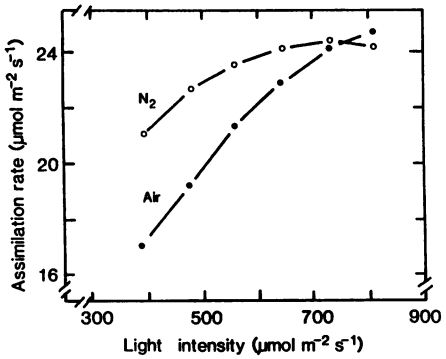

FIG. 2. Light response of assimilation rate in air or N_2. CO_2 was 500 μbar, leaf temperature was 15°C.

The dependence of O_2 insensitivity on light intensity was examined in more detail by comparing the light response of CO_2 assimilation in N_2 and in 210 mbar O_2 (Fig. 2). Whereas in N_2 photosynthesis was saturated at about 650 μmol photons m^{-2} s^{-1}, it was not saturated in 210 mbar O_2 even at 800 μmol m^{-2} s^{-1}. At high enough quantum flux (above 700 μmol m^{-2} s^{-1}), CO_2 assimilation was independent of the O_2 content of the atmosphere. In other experiments (not shown) we found that O_2 insensitivity depended upon the CO_2 partial pressure and temperature as well as light intensity. Under otherwise constant conditions, decreasing the CO_2 partial pressure or increasing the temperature restored the sensitivity of photosynthesis to O_2, as previously reported (3, 14 17, 18). O_2 insensitivity was observed whether N_2 or 21 mbar O_2 were used, indicating that O_2 insensitivity is not caused by changes in mitochondrial activity. To avoid potential mitochondrial interference caused by anaerobic conditions, all subsequent work was carried out with 21 mbar O_2 instead of N_2.

The development of O_2 insensitivity correlated with the appearance of oscillations in the rate of photosynthesis immediately

after switching to 21 mbar O_2. These oscillations appeared as light intensity was increased (Fig. 1). Similar results were obtained when the temperatue was lowered to induce O_2 insensitivity (not shown). When a leaf was treated so that it regained sensitivity to O_2 over a period of time, the oscillations decreased (Fig. 3).

The conditions required to demonstrate O_2 insensitivity (and the associated oscillations) in a given leaf depended on the conditions in which the plants were grown. O_2 insensitivity occurred when leaves grown at approximately 400 μbar CO_2 and 22°C were assayed at 550 μbar CO_2 and 15°C (Figs. 1–4). O_2 insensitivity was more pronounced when the assay temperature was below the growth temperature. For example, spinach grown in a growth room kept at 22°C showed large oscillations and O_2 insensitivity at 15°C, but much smaller oscillations and sensitivity to O_2 at 18°C, while plants grown in a greenhouse where the growth temperature was often above 25°C still showed O_2 insensitivity and very large oscillations at 18°C (data not shown).

If O_2 insensitivity of net CO_2 assimilation is associated with a limitation in carbon metabolism, plants kept in darkness for 24 h (to deplete carbohydrate pools) might respond differently from plants measured at the end of the normal 9 h photoperiod. Upon switching to low O_2, a carbohydrate-starved leaf showed a large initial inhibition but relatively small oscillations (Fig. 3). During this experiment the gas was switched between air and low O_2 every 20 min for 4 h. After 22 min in the light the oscillations were very small and substantial sensitivity to O_2 had been regained. The same leaf was assayed the next evening after a normal 9 h photoperiod. At the start of the experiment the initial inhibition was again very large but the oscillations were much more pronounced. After switching between 210 and 21 mbar O_2 for 200 min the oscillations were reduced but they were still greater than those which occurred in the leaf measured after 24 h darkness.

The recovery of sensitivity to O_2 during the experiment of Figure 3 is replotted in Figure 4, showing how the rates achieved

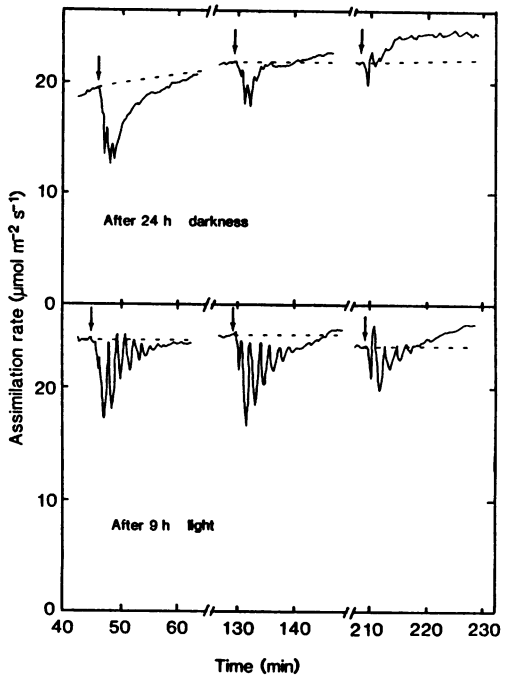

FIG. 3. Response of assimilation rate to 21 mbar O_2 in a leaf kept in darkness for 24 h and for the same leaf at the end of the subsequent 9 h photoperiod. CO_2 was 550 μbar and leaf temp was 15°C. The gas phase was switched from air to 2% O_2 at the arrows. Light intensity was 1500 μmol photons m^{-2} s^{-1}.

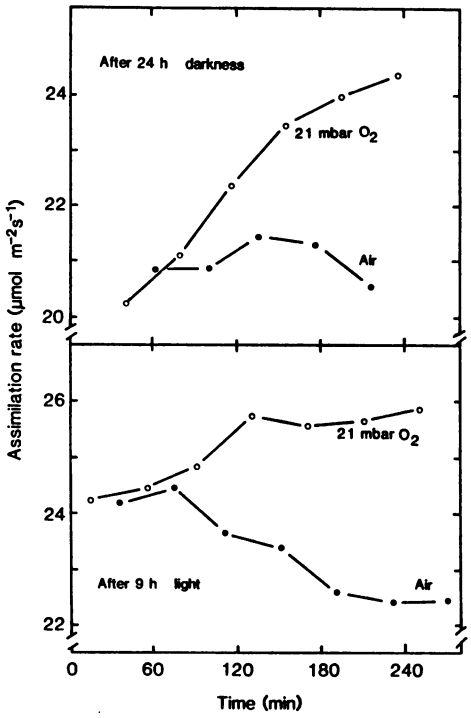

FIG. 4. Quasi-steady state rates of photosynthesis in air or 20 mbar O_2. The gas phase was switched from air to low O_2 or back every 20 min and the rate of CO_2 assimilation at the end of each 20 min period recorded. Leaf temp was 15°C and CO_2 was 550 μbar. Light intensity was 1500 μmol photons m^{-2} s^{-1}.

after 20 min in 21 or 210 mbar O_2 changed in the course of the experiments. Although O_2 sensitivity was restored in both experiments, this occurred for different reasons. With a predarkened plant the recovery of sensitivity to O_2 was due to an increasing rate of CO_2 assimilation in low O_2. In contrast, in the preilluminated plant, sensitivity was recovered primarily by a reduction of the rate in air which began after 1 h. The rate in low O_2 did not increase markedly. In both cases, the recovery of sensitivity to O_2 was accompanied by a decrease in the extent of the oscillations.

Measurements of Metabolite Levels. As a result of this characterization of O_2 insensitivity in spinach leaves, the following conditions were chosen for metabolite analysis: Leaves from greenhouse grown spinach were used after 4 to 10 h illumination, and were put into a high-speed freeze clamp gas exchange chamber while still attached to the rest of the plant. Illumination (1500 μmol m^{-2} s^{-1}) was provided by a quartz halogen lamp directed through light guides, leaf temperature was 18°C and CO_2 partial pressure was 550 μbar. After 20 to 30 min when the CO_2 assimilation rate was nearly constant, the O_2 content was lowered to 21 mbar. A typical CO_2 analyzer trace is shown in Figure 5. Lowering O_2 led to a 50% decrease in the rate of CO_2 assimilation. This was accompanied by large oscillations in the rate of CO_2 assimilation. During the next 15 min the rate of CO_2 assimilation rose again, but only to a rate similar to that in 210 mbar O_2. Switching back to 210 mbar O_2 showed that the rate of CO_2 assimilation had even drifted up during this time, indicating that the samples were taken before the decline noted in Figure 4 began.

Samples were taken for anaysis after the initial period in 210 mbar O_2, and after 1 min and 18 min in 21 mbar O_2. After switching to low O_2, the sharp decrease in CO_2 assimilation rate was accompanied by a decrease of the ATP/ADP ratio and a marked increase of P-glycerate, whereas dihydroxyacetone-P,

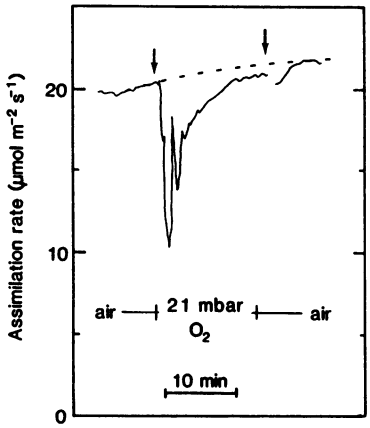

FIG. 5. Trace of CO_2 assimilation rate under conditions used for metabolite analysis. Condition one was after 25 min in air. Two was 60 s after switching to low O_2, and three was 18 min after switching to low O_2. CO_2 was 550 μbar, leaf temperature was 18°C and light level was 1500 μmol m^{-2} s^{-1}.

RuBP and Fru-6-P fell (Table I). Despite the decrease of dihydroxyacetone-P, Fru-1,6-P did not markedly change. Most of the cellular Fru-1,6-P is located in the stroma (Gerhardt, in prep.), of which a large portion may be bound to Rubisco, and not in aldolase equilibrium with dihydroxyacetone-P (4). Subcellular metabolite analysis obtained from another experiment indicated that the P-glycerate content in the stroma rose from 305 to 553 nmol (mg Chl)$^{-1}$ and that in the cytosol from 50 to 145 nmol mg^{-1} Chl after 1 min in 21 mbar O_2 (Table II). We were not able to assay subcellular adenylate levels, but one might expect that the decrease of the stromal ATP/ADP ratio after 60 s was at least as pronounced as the decrease of the overall ATP/ADP ratio.

In one experiment (Fig. 6) the kinetics of whole leaf metabolite changes during the initial 120 s after lowering of the O_2 were investigated in more detail. During this period there were very large fluctuations in the metabolite content. Part of the fluctuations may be due to variability among leaves since samples from separate leaves had to be taken for each time point. However, the changes involved were much larger than the standard error of the mean of four control samples from 210 mbar O_2. It remains to be elucidated to what extent rapid oscillatory events may be responsible for the observed fluctuations.

After 18 min in low O_2, the rate of CO_2 assimilation had recovered to about the initial value at 210 mbar O_2. However, the ATP/ADP ratio remained low (1.6) and P-glycerate high (Table I). The dihydroxyacetone-P content recovered and the contents of RuBP, Fru-6-P, Glc-6-P, and UDPGlc were now even slightly higher than those values found at 210 mbar O_2. Consequently the sum of the esterified phosphates measured after 18 min in 20 mbar O_2 was considerably higher than found at 210 mbar O_2.

Estimation of Fluxes. The relation between the net rate of CO_2 assimilation measured in gas exchange and the turnover of metabolites such as ATP and RuBP depends on the O_2 concentration, because these metabolites are also utilized in the oxidative photorespiratory cycle. In Table III we have used theoretical relationships (see "Materials and Methods") to estimate the required turnover of ATP and RuBP in 210 mbar O_2, and after 1 and 18 min in 21 mbar O_2. The use of ATP decreased 56% immediately after switching to 21 mbar O_2, and after 18 min in 21 mbar O_2 the turnover of ATP was still 17% below that in 210 mbar O_2. Similarly, switching to 21 mbar O_2 decreased the use of RuBP by 54% and 12% after 1 and 18 min, respectively. Because the pool of RuBP fell during the first min, the regeneration of RuBP was restricted by more than 54% during this time. Similar arguments apply to the generation of ATP.

DISCUSSION

O_2 Insensitivity. These results confirm previous reports that O_2-insensitive photosynthesis can be reproducibly observed when C_3 leaves photosynthesize in saturating light, elevated CO_2 and at moderate temperatures. For our material, these conditions were met at 15 to 18°C, about 800 μmol m^{-2} s^{-1} and 550 μbar CO_2. This insensitivity was not the result of the absence of photorespiration in 210 mbar O_2 in these conditions. Decreasing the light intensity, which should not alter the relative rates of carboxylation and oxygenation of RuBP, led to the reappearance of the expected sensitivity to O_2. Also, a post-illumination CO_2 release was found in 210 mbar O_2 in O_2-insensitive conditions. It appears that when these leaves were in saturating light and 550 μbar CO_2 they were unable to exploit the capacity for more photosynthesis made available when photorespiration is suppressed by low O_2.

The response to low partial pressure of O_2 is complex, as a new steady rate is not immediately achieved after transfer to low O_2. Instead, there is a large initial inhibition of CO_2 assimilation which is accompanied by the appearance of marked oscillations.

Table I. *Metabolite Analysis of Spinach Leaves Quick Frozen under Defined Conditions*

The partial pressure of CO_2 was 550 μbar, leaf temperature was 18°C, and the light intensity was 1500 μE m^{-2} s^{-1}. The results are the mean ± SE of 4 samples from separate leaves.

Metabolite	Condition		
	20 min 210 mbar O_2	1 min 21 mbar O_2	18 min 21 mbar O_2
	nmol mg^{-1} Chl		
ATP	60 ± 3	43 ± 6	44 ± 4
ADP	26 ± 3	37 ± 3	27 ± 4
AMP	7 ± 1	14 ± 1	9 ± 1
ATP + ADP + AMP	93 ± 7	94 ± 10	81 ± 9
ATP/ADP ratio	2.3	1.2	1.6
P-glycerate	203 ± 33	360 ± 27	322 ± 42
RuBP	98 ± 19	52 ± 3	113 ± 12
Dihydroxyacetone-P	33 ± 5	22 ± 2	30 ± 3
Fru-1,6-P	12 ± 2	12 ± 1	11 ± 2
Fru-6-P	80 ± 6	62 ± 7	89 ± 6
Glc-6-P	164 ± 4	144 ± 18	203 ± 19
UDPGlc	49 ± 4	60 ± 12	61 ± 12
Sum of organic phosphate measured	1037	1053	1209

Table II. *Subcellular Localization of PGA in Leaves in 550 μbar CO_2 and 210 mbar O_2 or 1 Min after Switching to 21 mbar O_2*
Leaf temperature was 18°C.

O_2	PGA	
	Stroma	Cytosol
mbar	*nmol mg^{-1} Chl*	
210	305	50
20	553	145

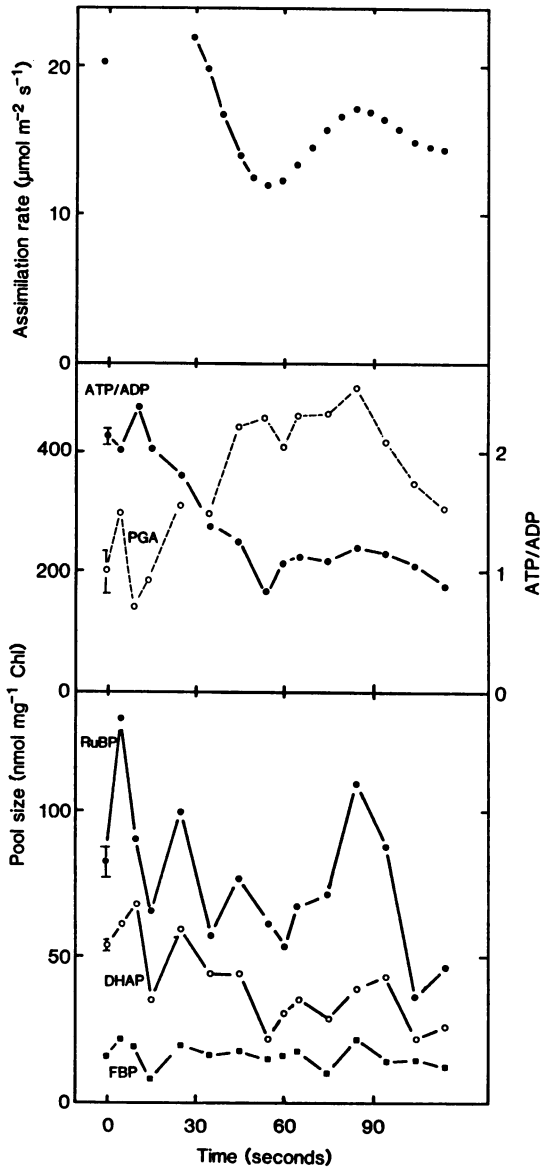

FIG. 6. Assimilation rate and metabolite pool sizes after switching to low O_2 at time zero. The error bars are ±SE of the mean of four samples taken without switching to low O_2.

After 15 to 20 min these oscillations disappear and photosynthesis recovers to a rate similar to that found at 210 mbar O_2. In all our experiments the appearance of O_2 insensitivity in steady state conditions was accompanied by the appearance of these transitory phenomena, suggesting these phenomena are related to one another.

What could be responsible for this response to a reduction in the partial pressure of O_2? One factor, as discussed in the introduction, is the possibility that the net rate of CO_2 fixation in O_2 insensitive conditions is limited by the utilization of phosphorylated intermediates, *e.g.* in synthesis of sucrose and starch. This imposes a ceiling on the maximal rate of photosynthesis. There could then be unused capacity for electron transport, RuBP regeneration, and Rubisco activity, which would be available to support the fluxes needed during photorespiration. However, a second possibility is that rapid photosynthesis in saturating light and CO_2 actually requires the presence of O_2, so that lowering O_2 decreases photorespiration, but also impinges directly on the rate of photosynthesis. This explanation would not apply however whenever it can be demonstrated that CO_2 sensitivity is lost concurrently with O_2 sensitivity (18).

These two explanations are not mutually exclusive and could even interact. Their contribution may be distinguished, however, by comparing fluxes and metabolite levels at 210 and 21 mbar O_2. If the rate of photosynthesis is limited by utilization of triose-P, then the levels of phosphorylated intermediates leading to sucrose or starch, and the general content of esterified phosphate should increase in low O_2. Further, the chloroplast metabolite pattern during O_2-insensitive photosynthesis in leaves should resemble that in isolated chloroplasts photosynthesizing at limiting Pi. (However, it is not necessary that dihydroxyacetone-P be high during triose-P utilization limited photosynthesis since the limitation ultimately leads to less ATP which would reduce the rate of triose-P production.) In contrast, if low O_2 directly restricts photosynthesis then such an accumulation of phosphorylated intermediates would not be expected. We will first consider the steady state condition and then the more complicated behavior during the transient.

Metabolites in Steady State in O_2-Insensitive Conditions. After 18 min at 21 mbar O_2 the rate of photosynthesis was almost the same as in 210 mbar O_2. Because photorespiration was reduced in low O_2 but the rate of CO_2 assimilation was similar to that in 210 mbar O_2, the activity of the carbon reduction cycle enzymes, of Rubisco, and the rate of electron transport decreased by 12–17% (Table III). However, there was a higher level of almost all the phosphorylated intermediates including RuBP (only marginal in this experiment, but see Ref. 19), P-glycerate, Glc-6-P, Fru-6-P, and UDPGlc in 21 mbar O_2 relative to 210 mbar O_2. As the total esterified Pi increases, Pi might be expected to decline. These results indicate that conversion of phosphorylated intermediates to end products and recycling of Pi may well limit the rate of photosynthesis in these O_2-insensitive conditions. But, are these changes large enough to cause the restriction of fluxes in the chloroplasts?

Direct evidence is still absent, as it has not yet been possible to measure the stromal and cytosolic Pi levels in leaves during photosynthesis because of the very high Pi in the vacuole (7). Using data from Table I, and published results it is, however, possible to evaluate whether the changes of esterified phosphate observed in our experiments could lead to significant changes of Pi in the stroma of the leaves. The amount of esterified phosphates measured in the whole leaf increased by 172 nmoles (mg Chl)$^{-1}$ 18 min after the leaf was brought from 210 mbar O_2 to 21 mbar O_2. Since phosphate transport from the vacuole appears to be on the order of hours (7, 16), the observed increase in organic phosphates should be accompanied by a parallel decline of Pi. Assuming a combined cytosolic and stromal space of 45 μl/mg Chl, this would be equal to a decrease of free Pi of almost 4 mM. Related to the levels of Pi (2–7 mM) found in isolated chloroplasts carrying out photosynthesis (11), such changes are very large.

Another approach is to ask whether much free Pi could remain in the stroma of leaves where over 1000 nmol Pi/mg Chl has already been esterified. Metabolite data from isolated chloroplasts may be used for a rough estimation of stromal Pi levels in leaves. Due to the counter exchange of the phosphate translocator

Table III. *Flux Rates Calculated from Figure 5 and Rubisco Activity Determined for the Experiment in Table I*

Activity	Condition		
	20 min 210 mbar O_2	1 min 21 mbar O_2	18 min 21 mbar O_2
CO_2 assimilation (μmol m^{-2} s^{-1})	21	10	21
RuBP flux (μmol m^{-2} s^{-1})	26	12	23
ATP flux (μmol m^{-2} s^{-1})	83	36	69
Rubisco activation (%)	86	68	79

(6), the total amount of phosphate and phosphorylated intermediates is kept about constant. In isolated spinach chloroplasts the total pool of Pi plus organic phosphates is usually in the range of 500 to 1000 nmol (mg Chl)$^{-1}$. By subcellular analysis of spinach leaves it was found that 60 to 80% of the total leaf content of organic phosphates is in the chloroplast stroma in the light (R Gerhardt, unpublished data) (see also Table II). Assuming a similar distribution of the total esterified phosphates in a leaf in our experiments, we estimate that the stroma would contain 620 to 830 nmol P-ester/mg Chl at 210 mbar O_2, and 720 to 960 nmol P-ester/mg Chl at 20 mbar O_2, indicating that stromal Pi could indeed be quite low in these conditions.

Further indirect evidence that low Pi limits photosynthesis in O_2-insensitive conditions is provided by the similar metabolite patterns found in leaves under low O_2 and in isolated chloroplasts photosynthesizing while Pi was limiting (11, 13). Both show elevated levels of RuBP and P-glycerate, an increase P-glycerate/triose-P ratio, and a decrease ATP/ADP ratio. Further the activation state of Rubisco decreased in leaves during O_2-insensitive photosynthesis (19), resembling the decrease found with isolated chloroplasts in low Pi (11).

While low Pi decreases the rate of photosynthesis, the precise mechanisms involved in this inhibition remain unclear, even in isolated chloroplasts. At least two aspects can be seen, involving restriction of Rubisco activity and of electron transport activity. The increased P-glycerate/triose-P ratio found in isolated chloroplasts in limiting Pi and in leaves in O_2-insensitive conditions shows how the delivery of energy from the thylakoids has been restricted in both cases. The ATP/ADP ratio declines in both, and this could be due to a restriction of ATP formation caused by Pi deficiency as proposed by Sharkey (18). However, it could also reflect a response of the ATP/ADP · Pi equilibrium to a decreased Pi level (9). The elevated level of RuBP observed after 18 min at 21 mbar O_2 (Table I) or in chloroplasts in limiting Pi concentration (11) indicates that Rubisco has decreased in its activity in both cases. This is in agreement with earlier findings that Pi is required for maximal activation of Rubisco (2, 11, 15). A control of Rubisco activity by Pi levels has been proposed as an important mechanism for adjusting the rate of carbon fixation to the rate of carbon utilization in order to avoid the total sequestration of Pi (11).

Transient Inhibition at Low Partial Pressure of O_2. Immediately after decreasing O_2 at 21 mbar, the rate of assimilation decreases by 50% and there are marked oscillations in the rate of CO_2 assimilation. One reason for this inhibition could be an imbalance in the recycling of Pi in the photorespiratory pathway. During photorespiration, Pi is released from 2-P-glycolate, and the photorespiratory glycerate which reenters the chloroplast is rephosphorylated by glycerate kinase (23). In effect, the Pi required in photorespiration is recycled without using the capacity for carbohydrate synthesis. When the partial pressure of O_2 is abruptly lowered, the release of Pi from 2-P-glycolate will be rapidly stopped, but a substantial amount of Pi will be needed to rephosphorylate the preexisting pool of photorespiratory carbon. This temporary imbalance in Pi metabolism could be one reason for the marked inhibition of CO_2 assimilation immediately after decreasing O_2.

The observation that transitions from 210 to 21 mbar O_2 can induce oscillations even under the nonphotorespiratory conditions of 5% CO_2 (21) indicates that the role of O_2 in pseudocyclic photophosphorylation can affect the transient behavior of photosynthesis. Such oscillations only occur at or near saturating light intensity, when Pi metabolism may be limiting photosynthesis (20, 22) and they can be abolished by feeding Pi or magnified by feeding mannose (20, 26). It may be that direct effects of O_2 on electron transport can restrict photosynthesis or induce oscillations when Pi metabolism is limiting or nearly limiting.

Limitation of Photosynthesis. These results show that the phenomenon of O_2 insensitivity can be at least partly explained by the suggestion that the recycling of Pi during the synthesis of carbohydrate limits the maximal rate of photosynthesis in saturating light and CO_2. However, the results do not exclude the possibility that O_2 may have additional influences on photosynthesis, particularly during transients in conditions when rapid photosynthesis is occurring.

These results, in conjunction with the preceding article (22) and studies of oscillatory phenomena by Walker, Sivak, and Osmond (20, 21, 26), suggest that the maximal rates of photosynthesis of C_3 plants can be limited by the ability of plants to convert triose-P to sucrose or starch.

Note Added in Proof. M. Miginiac-Maslow and A. Hoarau (1982 Z Pflanzenphysiol 107: 427–436) found that Pi sequestration leads to reduced total adenylate pools. The decline in total adenylate pool in this study (Table I) is, therefore, consistent with our hypothesis that the Pi pool is low under O_2.

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