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O_2-Insensitive Photosynthesis in C_3 Plants[1]

ITS OCCURRENCE AND A POSSIBLE EXPLANATION

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THOMAS D. SHARKEY
Biological Sciences Center, Desert Research Institute, P.O. Box 60220, Reno, Nevada 89506

ABSTRACT

Leaves of C_3 plants which exhibit a normal O_2 inhibition of CO_2 fixation at less than saturating light intensity were found to exhibit O_2-insensitive photosynthesis at high light. This behavior was observed in *Phaseolus vulgaris* L., *Xanthium strumarium* L., and *Scrophularia desertorum* (Shaw.) Munz. O_2-insensitive photosynthesis has been reported in nine other C_3 species and usually occurred when the intercellular CO_2 pressure was about double the normal pressure. A lack of O_2 inhibition of photosynthesis was always accompanied by a failure of increased CO_2 pressure to stimulate photosynthesis to the expected degree. O_2-insensitive photosynthesis also occurred after plants had been water stressed. Under such conditions, however, photosynthesis became O_2 and CO_2 insensitive at physiological CO_2 pressures. Postillumination CO_2 exchange kinetics showed that O_2 and CO_2 insensitivity was not the result of elimination of photorespiration.

It is proposed that O_2 and CO_2 insensitivity occurs when the concentration of phosphate in the chloroplast stroma cannot be both high enough to allow photophosphorylation and low enough to allow starch and sucrose synthesis at the rates required by the rest of the photosynthetic component processes. Under these conditions, the energy diverted to photorespiration does not adversely affect the potential for CO_2 assimilation.

Photosynthesis in plants having the C_3 pathway of photosynthesis is known to be inhibited by air levels of O_2 pressure when the partial pressure of CO_2 is below 1000 μbar. Present theory suggests that this is because (i) oxygenation of RuBP[2] leads to the release of CO_2 by the process of photorespiration, (ii) O_2 is a competitive inhibitor of CO_2 binding to RuBP carboxylase, and (iii) oxygenation of RuBP makes less RuBP available for carboxylation.

Recent advances in the theory and practice of gas exchange studies of intact leaves have resulted in the identification of gas exchange behavior which can be explained by the amount or activity of RuBP carboxylase (at high light and low CO_2) or the capacity to regenerate the CO_2 acceptor molecule, RuBP (at low light and/or high CO_2). When photosynthesis is limited by RuBP carboxylase activity, O_2 inhibition of photosynthesis occurs by mechanisms i and ii whereas, when RuBP regeneration limits photosynthesis, mechanisms i and iii are important (8). When CO_2 assimilation is limited by RuBP carboxylase activity, the inhibition of photosynthesis caused by air levels of O_2 will be roughly 40% while, under RuBP regeneration limitation, the O_2 inhibition will be only 30% (assuming $K_m^{CO_2}$= 300 μbar, $K_m^{O_2}$ = 215 mbar, $V_{o\,max}/V_{c\,max}$ = 0.21, C_i = 225 μbar) (9). Throughout this paper, I have compared observed O_2 responses to those expected under RuBP regeneration limited conditions, since these are more conservative estimates.

There have been a number of reports of no stimulation of photosynthesis and sometimes even an inhibition upon switching from air levels of O_2 to low O_2 pressure (29, 31). This unusual behavior is sensitive to temperature (5, 14, 15), CO_2 pressure, and light (18). In this paper, I show that as light is varied the inhibition of photosynthesis by O_2 is nearly constant over a range of CO_2 assimilation rates until the rate of CO_2 assimilation approaches a maximum, whereupon the O_2 inhibition becomes at first less severe, and then can even reverse. This O_2-insensitive limitation to photosynthesis also occurs in water-stressed plants. The postillumination burst of CO_2 was used to demonstrate that the lack of O_2 sensitivity was not the result of reduced photorespiration. Since the O_2-insensitive limitation of photosynthesis appears to set an upper limit on the rate of photosynthetic CO_2 assimilation, it was studied in a weedy species with a high rate of assimilation (*X. strumarium*) and a crop species that has a maximum assimilation rate of less than half that of *Xanthium* (*Phaseolus vulgaris*). No differences in the nature of O_2 insensitivity were noted, and results from both species are presented.

MATERIALS AND METHODS

Plant Material. *Phaseolus vulgaris* var Tendergreen (seeds from Northrup King), *Xanthium strumarium* L. (seeds from J.A.D. Zeevaart, Michigan State University), and *Scrophularia desertorum* (Shaw.) Munz (root cuttings obtained in Reno) were grown in 4-L plastic pots in potting soil (compost/sand/perlite, 2/1/1 by volume) in a greenhouse. The temperature was controlled at 27°C day, 15°C night; RH was 60%; and the photoperiod was extended to 18 h with 1 μmol photons m^{-2} s^{-1} from fluorescent tubes to keep the *Xanthium* from flowering.

Gas Exchange. Air was mixed from N_2, O_2, and 3% CO_2 in air using mass flow controllers (FC260, Tylan, Carson, CA). Some of this synthetic air passed through an aluminum chamber which had two fans for mixing the air, a glass window to admit light, and was temperature controlled by Peltier heating and cooling. The air flow was controlled by a mass flow controller. Some of the synthetic air and air from the leaf chamber was compared for water content and CO_2 content with a Binos IR gas analyzer (Leybold-Hereaus, Köln, West Germany). Cross sensitivity of the water analyzing section to CO_2 was reduced by optical filters while cross sensitivity of the CO_2 measuring section to H_2O was reduced by passing the measurement air stream through a copper coil in an ice bath. Additional 3% CO_2 in air was injected through another flow meter directly into the cham-

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[2] Abbreviations: RuBP, ribulose 1,5-bisphosphate; A, photosynthetic CO_2 assimilation rate; C_a, ambient CO_2 partial pressure; C_i, partial pressure of CO_2 inside the leaf; TPU, triose phosphate utilization; PGA, glycerate 3-phosphate.

ber to compensate photosynthetic depletion of CO_2 from the air stream. In this way, the CO_2 analyzer was used primarily as a null point detector.

Leaf temperature was measured with a copper-constantan thermocouple probe (SCPSS-020G-6; Omega Engineering Inc., Stamford, CT).

Calculations of evaporation, conductance to gas exchange, photosynthesis, and intercellular CO_2 pressure were done according to von Caemmerer and Farquhar (30).

'Light' is used throughout to describe photosynthetic photon flux (areal) density. Light was measured with a LiCor quantum sensor (190SB) and LI 188B. The unit of pressure used for gas pressure is bar because this has the same relative magnitude as mole fraction, the concentration. One bar is equal to 10^5 Pa.

Measurement of Postillumination CO_2 Exchange. The following method was devised to accurately resolve the very fast postillumination CO_2 exchange of leaves. A solenoid valve was put in the CO_2 airstream which compensated CO_2 assimilation. The solenoid valve was closed by a switch which was activated by closing a shutter on a Xenon arc lamp. As a result, the light could be cut off at exactly the same time that the CO_2 supply compensating photosynthesis was turned off. In this system, if the leaf neither lost nor gained CO_2 after the light was off there would be no change in the signal coming from the gas analyzer. Respiration would appear as an increase in the signal, postillumination burst would appear as an upward transient, and postillumination photosynthesis would appear as a downward transient.

RESULTS

Xanthium strumarium. The response of A to C_i at saturating light intensity is shown for a leaf of *X. strumarium* in Figure 1. Before water stress began, switching to low O_2 at 800 μbar C_i caused a reduction in A when a 10% stimulation should have occurred as a consequence of suppression of photorespiration (see Introduction). The results with the unstressed leaf show O_2 insensitivity only under nonphysiological CO_2 pressure. After 7 d of withholding water from the plant, A was reduced by over one-half at saturating C_i and the leaf exhibited O_2 insensitivity when C_i was below 300 μbar. In other experiments O_2 insensitivity after water stress occurred when C_a was 330 μbar (24). The imposed stress was relatively mild, causing the water potential to fall from −0.3 to −0.9 MPa. The apparent quantum yield fell from 0.09 to 0.06 mol CO_2 mol^{-1} incident quanta. This decline of the quantum yield by one third is less than the decline in maximum A of over one-half.

To test whether O_2 insensitivity could occur under physiological CO_2 concentration in an unstressed leaf, plants were grown under twice normal CO_2 pressure. After growth in 600 μbar CO_2, both *X. strumarium* and *P. vulgaris* exhibited O_2 insensitivity at 600 μbar C_a (data not shown) indicating that these plants may exhibit O_2 insensitivity under 'normal' conditions in the future.

Postillumination Burst of CO_2. Since the extent of photorespiratory O_2 fixation was important to this work, I measured the postillumination burst as a function of C_i under conditions (higher temperature) where the O_2-insensitive limitation did not occur (Fig. 2). There was a significant postillumination burst which was sensitive to C_i, as would be predicted by the competitive inhibition of photorespiration by CO_2. No postillumination assimilation was seen in this experiment, and photosynthesis was never CO_2 insensitive, indicating that it was also never O_2 insensitive (see below). Because postillumination exchange kinetics are only a rough measure of changes in pools of intermediates, it is meaningless to calculate rates of respiration, but the raw curves can be used to qualitatively indicate the presence of photorespiration.

The postillumination CO_2 exchange kinetics were determined after several of the gas exchange determinations shown in Figure 1. In both the unstressed and water-stressed condition, the presence of O_2 resulted in a postillumination burst not seen in the absence of O_2 even though photosynthesis was insensitive to O_2.

Light Dependence of O_2 Insensitivity. The phenomenon of O_2-insensitive photosynthesis was found to occur at high but not low light intensity (Fig. 3). In the left panel, the response of A to C_i at one-eighth full sunlight (~250 μmol m^{-2} s^{-1}) is shown at normal and low O_2 pressure. At all values of C_i, A in low O_2 was

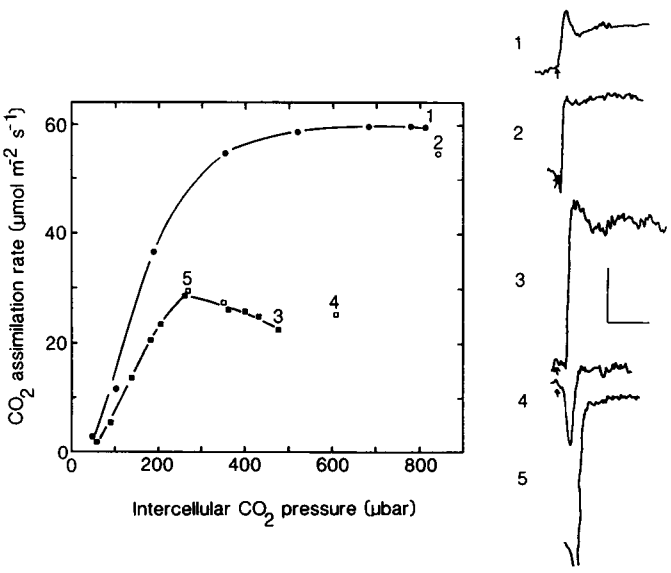

FIG. 1. Response of A to C_i before (○, ●) and after (□, ■) withholding water for 7 d in *X. strumarium*. The water potential measured with a pressure bomb on an adjacent leaf after the stress was −0.9 MPa. (○, □), Data points determined in 25 mbar O_2 pressure; all other points were determined in 180 mbar O_2 pressure. Postillumination CO_2 kinetics were determined for the numbered data points and are shown at the right. The apparent quantum yield before stress was 0.09 mol CO_2 mol^{-1} photons. After stress, the apparent quantum yield was 0.06. Leaf temperature was 25°C. The horizontal line by curve three represents 4 min, and the vertical line represents 50 mv of analyzer output.

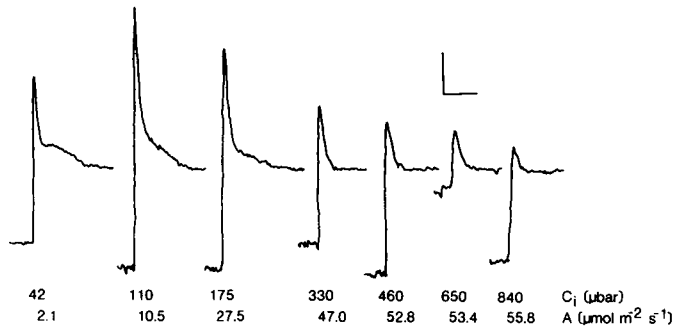

FIG. 2. Postillumination CO_2 exchange kinetics of a leaf of *X. strumarium* at various intercellular CO_2 pressures. Light and the CO_2 flow compensating assimilation were turned off simultaneously and for each tracing this was at the point where the signal began to increase. The conditions experienced just prior to when these tracings were made were 2000 μmol photons m^{-2} s^{-1} (400–700 nm) 180 mbar O_2, 27°C and 10.6 mbar leaf to air vapor pressure difference. In the upper right corner, the horizontal line represents 4 min and the vertical line represents 50 mv of CO_2 analyzer output. Analyzer output is slightly sensitive to CO_2. The curves were adjusted such that the final CO_2 level appears constant. The initial CO_2 level varies because the compensation of photosynthesis was not perfect. Dark respiration rate was not affected by C_i.

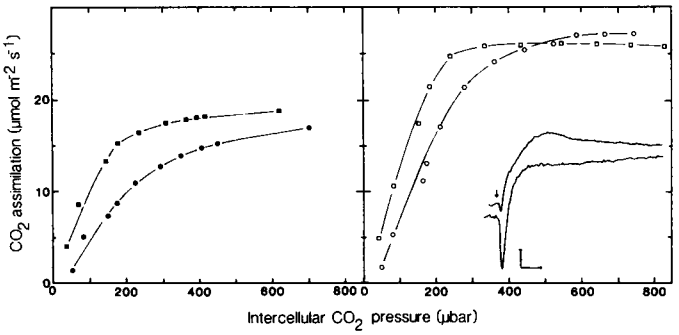

FIG. 3. Response of A to C_i at 250 μmol photons m^{-2} s^{-1} (left panel) and at 1000 μmol m^{-2} s^{-1} (right panel) in *P. vulgaris*. Responses were determined in 40 mbar O_2 (□, ■) and 180 mbar O_2 (○, ●). Inset in the panel are traces of the gas analyzer output when the light was turned off (indicated by the arrow). The upper trace is from a leaf in 180 mbar O_2 at 470 μbar C_i. The compensation CO_2 supply was turned off at the same time the light was turned off, the initial downward transient reflects postillumination CO_2 assimilation. In air levels of O_2, a postillumination burst of CO_2 can also be seen. The vertical bar indicates 20 mv of analyzer output and the horizontal bar indicates 2 min. Leaf temperature was 25°C.

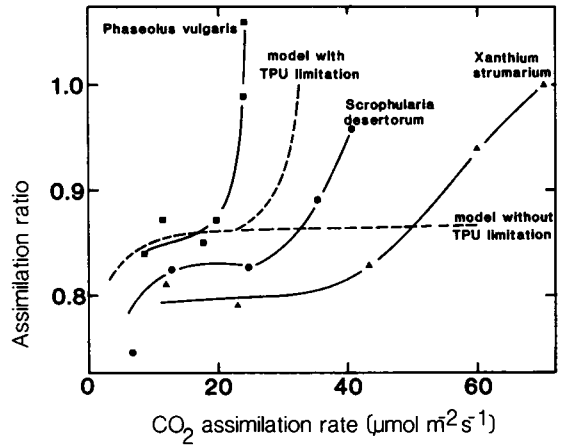

FIG. 4. Ratio of A at 180 mbar O_2 to the assimilation rate at 25 mbar O_2 (40 in the case of *P. vulgaris*) as a function of assimilation rate in 180 mbar O_2 and 500 μbar CO_2. A was varied by varying light.

higher than in normal O_2. In contrast, at 1000 μmol photons m^{-2} s^{-2} (which was saturating for this plant) above 500 μbar C_i, the assimilation rate in low O_2 pressure was less than A in normal O_2 pressure (Fig. 3, right panel). Because the results are expressed in terms of C_i, there was no influence of stomatal movements on the curves presented. The postillumination gas exchange of leaves under O_2-insensitive conditions was also examined (inset Fig. 3, right panel). The upper trace was obtained in normal O_2 pressure and the lower trace in low O_2 pressure. There was a postillumination burst of CO_2 in normal O_2 pressure and no burst in low O_2 pressure. The postillumination burst is relatively small because C_i was 500 μbar and because *Phaseolus* is much less active than *Xanthium*. The system response was 2 to 3 min, and so the maximum burst occurred approximately 2 min after the light was turned off. There was postillumination photosynthesis under low O_2 pressure.

The onset of O_2 insensitivity as A increases in response to light is shown in Figure 4. For the three species examined, the ratio of A at 21% O_2 (180 mbar) to A in 3% O_2 (C_i = 500 μbar) was between 0.8 and 0.9 at low rates of assimilation until some species-dependent rate of assimilation occurred. At higher rates of assimilation (caused by higher light), the ratio approached and sometimes exceeded 1.

The way that the ratio is expected to vary was modeled using the equation which describes assimilation under RuBP regeneration limited conditions (from Farquhar and von Caemmerer [8]):

$$A = \frac{(1 - 0.5\phi)\,R}{1 + \phi} - R_d \qquad (1)$$

where R is the rate of RuBP regeneration (varied between 5 and 40 μmol m^{-2} s^{-1}), R_d is the rate of respiration not associated with photosynthesis (mitochondrial respiration, taken to be 1.5 μmol m^{-2} s^{-1}), and ϕ is the ratio of oxygenation to carboxylation taken to be 0.105 in normal O_2 and 0.01 in low O_2 (8). A limitation (labeled TPU, see below) was imposed by assuming an arbitrary A that could not be exceeded. Once that rate was reached, as RuBP regeneration rate was increased, no increase in A was allowed so that the rate in normal O_2 pressure increased until it matched that in low O_2 pressure. To simulate the diverse capacities within the leaf, 20% of the cells were assumed to have a capacity for A of 24 μmol m^{-2} s^{-1}, 20% to have a capacity of 28, 20% to have a capacity of 32, 20% to have a capacity of 36, and 20% to have a capacity of 40. This causes the transition to O_2 insensitivity to be gradual instead of abrupt. The model without the TPU limitation did not reasonably describe the behavior of *P. vulgaris*, *S. desertorum*, or *X. strumarium*. The addition of a TPU limitation to the model caused the model to mimic the observed behavior. However, this simple model never predicts a ratio greater than 1.

Oxygen Insensitivity Is Correlated with CO_2 Insensitivity. The loss of O_2 sensitivity indicates that the oxygenase function of RuBP carboxylase no longer affected net CO_2 assimilation, even though postillumination CO_2 exchange showed that oxygenation still occurred. Under RuBP regeneration limited conditions, increasing CO_2 pressure has the same effect as decreasing O_2 pressure; that is, more RuBP is diverted away from oxygenation to carboxylation. Therefore, a loss of O_2 sensitivity allows one to predict a loss of CO_2 sensitivity. This correlation is evident in Figures 1 and 3, though the absence of stimulation upon switching to low O_2 pressure is much easier to measure than the absence of CO_2 sensitivity. The relationship between O_2 and CO_2 insen-

Table I. *Oxygen and CO_2 Sensitivity at Various Light Intensities for X. strumarium and P. vulgaris*

Sensitivity was measured as the effect of increasing C_i from 500 to 600 μbar (O_2 pressure = 180 mbar) or decreasing O_2 pressure from 180 to 120 mbar (C_i = 500 μbar). The slope between these points was divided by A at C_i = 500 μbar and O_2 = 180 mbar. Leaf temperature was 25°C.

Light	A	CO_2 Sensitivity	O_2 Sensitivity
μmol photons m^{-2} s^{-1}	*μmol CO_2 m^{-2} s^{-1}*	*bar^{-1}*	
Xanthium strumarium			
150	8.4	440	−1.34
240	11.8	513	−1.58
630	21.3	392	−1.30
800	33.1	361	−1.21
1300	45.1	136	−0.03
2000	48.0	−129	+0.84
Phaseolus vulgaris			
100	4.1	1285	−2.12
200	8.2	352	−1.15
350	12.8	412	−0.89
560	18.8	482	−0.68
930	21.5	63	−0.03
1500	21.6	49	+0.02

sitivity was specifically tested by measuring the relative O_2 sensitivity and CO_2 sensitivity of a leaf at various light levels (Table I). It was observed that a loss of O_2 sensitivity accurately predicted a loss of CO_2 sensitivity.

DISCUSSION

Occurrence of O_2 Insensitivity. O_2 insensitivity occurs at high light intensity, always at or near light saturation (Table I). For unstressed plants at 25°C, O_2 insensitivity occurs at high CO_2 pressure. This makes it sometimes difficult to distinguish between CO_2 suppression of photorespiration and O_2 insensitive photosynthesis. However in Figures 3 and 4, the expected O_2 sensitivity can be gauged at lower light intensity. The loss of O_2 sensitivity as light increases, with C_i held constant, cannot be ascribed to CO_2 suppression of photorespiration.

Water stress caused O_2 insensitivity to occur at much lower CO_2 pressure and assimilation rate (Fig. 1; Refs. 23 and 24). Low temperature (5–20°C) also causes O_2 insensitivity to occur at physiological CO_2 pressures (5; unpublished data). O_2 insensitivity under physiological conditions can also be induced by long periods at high light (3).

There is, therefore, an O_2- and CO_2-insensitive mode of C_3 photosynthesis which can be easily identified by measuring gas exchange characteristics of intact leaves and which appears to set the upper limit to the potential rate of photosynthesis at 25°C and below. This limitation occurs in water-stressed leaves, leaves at low temperature, and leaves of plants grown at high CO_2. Reports of O_2 insensitivity have been published in the past (3, 5, 10, 14, 15, 18, 25, 29, 31), but no concensus of the possible explanation has yet emerged.

The observation of a postillumination burst of CO_2 indicates that the loss of O_2 insensitivity is not the result of a loss of RuBP oxygenase activity. This result challenges conventional wisdom which says that O_2 sensitivity is a good indicator of photorespiration. When photosynthesis is RuBP carboxylase limited, the expected O_2 inhibition in air is 40%; when it is RuBP regeneration limited, the expected inhibition is 30%; and, under the conditions I have reported, the O_2 inhibition is zero. This variation in O_2 sensitivity does not indicate a variation in oxygenase/carboxylase ratio but rather the variation reflects the different ways in which photosynthesis can be limited.

Postillumination CO_2 uptake of O_2-insensitive leaves (Fig. 3) was found under the same conditions that have been shown to produce high concentrations of RuBP in leaves (4, 25). The very large RuBP pools under O_2-insensitive conditions would be more than sufficient to account for all of the postillumination photosynthesis observed.

A Possible Explanation of O_2 Sensitivity. Under O_2-insensitive conditions, increasing light, CO_2, or O_2 pressure does not increase A. This observation is consistent with the idea that A is limited by the rate of use of the products of photosynthesis rather than the availability of light or CO_2. Under O_2-insensitive conditions, switching from normal to low O_2 suppresses photorespiration but does not stimulate CO_2 fixation. Therefore, in low O_2, the rate of carboxylation must be reduced. This could be achieved by allowing the RuBP pool to fall but Sharkey and Badger (25) have shown that low O_2 induced large RuBP pools. The alternative is deactivation of RuBP carboxylase, which has been demonstrated recently (Sharkey, Seemann, and Berry, unpublished). There is no need to postulate differential effects on the oxygenase and carboxylase functions, only that the activation state of the enzyme should vary in such a way as to keep the net CO_2 assimilation rate constant.

One treatment which can induce the loss of O_2 sensitivity is feeding of hexoses which sequester phosphate (11). However, the large amount of Pi in vacuoles of photosynthetic cells makes it unlikely that a plant grown under luxuriant nutrient conditions would become limited because of phosphate nutrition.

I propose an alternative explanation. As the rate of CO_2 assimilation increases, starch and sucrose synthesis must increase as well. If not, triose-P and PGA will build up and Pi will decline. These changes in pool size will stimulate starch and sucrose synthesis. However, there is a limit to how far the Pi pool can fall before it begins to limit photophosphorylation. Once this limit is reached, CO_2 will be assimilated at the rate at which starch and sucrose synthesis can metabolize triose-P, regardless of whether oxygenation occurs or not. At this limit, the ratio of starch to sucrose synthesis is between 1 and 2 and is independent of O_2 pressure in *Phaseolus* (26).

Although the flux through the Pi pool limits photosynthesis, the pool size must be kept small in order to keep starch and sucrose synthesis maximally stimulated. For this reason, deactivation of RuBP carboxylase and concomitant buildup of RuBP probably allows a greater rate of CO_2 assimilation to occur than if RuBP supply limited carboxylation. Since this limitation depends on the balance between the capacities for photophosphorylation and starch plus sucrose synthesis, an increase in either capacity will alleviate this limitation; at the same time, damage to either capacity will reduce the capacity of photosynthesis.

If this argument is to be convincing, it must be shown that there exists a Pi concentration at which both photophosphorylation and TPU are sensitive to changes in pool size.

Starch formation is sensitive to Pi because the enzyme ADP glucose pyrophosphorylase is inhibited by Pi. The Pi inhibition is counteracted by PGA but, for tobacco, even at 9 mM PGA, 0.5 mM Pi lowered the activity of ADP glucose pyrophosphorylase by almost 50% (20). A range of PGA/Pi ratios, 5 to 0.6, causing a 50% inhibition have been reported for various species.

Sucrose synthesis also decreases with increasing Pi. The specific fructose 6-P 2-kinase which produces fructose 2,6-bisP is responsible, in part, for the sensitivity of sucrose synthesis to Pi (6). Sucrose phosphate synthase is also inhibited by Pi (2, 7) which could lead to the buildup of hexose monophosphates. Hexose monophosphates could activate a cascade of events, stimulating fructose 2,6-bisP synthesis which inhibits cytoplasmic FBPase (28). The net effect of this cascade of events would be to reinforce the Pi inhibition of sucrose synthesis.

Although the Pi concentration must be low for maximum starch and sucrose synthesis, it must be high for maximum photophosphorylation rates. Many recent estimates of the effective K_m^{Pi} of the coupling factor are between 400 and 800 μM (*e.g.* 1, 21). Therefore, when the Pi concentration in the chloroplast is 4 mM, photophosphorylation is only 90% of maximum (assuming Michaelean kinetics) and is affected positively by increasing Pi. If the Pi concentration were 1 mM and the K_m 800 μM, photophosphorylation would be only 55% of maximum.

The Pi concentration in the stroma is often taken to be 4 mM from the work of Santarius and Heber (19). However, these measurements were made after only 2 min of light during which time photosynthesis probably had not achieved its maximum rate. Chloroplasts from 'rapidly' fractionated oat protoplasts had 3 mM Pi in the experiments of Hampp *et al.* (10). Because of the Pi sensitivity of starch synthesis and enzyme activation (12, 13), I believe that the Pi concentration inside the chloroplast can often be as low as 1 mM, sometimes even less.

Hence, there is a Pi concentration at which both TPU and photophosphorylation respond to changes in the Pi concentration and, what is more, there are indications that this is the Pi concentration (approximately 3 mM or less) that occurs inside chloroplasts during photosynthesis. The compartmentation of the processes involved has been discussed in detail elsewhere (22).

Why should water stress cause the O_2 insensitivity of photosynthesis to occur at lower assimilation rate? Several groups have

shown photophosphorylation to be sensitive to mild water stress (16, 17, 24, 32). If the photophosphorylation capacity were decreased, then a higher Pi concentration would be required for a given rate of phosphorylation, regardless of whether the effect of water stress was on the K_m, k_{cat}, or number of active enzyme sites. The higher Pi level required for photophosphorylation would limit the potential rate of TPU, limiting the maximum rate of CO_2 assimilation.

Finally, can this mechanism explain the decline in photosynthesis seen upon switching to low O_2 or to higher CO_2? The simple model presented in Figure 4 does not predict this behavior. However, if the rate of oxygenation contributed to establishing the Pi level at exactly the optimum concentration because of the release of Pi by glycolate-P phosphatase, then reducing oxygenation by either increasing CO_2 or decreasing O_2 pressure could cause the Pi concentration to be below optimum and so the rate of photophosphorylation would be lowered. I do not believe, however, that oxygenation serves an essential role in regulating the Pi level in chloroplasts, only that putting leaves under conditions that they have not experienced (low O_2) can cause a metabolic imbalance leading to unusual O_2 and CO_2 responses.

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