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Communication

Low Oxygen Inhibition of Photosynthesis Is Caused by Inhibition of Starch Synthesis[1]

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ABSTRACT

Photosynthesis of C_3 plants is occasionally inhibited upon switching from normal to low partial pressure of O_2. Leaves of *Solanum tuberosum* exhibited this effect reproducibly under saturating light and 700 microbars of CO_2. We determined the partitioning of recent photosynthate between starch and sucrose and measured the concentration of hexose monophosphates in the stroma and cytosol after nonaqueous fractionation. The reduction in the rate of photosynthesis upon switching to low partial pressure of O_2 was caused by reduced starch synthesis. The concentration of hexose monophosphates in the stroma fell and the glucose 6-phosphate to fructose 6-phosphate to fructose 6-phosphate ratio fell from 2.7 to 1.3, indicating an inhibition of phosphoglucoisomerase as described by K-J Dietz ([1985] Biochim Biophys Acta 839: 240–248). The concentration of hexose monophosphates in the cytosol increased, ruling out a sucrose synthesis limitation by reduced transport from the chloroplast as the explanation for low O_2 inhibition of photosynthesis.

Oxygen usually inhibits photosynthesis of C_3 plants because of photorespiration. Sometimes, photosynthesis of C_3 plants can be insensitive to O_2; switching to low O_2 partial pressure causes no change in the rate of photosynthesis (11). Oxygen insensitivity is believed to result from a feedback limitation of photosynthesis (15). Occasionally, photosynthesis is inhibited by switching to low O_2 partial pressure (1, 2, 7, 8, 16). This is called O_2 stimulation of photosynthesis (2, 17).

A number of ideas have been offered to explain the cause of low O_2 inhibition of photosynthesis. Viil *et al.* (17) and McVetty and Canvin (8) suggest that regulation of the redox status of the electron carriers of the photosynthetic electron transport chain may cause the O_2 stimulation of photosynthesis. Leegood and Furbank (7) treat the phenomenon as an extension of O_2 insensitive photosynthesis, and suggest that very low concentrations of cytosolic phosphate restrict the capacity for export of triose phosphate from the chloroplast to the cytosol, thereby restricting sucrose synthesis under low O_2 partial pressure. Sharkey *et al.* (14) suggest that the high PGA[2] level which occurs during feedback limited photosynthesis would inhibit starch synthesis by inhibiting phosphoglucoisomerase as first reported by Dietz (3).

The mechanism of reduced starch synthesis described by Dietz (3) works like this. PGA inhibits phosphoglucoisomerase, the enzyme necessary for conversion of F6P to G6P. At low rates of photosynthesis this effect is overshadowed by the stimulatory effect of PGA on ADPglucose pyrophosphorylase (9). However, as the rate of photosynthesis increases, and the concentration of PGA increases, the inhibition of phosphoglucoisomerase is observed as a displacement from equilibrium of the G6P/F6P ratio. While this ratio is usually 3, it falls to 1.3 in chloroplasts at high rates of photosynthesis (3, 5). Dietz showed that starch accumulation was reduced in low O_2 concentration when the G6P/F6P ratio was low.

The three explanations for low O_2 inhibition of photosynthesis can be distinguished on the basis of their effect on partitioning to starch and sucrose. If electron transport effects are responsible, then little effect on partitioning between starch and sucrose is expected. If inhibited sucrose synthesis is responsible, then it is expected that less photosynthate will be partitioned into sucrose and more into starch. But if the effect described by Dietz (3) is responsible, then less photosynthate is expected in starch at low O_2 partial pressure than at normal O_2 partial pressure.

We have measured the partitioning of recent photosynthate between starch, sucrose, and the ionic fraction in potato leaves in normal and low O_2 partial pressure under conditions which caused the low O_2 inhibition of photosynthesis. We also measured the concentration of F6P and G6P in the stroma and cytosol of these leaves. The results indicate that low O_2 inhibition of photosynthesis is caused by an inhibition of chloroplastic phosphoglucoisomerase. Some of these data have appeared in a preliminary form (12).

MATERIALS AND METHODS

Plant Culture

Potato plants (*Solanum tuberosum* cv Russet Burbank) were grown in a growth chamber in 4 L pots containing a soil:peat:perlite:rice hull (3:3:3:2) mix. Plants were grown under a 12 h photoperiod with 24°C/17°C day/night temperature, 60% RH with a photon flux density of 500 μmol m^{-2} s^{-1}. The plants were fertilized 5 times per week with Hoagland solution B (6). The plants were 4 to 6 weeks old at the time of these measurements.

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[2] Abbreviations: PGA, 3-phosphoglycerate; F6P, fructose 6-phosphate; G6P, glucose 6-phosphate.

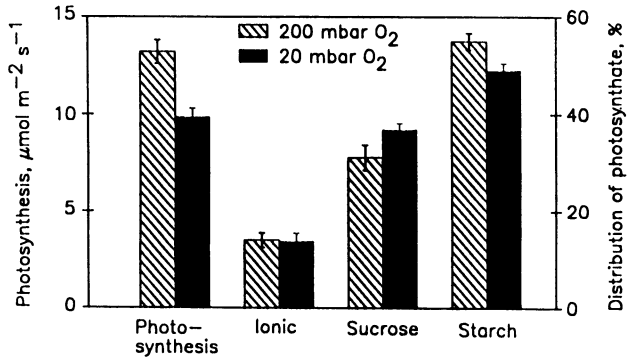

Figure 1. Photosynthesis and partitioning of recent photosynthate in potato leaves held in normal or low O_2. Data are reported as average ± SE (n = 12). The data were obtained during three different periods through the summer of 1988.

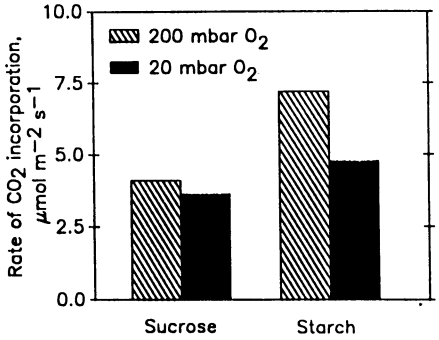

Figure 2. Rate of starch and sucrose synthesis determined by multiplying the rate of photosynthesis by the proportion of label in each fraction using the data of Figure. 1.

Table I. *Subcellular Distribution of Hexose Monophosphates and Concentration of PGA in Potato Leaves Harvested in Normal or Low O_2 Partial Pressure*

The O_2 stimulation was measured in each of the five leaves which made up each sample and was calculated as $100 \times (A_{200}-A_{20})/A_{20}$ where A_{200} is CO_2 assimilation in 200 mbar and A_{20} is CO_2 assimilation in 20 mbar O_2. The CO_2 partial pressure was 700 μbar.

O_2	O_2 Stimulation	PGA	Stroma		Cytosol	
			G6P	F6P	G6P	F6P
mbar	%	*nmol mg^{-1} Chl*				
200	19 ± 6	56	30	11	54	22
20	15 ± 3	160	8	6	137	29

Conditions for Observing Low Oxygen Inhibition

Because it is hard to reproduce from day to day, the study of this phenomenon is difficult. During an unrelated study of potatoes, we consistently obtained the low O_2 inhibition of photosynthesis using standard assay conditions of 750 μmol photons m^{-2} s^{-1}, leaf temperature of 22.5°C, and high CO_2 (700 μbar). Leaves which did not exhibit at least 10% inhibition of photosynthesis upon switching to low CO_2 were not used. Every leaf used was tested, and we report the average inhibition observed in the leaves used for nonaqueous fractionation.

Partitioning

Partitioning was assessed by feeding $^{14}CO_2$ to leaves photosynthesizing in either normal or low O_2 partial pressure. The CO_2 partial pressure during feeding was 700 μbar. Photosynthesis was measured as depletion of CO_2 in an air stream passing over the leaf as described by Vassey and Sharkey (16). The air stream was mixed from N_2, O_2, and 5% CO_2 in air. To label, the CO_2 supply to the gas exchange system was switched to a small tank containing 5% $^{14}CO_2$ (0.1 Ci mol^{-1}). After feeding for 10 min, the unlabeled CO_2 was switched back into the system for a 5 min chase. If the rate of photosynthesis after the chase differed from the rate before the chase by more than 10%, the leaf was discarded. Leaves were frozen and stored by −80°C until analyzed. For analysis, the leaves were extracted as described in Sharkey *et al.* (13). The neutral, soluble fraction was presumed to be sucrose, the insoluble fraction made soluble by amyloglucosidase digestion was presumed to be starch.

Nonaqueous Fractionation

The concentration of F6P and G6P in the stroma and cytosol was measured after nonaqueous fractionation of freeze-dried leaf material as described by Gerhardt and Heldt (4). Five 8 cm^2 leaf samples were combined for each measurement. Glucose 6-P was measured by measuring the reduction of NADP by G6P dehydrogenase. Fructose 6-P was measured by adding phosphoglucoisomerase. Other details concerning metabolite measurements are given in Seemann and Sharkey (10). Interpretation of the data was done as described by Gerhardt and Heldt (4). All chemicals and enzymes were obtained from Sigma Chemical Co.

RESULTS

Photosynthesis was reduced in low O_2 partial pressure relative to normal O_2 partial pressure in the plants used in this study (Fig. 1). About 15% of the recent photosynthate was in the ionic fraction in leaves regardless of O_2 partial pressure. The proportion of recent photosynthate in sucrose was higher in low O_2 while the proportion in starch was lower in low O_2. By multiplying the proportion of label in each fraction by the rate of photosynthesis at either high or low O_2, we calculated the rate of sucrose and starch synthesis. The rate of sucrose synthesis was almost the same in low and normal O_2 partial pressure, but the rate of starch synthesis was substantially reduced in low O_2 (Fig. 2).

The partitioning data indicated that inhibition of phosphoglucoisomerase reduced starch synthesis and so caused the low O_2 inhibition of photosynthesis. We tested this conclusion by measuring the concentration of PGA and stromal G6P and F6P concentration. The leaves used for this measurement exhibited an average stimulation of photosynthetic rate of 17% upon switching from low to normal O_2 (Table I). The concentration of PGA tripled upon switching to low O_2. Both stromal G6P and stromal F6P fell upon switching to low O_2, and the G6P to F6P ratio fell from 2.7 to 1.3. In the cytosol the concentration of G6P more than doubled (Table I).

DISCUSSION

Starch synthesis was dramatically reduced in low O_2. The high concentration of PGA, and the low G6P to F6P ratio in leaves in low O_2 support the theory that PGA inhibition of phosphoglucoisomerase can inhibit starch synthesis (3). The reduced partitioning into starch and the increased concentrations of hexose monophosphates in the cytosol are not consistent with the suggestion that a low phosphate concentration in the cytosol reduces the amount of carbon exported from the stroma to the cytosol thereby restricting sucrose synthesis (7). It is possible that spinach leaves in low temperature exhibit the low O_2 inhibition of photosynthesis for different reasons than do potato leaves at normal temperature. We were unable to obtain low temperature inhibition of photosynthesis in spinach leaves at low temperature.

The results do not support the hypothesis that the change in O_2 partial pressure affects photosynthetic electron transport. Electron transport effects are also ruled out by the observation that reversed O_2 sensitivity is correlated with reversed CO_2 sensitivity (11), indicating that it is not the low O_2 which causes the inhibition of starch synthesis but the mismatch between the production and consumption of triose phosphates.

It is difficult to imagine the adaptive significance of this regulation. It may be one component of the regulation of partitioning of recent photosynthate. Except when plants are feedback limited, reductions in starch synthesis will be compensated by increases in sucrose synthesis. In the experiments reported by Dietz (3), starch synthesis was inhibited even though the rate of photosynthesis was stimulated in low O_2. However, it also may be an anomolous response to nonphysiological conditions, with no adaptive significance. In any case, the study of this phenomenon has helped elucidate the regulation of carbon metabolism.

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LITERATURE CITED

1. **Brown RH, Boulton JH, Evans PT** (1986) Oxygen stimulation of apparent photosynthesis in *Flaveria linearis*. Plant Physiol **81:** 212–215
2. **Cornic G, Louason G** (1980) The effects of O_2 on net photosynthesis at low temperature (5°C). Plant Cell Environ **3:** 149–157
3. **Dietz K-J** (1985) A possible rate limiting function of chloroplast hexosemonophosphate isomerase in starch synthesis of leaves. Biochim Biophys Acta **839:** 240–248
4. **Gerhardt R, Heldt HW** (1984) Measurement of subcellular metabolite levels in leaves by fractionation of freeze-stopped material in nonaqueous media. Plant Physiol **75:** 542–547
5. **Gerhardt R, Stitt M, Heldt HW** (1987) Subcellular metabolite levels in spinach leaves. Regulation of sucrose synthesis during diurnal alterations in photosynthetic partitioning. Plant Physiol **83:** 399–407
6. **Hoagland DR, Arnon DI** (1938) The water culture method for growing plants without soil. Univ Calif Agric Exp Stan Circ 347, Berkeley
7. **Leegood RC, Furbank RT** (1986) Stimulation of photosynthesis by 2% O_2 at low temperatures is restored by phosphate. Planta **168:** 84–93
8. **McVetty PBE, Canvin DT** (1981) Inhibition of photosynthesis by low oxygen concentrations. Can J For Res **59:** 721–725
9. **Preiss J, Robinson N, Spilatro S, McNamara K** (1985) Starch synthesis and its regulation. In RI Heath, J Preiss, eds, Regulation of Carbon Partitioning in Photosynthetic Tissue. American Society of Plant Physiologists, Rockville, MD, pp 1–26
10. **Seemann JR, Sharkey TD** (1986) Salinity and nitrogen effects on photosynthesis, ribulose-1,5-bisphosphate carboxylase and metabolite pool sizes in *Phaseolus vulgaris* L. Plant Physiol **82:** 555–560
11. **Sharkey TD** (1985) O_2-insensitive photosynthesis in C_3 plants. Its occurrence and a possible explanation. Plant Physiol **78:** 71–75
12. **Sharkey TD** (1989) Feedback limitation of photosynthesis and the physiological role of ribulose bisphosphate carboxylase carbamylation. Bot Mag Tokyo (in press)
13. **Sharkey TD, Berry JA, Raschke K** (1985) Starch and sucrose synthesis in *Phaseolus vulgaris* as affected by light, CO_2, and abscisic acid. Plant Physiol **77:** 617–620
14. **Sharkey TD, Kobza J, Seemann JR, Brown RH** (1988) Reduced cytosolic fructose-1,6-bisphosphatase activity leads to loss of O_2 sensitivity in a *Flaveria linearis* mutant. Plant Physiol **86:** 667–671
15. **Sharkey TD, Stitt M, Heineke D, Gerhardt R, Raschke K, Heldt HW** (1986) Limitation of photosynthesis by carbon metabolism. II O_2 insensitive CO_2 uptake results from limitation of triose phosphate utilization. Plant Physiol **81:** 1123–1129
16. **Vassey TL, Sharkey TD** (1989) Mild water stress of *Phaseolus vulgaris* plants leads to reduced starch synthesis and extractable sucrose phosphate synthase activity. Plant Physiol **89:** 1066–1070
17. **Viil J, Laisk A, Oja V, Parnik T** (1977) Enhancement of photosynthesis caused by oxygen under saturating irradiance and high CO_2 concentrations. Photosynthetica **11:** 251–259