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Leaf Phosphate Status, Photosynthesis, and Carbon Partitioning in Sugar Beet

I. Changes in Growth, Gas Exchange, and Calvin Cycle Enzymes

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ABSTRACT

Sugar beets (*Beta vulgaris* L. cv F58-554H1) were cultured hydroponically for 2 weeks in growth chambers with two levels of orthophosphate (Pi) supplied in half strength Hoagland solution. Low-P plants were supplied with 1/20th of the Pi supplied to control plants. With low-P treatment, the acid soluble leaf phosphate and total leaf P decreased by about 88%. Low-P treatment had a much greater effect on leaf area than on photosynthesis. Low-P decreased total leaf area by 76%, dry weight per plant by 60%, and the rate of photosynthesis per area at light saturation by 35%. Low-P treatment significantly decreased the total extractable activity of phosphoglycerate kinase (by 18%) and NADP-glyceraldehyde-3-phosphate dehydrogenase (by 16%), but did not decrease the total activities of ribulose-1,5-bisphosphate (RuBP) carboxylase (RuBPCase) and ribulose-5-phosphate kinase. Low-P treatment decreased the initial activities of three rate-limiting Calvin cycle enzymes, but had no effect on the initial activity of RuBPCase. Furthermore, low-P treatment significantly increased the total extractable activities of fructose-1,6-bisphosphatase (by 61%), fructose-1,6-bisphosphate aldolase (by 53%), and transketolase (by 46%). The results suggest that low-P treatment affected photosynthetic rate through an effect on RuBP regeneration rather than through RuBPCase activity and that the changes in Calvin cycle enzymes with low-P resulted in an increased flow of carbon to starch.

Several studies in recent years have indicated that the level of Pi in leaves may regulate photosynthesis and carbon partitioning. Leaf Pi concentration is believed to influence the photosynthetic rate by way of the operation of the Pi-translocator, an antiporter located in the inner membrane of the chloroplast envelope, which facilitates the counter-exchange of Pi, triose phosphate,[1] and PGA (9, 12, 16, 29). The Pi-translocator permits the transport of triose phosphate from the stroma to the cytosol in a one-to-one stoichiometric exchange for Pi (9, 12). Since one molecule of Pi must be made available for incorporation into triose phosphate for every three molecules of CO_2 fixed, Pi must be generated in large amounts to maintain carbon fixation. Much of this Pi derives from the synthesis of sucrose from triose phosphate, *i.e.* Pi is released due to the action of cytosolic FBPase and sucrose-P phosphatase and pyrophosphatase after UDPG formation. Also, some Pi will be released within the stroma as triose phosphate is utilized in starch synthesis, but starch synthesis is usually slower (by a factor of 3 to 4) than the maximal rate of CO_2 fixation (12).

Since Pi, triose phosphate, and PGA are exchanged through the Pi translocator, changes in the Pi concentration outside the chloroplast may affect the Calvin cycle by changing the levels of phosphorylated intermediates within the chloroplast (29). In addition, Pi may also affect Calvin cycle enzymes through their level of activation. Heldt *et al.* (13) indicated that Pi is a prerequisite for carbamylation of RuBPCase. The activation of FBPase and of SBPase is strongly inhibited by Pi concentrations in the range of 5 to 10 mM Pi. Another Calvin cycle enzyme, the light-activated form of Ru5P kinase, is inhibited by the monovalent ionic species of Pi (see ref. 16 for review). The decrease in the concentration of stromal Pi that occurs upon illumination (16) is therefore likely to enhance the activity of the cycle.

The view that Pi is an important regulator of the rate of photosynthesis and of the partitioning of triose phosphates between starch biosynthesis and sucrose biosynthesis is to a large extent based on research carried out with *in vitro* systems. Much of the earlier research involved the use of isolated chloroplasts, enzyme systems, protoplasts, and detached leaves or leaf discs fed with mannose to induce P deprivation (16).

More recently, research has been carried out with P-deficient plants (1, 6–8, 10, 19, 20–22). In this approach, the changes in the physiology and biochemistry of leaves were monitored in response to nutritionally induced variation in leaf Pi status. In these studies, low leaf phosphate decreased photosynthesis and/or increased the flux of carbon toward starch synthesis, changes that are in some respects consistent with the *in vitro* studies. However, the physiological and biochemical basis of these nutritional effects of low leaf Pi on photosynthesis and carbon partitioning have not been fully

[1] Abbreviations: triose phosphate, dihydroxyacetone phosphate + glyceraldehyde-3-phosphate; ADPG, adenosine 5′-diphosphoglucose; C_i, internal partial pressure of CO_2; FBP, fructose 1,6-bisphosphate; G3P, glyceraldehyde-3-phosphate; PEP, phospho*enol*pyruvate; PGA, 3-phosphoglycerate; P_{max}, photosynthetic CO_2 uptake measured at light and CO_2 saturation; Ru5P, ribulose-5-phosphate; RuBP, ribulose 1,5-bisphosphate; RuBPCase, ribulose 1,5-bisphosphate carboxylase/oxygenase; SBP, sedoheptulose 1,7-bisphosphate; UDPG, uridine 5′-diphosphoglucose.

evaluated. The objective of this series of papers is to provide such an evaluation. In this paper, we show that low phosphate effects on photosynthesis were not mediated through changes in the activity of RuBPCase but were associated with significant changes in the activities of six other Calvin cycle enzymes.

MATERIALS AND METHODS

Plant Culture

Sugar beets (*Beta vulgaris* L. cv F58-554H1) were cultured hydroponically in growth chambers at 25°C, 500 μmol·m^{-2}·s^{-1} PFD (400–700 nm) and a 16 h photoperiod (20). Sugar beet seedlings were grown for 2 weeks after sowing in sand with half-strength Hoagland solution. They were then transplanted (24 plants per 15 L container) into a culture solution containing (mM): 2.5 $Ca(NO_3)_2$, 1.0 KH_2PO_4, 3.0 KNO_3, 1.0 $MgSO_4$, and 0.5 NaCl, and (μM) 23.1 H_2BO_3, 4.6 $MnCl_2$, 0.38 $ZnSO_4$, 0.16 $CuSO_4$, 0.052 H_2MoO_4, and 44.8 $FeSO_4$ (as ferric-sodium EDTA complex). After 1 week, the plants were transferred to solutions with the same composition except for KH_2PO_4, which was replaced with different concentrations of $Ca(H_2PO_4)_2$. The low phosphate treatment (hereafter referred to as 'low-P treatment') was obtained by growing the plants at a Pi concentration of 0.05 compared to 1.0 mM for the control (20). The pH was maintained at about 6.0 by addition of solid $CaCO_3$. The containers were topped up with deionized water as required and were aerated continuously. Plants were grown for 2 weeks and the measurements were carried out using recently expanded leaves.

Leaf Gas Exchange

The rate of photosynthetic CO_2 uptake per unit leaf area, leaf conductance and the internal partial pressure of CO_2 (C_i, Pa) of individual attached leaves were determined over a range of ambient CO_2 concentrations using open flow gas exchange as described previously (25). The measurements were made by exposing the leaf initially to an ambient CO_2 concentration of about 100 Pa for 1 h at 21 kPa O_2 and subsequently lowering the ambient CO_2 concentration to successive levels with 30-min periods at each ambient CO_2 level. Leaf temperature was maintained at 25 ± 0.5°C and RH was 60%. PFD was held either at a constant level or increased gradually to achieve light saturation at each CO_2 level.

Enzyme Assays

RuBPCase (EC 4.1.1.39) was assayed according to Salvucci and Anderson (24) with some modifications. Leaves were illuminated for 1 h at the growth chamber PFD. One leaf disc (3.88 cm^2) was removed directly from the attached leaf using a leaf punch machine and frozen in liquid N_2. The frozen leaf disc was placed in 2 mL of ice-cold extraction medium (100 mM Tricine-NaOH [pH 8.0], 10 mM $MgCl_2$, 1 mM EDTA, 10 mM DTT, 10 mM Na-ascorbate, 0.5% BSA, and 1% w/v PVP-40 at 0°C) and ground in a prechilled mortar and pestle. The resulting homogenate was used for the assay of initial and total activity. The initial RuBPCase activity was determined by adding 25 μL of extract to 450 μL of the assay medium (200 mM Tricine-NaOH [pH 8.0], 20 mM $MgCl_2$, 1 mM DTT, 20 mM $NaH^{14}CO_3$, 1.9×10^{10} Bq/mol) to which 25 μL of 15 mM RuBP had been added. The reaction was run at 25°C for 1 min, then stopped with 0.1 mL of 6 N acetic acid. Total inducible activity was determined by adding 25 μL of the extract to 450 μL of the assay solution (without added RuBP), incubating at 25°C for 10 min to fully activate the RuBPCase with CO_2 and Mg^{2+}, then adding 25 μL of 15 mM RuBP to initiate the reaction. Acid stable ^{14}C was determined by liquid scintillation counting.

For the assay of all other enzymes of the Calvin cycle, crude homogenates from leaf discs frozen in liquid N_2 at 1 h after illumination at the growth chamber PFD were prepared by grinding (in a prechilled mortar and pestle with liquid N_2) one leaf disc (3.88 cm^2) with 2 mL of extraction buffer (100 mM Hepes-NaOH [pH 8.0], 10 mM $MgCl_2$, 0.4 mM EDTA, 1% PVP, 100 mM Na-ascorbate, 0.1% BSA at 0–4°C). For the determination of total extractable activity of the enzymes, 50 mM DTT was included in the extraction medium. Initial activity was determined in the absence of DTT both in the extraction and assay medium. The extract was spun in a microfuge (Eppendorf: model 5414) for 3 min (0–2°C) and the supernatant retained for enzyme assays.

Enzyme activities were determined spectrophotometrically using a DW-2C spectrophotometer (SLM.AMINCO, Urbana, IL), according to the referenced procedures, with some modifications. The compositions of the assay media for the respective enzymes are as follows. Ru5P kinase (EC 2.7.1.19) (14): 30 mM Hepes-NaOH (pH 8.0), 10 mM $MgCl_2$, 5 mM DTT, 2 mM ATP, 2 mM PEP, 0.4 mM Ru5P, 0.3 mM NADH, 2 units per mL of lactic dehydrogenase (EC 1.1.1.27), and pyruvate kinase (EC 2.7.1.40); the reaction was initiated by adding leaf extract. PGA kinase (EC 2.7.2.3) (11): 30 mM Hepes-KOH (pH 7.8), 5 mM $MgCl_2$, 1 mM NaF, 1 mM KH_2PO_4, 5 mM DTT, 2 mM ATP, 4 mM PGA, 0.3 mM NADH, and 4 units per mL of NAD-G3P dehydrogenase (EC 1.2.1.12) and triose phosphate isomerase (EC 5.3.1.1); the reaction was initiated by adding ATP. NADP-G3P dehydrogenase (EC 1.2.1.13) (15): 30 mM Hepes-KOH (pH 8.0), 4 mM PGA, 5 mM ATP, 10 mM $MgCl_2$, 0.6 mM NADPH, 1 mM NaF, 1 mM KH_2PO_4, 5 mM DTT, 20 units per mL of PGA kinase, and 10 units per mL of triose phosphate isomerase; the reaction was initiated by adding ATP. FBP aldolase (EC 4.1.2.7) (23): 30 mM Hepes-KOH (pH 7.6), 10 mM FBP, 0.25 mM NADH, and 2 to 4 units per mL each of α-glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) and triose phosphate isomerase; the reaction was initiated by adding FBP. FBPase (EC 3.1.3.11) (14): 30 mM Hepes-KOH (pH 8.2), 5 mM $MgCl_2$, 5 mM DTT, 0.5 mM NADP, 5 mM FBP, and 2 to 4 units per mL each of glucose-6-P dehydrogenase (EC 1.1.1.49) and phosphoglucose isomerase (EC 5.3.1.9); reaction was initiated by addition of FBP, and the rate was taken 10 to 15 min after start of assay. SBPase (EC 3.1.3.37) (30): 30 mM Hepes-KOH (pH 8.2), 10 mM $MgCl_2$, 20 mM KCl, 0.1 mM ATP, 1 mM PEP, 0.2 mM NADH, 5 mM DTT, 0.1 mM SBP, 6 units each pr mL of pyruvate kinase and lactic dehydrogenase, and 2 units of fructose-6-phosphate kinase (EC 2.7.1.11); reaction

was initiated by addition of leaf extract. Triose phosphate isomerase (EC 5.3.1.1) (2): 30 mM Hepes-NaOH (pH 7.8), 0.25 mM NADH, 2 to 4 units per mL of α-glycerol 3-P dehydrogenase (EC 1.1.1.8), and 0.1 mM G3P; reaction was initiated by addition of G3P. Transketolase (EC 2.2.1.1) (3): 30 mM Hepes-KOH (pH 7.9), 0.25 mM NADH, 3 mM $MgCl_2$, 0.5 mM xylulose 5-P, 0.5 mM ribose 5-P, 0.12 μM cocarboxylase, and 2 to 4 units per mL each of triose phosphate isomerase and α-glycerol-3-phosphate dehydrogenase; reaction was initiated by the addition of leaf extract.

The percent activation of Calvin cycle enzymes was determined from the ratio of initial activity to total inducible activity × 100.

Leaf Area and Dry Weights

Leaf area measurements were made using a Decagon leaf area meter. The plants were harvested 5 weeks after planting and were separated into leaf blades, petioles, storage root, and fibrous roots. The tissue was dried at 70°C to constant weight.

Leaf P, Chl, and Soluble Protein

Acid soluble and total leaf P was estimated as described in Fredeen *et al.* (10). Leaf Chl content was determined in 80% acetone as described in Abadia *et al.* (1). Soluble leaf protein was determined by the method of Bradford (5).

Chemicals

All compounds used were purchased from Sigma Chemical Co. with the exception of $NaH^{14}CO_3$ (Amersham).

RESULTS AND DISCUSSION

Effects of Low-P Treatment on Plant Growth and Leaf Gas Exchange

Low-P treatment had a greater impact on plant biomass than on the rate of photosynthesis/area. When plants were supplied with 1/20 of the amount of Pi supplied to control plants (the 'low-P' treatment), the acid soluble leaf P and total leaf P decreased by about 89% in a period of 2 weeks (Table I). This reduction in leaf P correlated with a 60% reduction in total plant dry weight while the rate of photosynthesis/area (at ambient or saturating CO_2 levels) decreased 35% at light saturation (Table I) and much less at lower light levels (see below). Similar observations showing that low-P treatment had a much greater effect on plant growth than on photosynthesis have been made for other plant species (for review see ref. 4).

The effect of low-P on growth was mediated by an effect on leaf expansion which decreased 76% (Table I). This effect occurred through an effect on the rate of expansion of individual leaves, not on the rate of leaf emergence (data not shown). Radin and Eidenbock (18, 19) suggest that the decline in leaf elongation rate with P deficiency is a consequence of decreased hydraulic conductance leading to reduced water transport and therefore turgor necessary for leaf cell enlargement. Another possibility is that the effect of low-P treatment on leaf expansion may be mediated by growth hormones (17).

Changes in leaf expansion with leaf phosphate status have been shown to be correlated with changes in leaf epidermal cell area (18). Since leaf epidermal cell expansion is apparently a critical process controlling the expansion of the sugar beet leaf blade (28), and since low-P leads to dramatic decreases in Pi concentrations in the upper epidermis (27), the effect of low-P on leaf expansion may also have been mediated through the supply of Pi to the epidermal cells.

Another interesting difference between low-P and control plants is that low-P treatment significantly decreased the accumulation of dry matter in leaf blades, petioles, and storage roots ($P < 0.05$, Fig. 1) while the dry matter accumulation in fibrous roots was not significantly affected (Fig. 1). Low-P treatment decreased the shoot/root ratio by 37%. This is in accord with the findings of other researchers who have shown that low-P may increase fibrous root growth in relation to the growth of other plant parts (4, 17).

Low-P treatment also changed the appearance and morphology of sugar beet plants. Compared to the controls, low-P leaves were smaller, thicker, darker-green with a glossy upper leaf surface and shorter petioles; low-P storage roots were smaller and fibrous roots were longer. Soluble leaf protein did not increase significantly while Chl increased by 23% ($P < 0.05$) and dry weight/area by 30% ($P < 0.05$) (Table I). Despite the significant increase in leaf dry weight/area, respiration/area did not increase (Table I). The increase in weight per area with low-P treatment was most likely the result of increases in cell wall material as well as storage carbohydrates, especially starch.

Low-P treatment affected photosynthesis much less at low than high PFD (Fig. 2A) Photosynthesis at very low PFD, such as that used to measure quantum yield, was virtually unaffected by low-P (1). Because of the higher Chl content per area in low-P leaves, low-P treatment caused a greater reduction in photosynthesis/Chl than in photosynthesis/area (*i.e.* −47% compared to −35%, respectively ($P < 0.05$), Table I). This effect may have been due partly to increased amounts of antenna Chl per reaction center in low-P leaves (1).

Low-P treatment decreased conductance in high light by 50% ($P < 0.05$) and in low light by 81% ($P < 0.05$) (Fig. 2B; see also ref. 26). This resulted in slightly lower intercellular CO_2 partial pressures in low-P leaves at each PFD level (Fig. 2C). The rate of photosynthesis declined significantly with low-P ($P < 0.05$) at all values of intercellular CO_2 partial pressure (Fig. 3). These results indicate that the major effect of low-P treatment on photosynthesis was on the enzymic reactions occurring within the chloroplast. This observation is consistent with the findings of Brooks (6) with spinach.

Brooks *et al.* (6, 7) concluded that P deficiency diminishes photosynthesis by decreasing RuBP regeneration. Abadia *et al.* (1) found that low-P treatment had relatively small effects on thylakoid composition and photosynthetic electron transport, and even smaller effects on photosynthetic quantum yield. We conclude therefore, as did Brooks *et al.* (6, 7), that the RuBP regeneration capacity of low-P leaves was not limited by photochemical capacity.

Effects of Low-P Treatment on the Activities of Calvin Cycle Enzymes

Low-P treatment resulted in significant increases in the activities of some enzymes while significantly decreasing oth-

Table I. *Influence of Low-P Treatment on Certain Gas Exchange and Growth Characteristics of 5-Week-Old Sugar Beet Plants*

Low-P plants were supplied with 1/20th of the Pi supplied to the control plants for 2 weeks. Values are mean ± SD for at least 3 replications.

Characteristics (units)	Treatment		Percent of control
	Control	Low-P	
Photosynthesis/area (μmol CO_2 m^{-2} s^{-1})	30.5 ± 0.1	19.9 ± 1.9	65
Photosynthesis/Chl (nmol CO_2 mg^{-1} Chl s^{-1})	64.2 ± 0.2	33.9 ± 3.2	53
P_{max}/area (μmol CO_2 m^{-2} s^{-1})	47.3 ± 0.6	31.4 ± 1.4	66
P_{max}/Chl (nmol CO_2 mg^{-1} Chl s^{-1})	99.7 ± 1.4	53.4 ± 2.5	54
Respiration (μmol CO_2 m^{-2} s^{-1})	−1.6 ± 0.1	−1.4 ± 0.1	87
Leaf Chl (mg m^{-2})	427.5 ± 43.9	528.3 ± 63.1	123
Leaf protein (mg cm^{-2})	0.7 ± 0.1	0.8 ± 0.1	114
Leaf Pi (mmol m^{-2})	8.9 ± 0.6	1.0 ± 0.2	11
Total leaf P (mmol m^{-2})	12.4 ± 0.6	1.4 ± 0.1	11
Leaf area (dm^2 $plant^{-1}$)	21.7 ± 3.2	5.3 ± 0.9	24
Specific leaf dry weight (mg cm^{-2})	3.6 ± 0.2	4.7 ± 0.5	130
Total dry matter (g $plant^{-1}$)	17.2 ± 4.8	6.9 ± 2.2	40

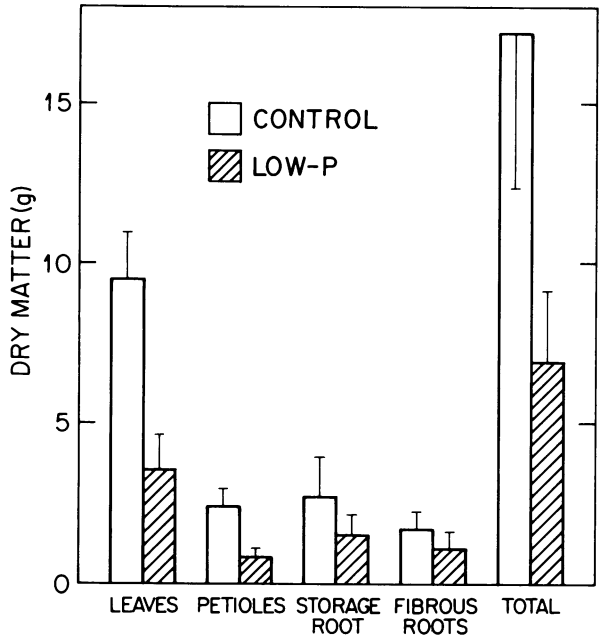

Figure 1. Influence of low-P treatment on the dry matter distribution between plant parts of 5-week-old sugar beet plants. Low-P plants were supplied with 1/20th of the Pi supplied to the control plants for 2 weeks. Values are mean ± SD of 12 plants.

ers. Low-P treatment significantly increased the total activity of FBPase by 61%, FBP aldolase by 53%, transketolase by 46% ($P < 0.05$), while the increase in SBPase (24%) and triose phosphate isomerase (20%) were not significant (Table II). Calvin cycle enzymes significantly decreased by low-P ($P < 0.05$) included PGA kinase (−18%) and NADP-G3P dehydrogenase (−16%) (Table II). Low P treatment, however, had no significant effects on the total activities of Ru5P kinase and RuBPCase (Table II).

These results show that low-P treatment not only affected the total activities of most Calvin cycle enzymes but also significantly affected the initial activities of three potentially

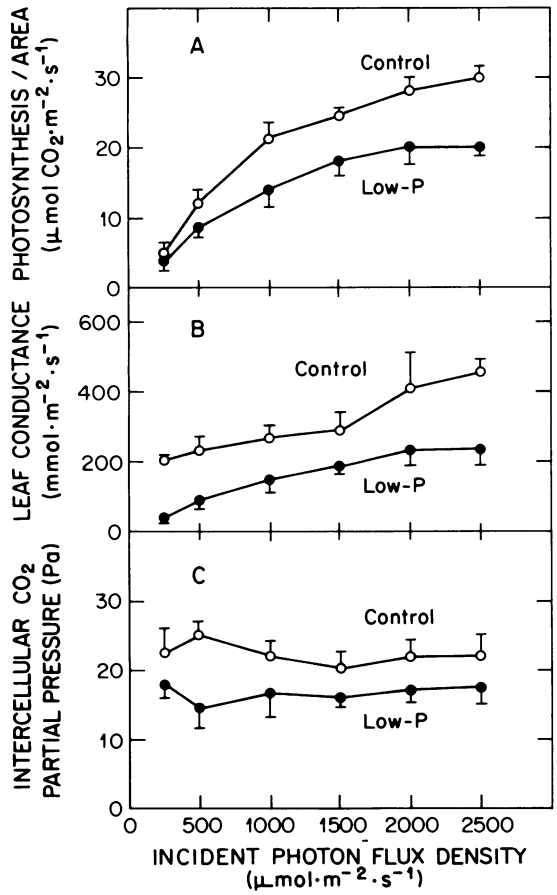

Figure 2. Influence of low-P treatment on the relationship with PFD for the rate of photosynthesis (A), leaf conductance (B), and intercellular CO_2 partial pressure (C). Photosynthesis was measured as leaf CO_2 uptake at 25°C, air levels of CO_2 (30 Pa) and O_2 (21 kPa). Low-P plants were supplied with 1/20th of the Pi supplied to the control plants for 2 weeks. Values are mean ± SD of three experiments. (○), control; (●), low-P.

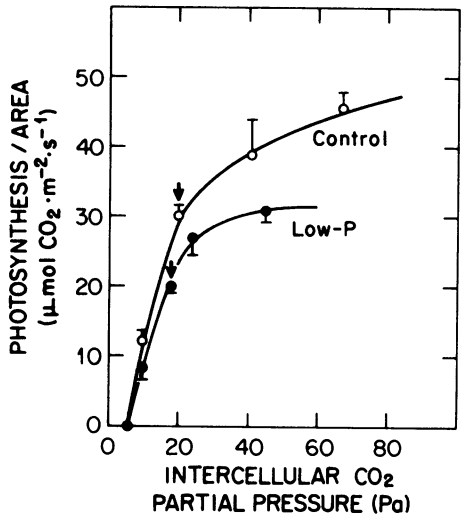

Figure 3. Influence of low-P treatment on the relationship of photosynthesis/area to intercellular CO_2 partial pressure at air levels of O_2 (21 kPa). PFD of 2500 μmol·m^{-2}·s^{-1} was used. (↓), Measurement at 30 Pa of external CO_2 concentration. Low-P plants were supplied with 1/20th of the Pi supplied to the control plants for 2 weeks. Values are mean ± SD of three experiments. (○), control; (●), low-P.

rate-limiting enzymes ('initial activity' is assumed to represent *in vivo* activity). When leaves were illuminated at growth chamber PFD (500 μmol·m^{-2}·s^{-1}), the initial activities of NADP-G3P dehydrogenase, PGA kinase, and Ru5P kinase were significantly decreased by 77, 32, and 31%, respectively ($P < 0.05$), compared to the control values while the initial activity of RuBPCase was not significantly affected (Table II). This report is the first to provide percent activation (initial activity/fully induced activity × 100) for all the potentially regulatory enzymes of the Calvin cycle in intact leaves for any plant species (Table II). Low-P treatment significantly decreased the percent activation of NADP-G3P dehydrogenase and Ru5P kinase ($P < 0.05$).

Low-P treatment had no significant effect on the percent activation of SBPase. In an earlier study, however, low-P treatment reduced both total and initial activities of SBPase (21). This discrepancy may have been due to differences in technique. Our current procedure is to freeze leaf discs in liquid N_2 and then rapidly extract and assay the Calvin cycle enzymes. This technique gives higher rates of enzyme activity compared to our preliminary study (21).

Brooks *et al.* (6, 7) concluded that low-P effects on photosynthesis could be mediated by RuBP regeneration and/or by RuBPCase activation. Since low-P treatment had no significant effects on RuBPCase activation, the decrease in the rate of photosynthesis was almost certainly due to a decrease in RuBP regeneration. Earlier work showed that low-P treatment substantially decreased RuBP levels in spinach (6, 7) and sugar beet (21). The decrease in RuBP regeneration could have resulted from diminished initial activities of PGA kinase, NADP-G3P dehydrogenase, and Ru5P kinase. Alternatively, however, a greater proportion of photosynthetic carbon may have been diverted from RuBP formation to starch formation. The increased activities of FBP aldolase and FBPase together with an increase in the activity of ADPG pyrophosphorylase (22) suggests that the capacity for starch synthesis increased in low-P leaves.

The increases in both FBPase and SBPase activities associated with low-P are in accord with the observation that the activities of the phosphatases increase in response to P deficiency (4). The increase in initial and total activities of FBPase

Table II. *Effect of Low-P Treatment on the Initial and Total Activities of Certain Calvin Cycle Enzymes from Leaves of 5-Week-Old Sugar Beet Plants*

Plants were dark adapted for 8 h prior to illumination for 1 h in the growth chamber at 500 μmol·m^{-2}·s^{-1} PFD. Percent activation was calculated from the ratio of initial activity to fully inducible total activity. Low-P plants were supplied with 1/20th of the Pi supplied to the control plants for 2 weeks. Values are mean ± SD for at least 3 replications.

Enzyme	Activity	Treatment			
		Control		Low-P	
		μmol m^{-2} s^{-1}	% activation	μmol m^{-2} s^{-1}	% activation
RuBPCase	Initial	123.6 ± 14.6	82	114.2 ± 9.0	73
	Total	150.9 ± 23.4		156.5 ± 27.7	
PGA kinase	Initial	228.5 ± 37.9	78	155.4 ± 2.9	65
	Total	292.7 ± 48.0		240.4 ± 11.7	
NADP-G3P dehydrogenase	Initial	56.6 ± 18.5	34	13.3 ± 8.5	10
	Total	164.4 ± 25.3		138.1 ± 10.0	
FBPase	Initial	3.2 ± 0.8	33	6.2 ± 0.7	39
	Total	9.7 ± 2.1		15.7 ± 0.6	
SBPase	Initial	8.1 ± 0.6	62	10.1 ± 1.2	82
	Total	9.9 ± 1.5		12.3 ± 0.1	
Ru5P kinase	Initial	73.9 ± 49.2	34	50.8 ± 24.9	23
	Total	216.6 ± 24.9		218.6 ± 19.0	
Triose-P isomerase	Total	665.8 ± 62.2		801.2 ± 110.4	
FBP aldolase	Total	41.7 ± 1.7		63.8 ± 9.5	
Transketolase	Total	21.0 ± 2.1		30.7 ± 0.8	

and SBPase may form part of an adaptive mechanism to enable plants to survive periods of P stress. Such an adaptive strategy would have the effect of increasing the availability of Pi for photosynthesis (and other important leaf functions) at times when the phosphate supplies were low.

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