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Dependence of Photosynthesis of Sunflower and Maize Leaves on Phosphate Supply, Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Activity, and Ribulose-1,5-Bisphosphate Pool Size

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ABSTRACT

Sunflower (*Helianthus annuus* L. cv Asmer) and maize (*Zea mays* L. cv Eta) plants were grown under controlled environmental conditions with a nutrient solution containing 0, 0.5, or 10 millimolar inorganic phosphate. Phosphate-deficient leaves had lower photosynthetic rates at ambient and saturating CO_2 and much smaller carboxylation efficiencies than those of plants grown with ample phosphate. In addition, phosphate-deficient leaves contained smaller quantities of total soluble proteins and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) per unit area, although the relative proportions of these components remained unchanged. The specific activity of Rubisco (estimated in the crude extracts of leaves) was significantly reduced by phosphate deficiency in sunflower but not in maize. Thus, there was a strong dependence of carboxylation efficiency and CO_2-saturated photosynthetic rate on Rubisco activity only in sunflower. Phosphate deficiency decreased the 3-phosphoglycerate and ribulose-1,5-bisphosphate (RuBP) contents of the leaf in both species. The ratio of 3-phosphoglycerate to RuBP decreased in sunflower but increased in maize with phosphate deficiency. The calculated concentrations of RuBP and RuBP-binding sites in the chloroplast stroma decreased markedly with phosphate deficiency. The ratio of the stromal concentration of RuBP to that of RuBP-binding sites decreased in sunflower but was not affected in maize with phosphate deficiency. We suggest that a decrease in this ratio made the RuBP-binding sites more vulnerable to blockage or inactivation by tight-binding metabolites/inhibitors, causing a decrease in the initial specific activity of Rubisco in the crude extract from phosphate-deficient sunflower leaves. However, the decrease in Rubisco specific activity was much less than the decrease in the RuBP content in the leaf and its concentration in the stroma. A large ratio of RuBP to RuBP-binding sites may have maintained the Rubisco-specific activity in phosphate-deficient maize leaves. We conclude that the effect of phosphate deficiency is more on RuBP regeneration than on Rubisco activity in both sunflower and maize.

In a previous study (7), we showed that the decrease in A[1] by leaves of phosphate-deficient sunflower, maize, and wheat plants was due to inhibition of nonstomatal processes rather than to stomatal factors despite a decrease in stomatal conductance. The carboxylation efficiency and the apparent quantum yield for CO_2 assimilation were two parameters strongly affected by phosphate deficiency, leading to an increase in the relative mesophyll limitation of photosynthetic rate. Similar effects on carboxylation efficiency and quantum yield in phosphate-deficient leaves of spinach, soybean, and sugar beet have been observed in other studies (4, 11, 19).

Under conditions of a nonlimiting supply of RuBP, the amount and specific activity of Rubisco determine carboxylation efficiency (24), defined as the initial slope of the curve relating A to C_i, *i.e.* dA/dC_i when $A = 0$ (10). Phosphate-deficient leaves produce smaller amounts of RuBP and Rubisco (4, 19), and the Rubisco has a lower specific activity (11) than do leaves grown with adequate phosphate. Low specific activity may be a direct result of phosphate deficiency because Pi ions are known to activate many Calvin cycle enzymes. For example, Parry *et al.* (16) showed that Pi is required for the full activation of Rubisco *in vitro,* probably by facilitating carbamylation of Rubisco (6). However, Rao *et al.* (18) reported that the effect of phosphate deficiency on photosynthetic rate acted through RuBP regeneration rather than Rubisco activity. Assessment of the regulation of photosynthesis in phosphate-deficient leaves, therefore, requires understanding of the carboxylation reaction and the autocatalytic Calvin cycle.

Both ATP and NADPH, which are products of the light-driven reactions of the thylakoids and are required by different reactions of the Calvin cycle, affect the synthesis of RuBP and thus influence the rate of carboxylation as do the amount and activity of Rubisco. In the present investigation, we analyzed the relative importance of Rubisco activity and RuBP regeneration as causes of the reduced carboxylation efficiency of sunflower and maize plants grown under continuous phosphate deficiency.

MATERIALS AND METHODS

Sunflower (*Helianthus annuus* L. cv Asmer) and maize (*Zea mays* L. cv Eta) were grown under controlled environmental conditions in plastic pots containing washed sand, as previously described (7). After germination, the plants were irrigated daily with modified nutrient solution that contained

[1] Abbreviations: A, net CO_2 assimilation rate; RuBP, ribulose-1,5-bisphosphate; C_i, leaf internal CO_2 partial pressure; A_{max}, CO_2-saturated photosynthetic rate; 3PGA, 3-phosphoglycerate.

0, 0.5, or 10 mM Pi. The flux of photosynthetically active radiation (400–700 nm) was 350 to 400 μmol quanta m^{-2} s^{-1} for 16 h a day, RH 75 to 85%, and air temperature 22°C day and 20°C night. Measurements were made on the third, fully expanded leaf.

Gas Exchange

A, by intact leaves, was determined as described by Lawlor *et al.* (12) using an open gas exchange system at saturating light intensity (1200 μmol quanta m^{-2} s^{-1}). The response of *A* to C_i was measured and an asymptotic function fitted because it gave high R^2 values (7). There was no evidence of "stomatal patchiness" in leaves of sunflower (7) or soybean (11) plants grown with different levels of phosphate nutrition. Therefore, carboxylation efficiency (10) was calculated by taking the first order differential dA/dC_i at $A = 0$. A_{max} was calculated from the $A \cdot C_i$ response function.

Protein and Rubisco Assay

After the gas exchange rates were measured at an ambient CO_2 concentration of 35 Pa, the leaves (which had been illuminated for at least 2 h) were immediately freeze clamped (12). The Rubisco was extracted at 0 to 4°C from a known area of leaf in 4 mL of a buffer (100 mM Bicine-NaOH [pH 8.0], 20 mM $MgCl_2$, 1 mM EDTA [sodium salt], 50 mM mercaptoethanol, two to three drops 40 mM PMSF, and 0.02 g acid-washed PVP) and assayed according to the method of Joseph *et al.* (8). The initial specific activity of Rubisco was determined immediately from the amount of ^{14}C fixed into acid-stable products as described by Lorimer *et al.* (14). The reaction was carried out for 45 s in a stoppered O_2 electrode vessel (Hansatech Ltd, Kings Lynn, U.K.) containing the assay medium (100 mM Bicine, 20 mM $MgCl_2$, 0.33 mM RuBP, and 10 mM $NaH^{14}CO_3$ [1.85 MBq mL^{-1}]), at 25°C. At the end of 30 and 45 s, 100 μL of the reaction mixture was taken and rapidly injected into a scintillation vial containing 300 μL 4 N HCl. To measure the total carboxylase activity of Rubisco, the extract was preincubated in the assay medium containing no RuBP but with or without 10 mM Pi before adding RuBP to start the reaction (16). The preincubation was done at two different temperatures (25 and 40°C) and for two different durations (4 or 30 min) with the objective of activating Rubisco by facilitating the carbamylation of the RuBP-binding sites before actually starting the assay. An aliquot of the crude extract was used to determine both the total soluble proteins (3) and the amount of Rubisco, the latter by separating the subunits on a 15% SDS-PAGE based on the modified method of Servaites *et al.* (21). Freeze-dried purified Rubisco from sunflower and maize leaves was used as standards.

Determination of RuBP and 3PGA

RuBP and 3PGA were extracted in 3% (v/v) perchloric acid from leaf samples freeze clamped immediately after gas exchange measurements. The amount of RuBP was determined by ^{14}C incorporation into acid-stable products using purified Rubisco and $H^{14}CO_3^-$ as described by Badger *et al.* (2). The reaction was carried out in 625 μL total volume containing 500 μL assay medium (100 mM Bicine-KOH [pH 8.2], 20 mM $MgCl_2$, 14 mM $NaH^{14}CO_3$ [1.85 MBq mL^{-1}]), 25 μL purified and activated Rubisco (50 μg), and 100 μL extract. Replicated assays were run at 25°C for 1 h and terminated by adding 1 mL of 4 N HCl. The radioactivity incorporated into acid-stable products was then determined as described earlier (14). 3PGA was determined according to the method of Usuda (23). The reaction was run in a total volume of 1025 μL containing 625 μL assay medium (60 mM Hepes [pH 8.0], 20 mM $MgCl_2$, 0.2 mM NADH, 5 mM ATP, 5 mM phosphocreatine, 10 units creatine phosphokinase [EC 2.7.3.2.], 5 units NAD-glucose-3-phosphate dehydrogenase [EC 1.2.1.12], and 5 units PGA-kinase [EC 2.7.2.3.]) and 400 μL extract. The recovery of the above two metabolites was >80%. The data are not corrected for recovery.

Leaf Chl and Pi Contents

Leaf Chl was determined according to the method of Arnon (1) and Pi according to the method of Kitson and Mellon (9).

RESULTS

Gas Exchange

Leaves of plants grown without Pi had significantly smaller *A* at ambient and saturating CO_2 partial pressures than did those grown with 10 mM Pi when measured at saturating light intensity (Table I). In sunflower, *A* decreased by about 82% and A_{max} by 80% and in maize *A* was reduced by 86% and A_{max} by 83% with phosphate deficiency. Phosphate deficiency markedly decreased the carboxylation efficiency also (Table I); the reduction was 62% in sunflower and 79% in maize.

Table I. *Photosynthetic A at Ambient CO_2 (35 Pa), A_{max}, and the Carboxylation Efficiency (dA/dC_i) of Sunflower and Maize Plants Grown with 10, 0.5, or 0 mM Pi*

[Pi] in the Nutrient Solution	Sunflower			Maize		
	A	A_{max}	dA/dC_i	*A*	A_{max}	dA/dC_i
mM	μmol m^{-2} s^{-1}		μmol m^{-2} s^{-1} Pa^{-1}	μmol m^{-2} s^{-1}		μmol m^{-2} s^{-1} Pa^{-1}
10.0	28.2	41.2	2.09	23.7	26.2	7.81
0.5	15.3	26.5	1.03	14.1	15.1	4.03
0.0	5.2	8.4	0.79	3.2	4.5	1.66
$LSD_{(P=0.05)}$	3.7	4.1	0.21	2.8	2.6	0.63

Soluble Proteins and Rubisco

The [Pi] in the leaf water was calculated from simultaneous measurements of Pi and leaf water content (7). The lower the concentration of phosphate supplied in the nutrient solution, the lower the calculated [Pi] in the leaf water. Low [Pi] gave smaller amounts of total leaf soluble proteins and Rubisco (Fig.1, A and B); however, the proportion of protein as Rubisco remained relatively unchanged at 40 to 44% in sunflower and 34 to 37% in maize (Fig. 1C).

The initial specific activity of Rubisco extracted from the two species grown with three different levels of phosphate nutrition is shown in Table II. The lower the [Pi] in the nutrient solution, the lower the initial specific activity of sunflower Rubisco. However, there was no effect of Pi nutrition on the initial specific activity of Rubisco from maize leaves.

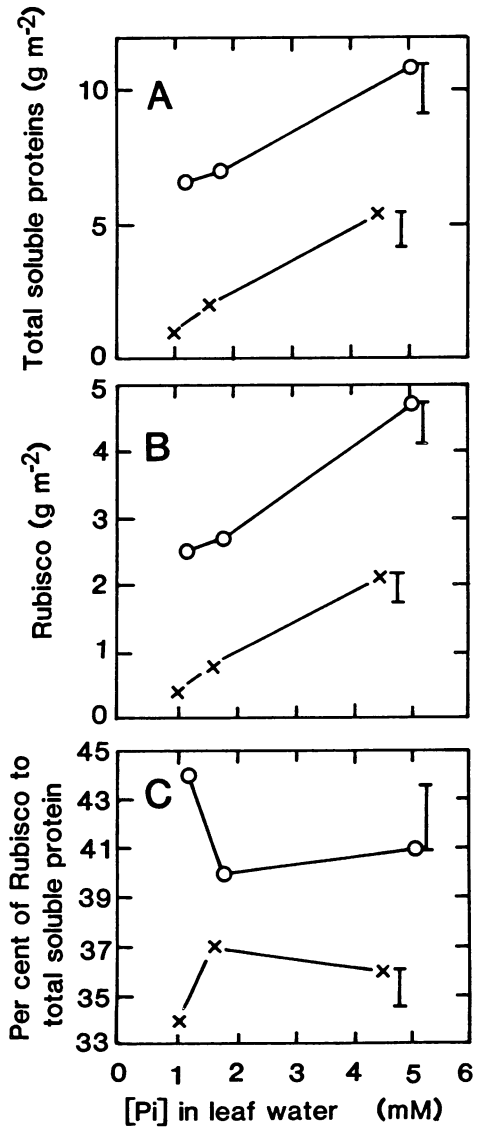

Figure 1. The relation between [Pi] in leaf water and the total soluble proteins (A) and Rubisco content (B) and the percentage of total soluble protein as Rubisco (C) in sunflower (O) and maize (X). Bars, LSD between mean values for each species at P = 0.05.

Table II. *Initial Specific Activity of Rubisco in Crude Extracts from Leaves of Sunflower and Maize Plants Grown with 10, 0.5, and 0 mM Pi and the Effect on the Total Activity of Incubating Crude Extracts with Mg^{2+} and HCO_3^- for Different Periods at 25°C*

[Pi] in the Nutrient Solution	Initial Specific Activity	Total Activity after Preincubating at 25°C for	
		20 min	40 min
mM	*μmol CO_2 mg^{-1} Rubisco min^{-1}*		
Sunflower			
10.0	1.22	1.06 (13)[a]	0.95 (22)
0.5	0.98	0.95 (3)	0.72 (27)
0.0	0.79	0.35 (56)	0.31 (61)
$LSD_{(P=0.05)}$	0.13	0.12	0.15
Maize			
10.0	1.15	1.09 (5)	1.06 (8)
0.5	1.05	0.89 (15)	0.67 (36)
0.0	1.02	0.99 (3)	0.92 (10)
$LSD_{(P=0.05)}$	NS[b]	NS	0.21

[a] Figures in parentheses indicate percentage of reduction from the initial specific activity. [b] Not significant at P = 0.05.

The effect of preincubating the crude extracts of Rubisco at 25°C with Mg^{2+} and $H^{14}CO_3$ on the total activity is also given in Table II. Preincubation for 20 or 40 min considerably reduced the total activity of Rubisco from sunflower compared with the initial specific activity. The reduction was most prominent with the phosphate-deficient treatment; after 20 min, total activity was 56% less than the initial specific activity with the phosphate-deficient treatment but only 13% less with the phosphate-sufficient treatment. Changes in total activity with time and phosphate treatment were less marked in maize.

The total activity of Rubisco extracted from sunflower decreased with the temperature of preincubation, independently of the phosphate treatment (Table III). The higher the

Table III. *Initial Specific Activity of Rubisco in Crude Extracts of Leaves from Sunflower and Maize Plants Grown with 10 or 0 mM Pi and the Effects of Temperature of Incubation of Crude Extracts with Mg^{2+} and HCO_3^- on the Total Activity*

[Pi] in the Nutrient Solution	Initial Specific Activity	Total Activity at the End of 30 min Preincubation at	
		25°C	40°C
mM	*μmol CO_2 mg^{-1} Rubisco min^{-1}*		
Sunflower			
10.0	1.61	1.27 (21)[a]	0.84 (48)
0.0	0.62	0.51 (18)	0.35 (44)
$LSD_{(P=0.05)}$	0.41	0.38	0.33
Maize			
10.0	0.99	0.96 (3)	0.91 (8)
0.0	1.00	0.99 (1)	0.99 (1)
$LSD_{(P=0.05)}$	NS[b]	NS	NS

[a] Figures in parentheses indicate percentage of reduction from the initial specific activity. [b] Not significant at P = 0.05.

temperature of preincubation, the greater the reduction in total activity in sunflower. However, total activity of Rubisco from maize did not vary with the temperature of preincubation (25 or 40°C). The amount of Rubisco in the crude extract from both species, detected after separation by SDS-PAGE, remained unchanged after preincubation at 25 or 40°C for 30 min (Table IV).

The effect of 4-min activation with 10 mM Pi in the preincubation medium on the total activity of Rubisco is given in Table V. With Pi, total activity increased by 20% in leaves of phosphate-sufficient sunflower plants, but without Pi, it increased by only 14%. In the sunflower leaves grown with low phosphate, total activity decreased after 4 min of preincubation independently of Pi. In maize, total activity of Rubisco extracted from phosphate-deficient leaves was significantly reduced, particularly when Pi was present in the incubation medium. Including Pi in the assay medium did not affect the total activity of Rubisco in phosphate-sufficient maize leaves; however, when no Pi was present in the incubation medium, total activity was significantly increased in Pi-sufficient maize leaves.

The carboxylase activity has been related to the gas exchange characteristics. In sunflower, both dA/dC_i and A_{max} were strongly correlated with the initial specific activity of Rubisco (Fig. 2A). From the initial specific activity and amount of Rubisco per unit leaf area, the expected maximum carboxylase activity of the leaf was estimated. This was always higher than the observed A_{max}, but there was clearly a positive relation between them in both sunflower and maize (Fig. 2B).

RuBP and 3PGA

There was less RuBP and 3PGA in the leaf with decreases in [Pi] in both species (Fig. 3, A and B). In sunflower leaves, 3PGA content decreased 4.7-fold and RuBP 4.3-fold, thus decreasing the ratio of 3PGA to RuBP (Fig. 3C) when [Pi] decreased from 5.1 to 1.2 mM. In maize, 3PGA decreased 2.9-fold and RuBP 4.1-fold when [Pi] decreased from 4.5 to 1 mM. This increased the 3PGA/RuBP ratio at low [Pi] in maize (Fig. 3C). The ratio was always higher in maize than in sunflower.

The concentration of RuBP-binding sites in the stroma was calculated from the amounts of Rubisco and total Chl per unit leaf area, based on the relation between volume of stroma and Chl content of 30 μL/mg Chl (2, 15) and assuming a molecular mass of 555 kD for Rubisco and eight RuBP-binding sites per molecule. The concentration of RuBP-binding sites in the stroma decreased with decreasing [Pi] in both species (Fig. 4A). Between the highest and the lowest [Pi], the stromal concentration of RuBP-binding sites decreased about 1.5-fold in sunflower and 2.3-fold in maize. Similarly, the stromal concentration of RuBP was calculated. In sunflower, RuBP concentration in the stroma decreased by 2.7-fold and in maize by 2.6-fold (Fig. 4B) between the highest and lowest [Pi]. The ratio of RuBP to RuBP-binding sites was smaller in sunflower than in maize (Fig. 4C), and this ratio decreased with Pi deficiency in the former but not in the latter.

Table IV. *Rubisco Content of Crude Extracts from Leaves of Sunflower and Maize Plants Grown with 10 or 0 mM Pi Immediately after Extraction and after Incubation with Mg^{2+} and HCO_3^- at 25°C and 40°C for 30 min*

[Pi] in the Nutrient Solution	Amount of Rubisco in the Extract		
	Immediately after extraction	After 30 min preincubation	
		at 25°C	at 40°C
mM	μg Rubisco μL^{-1}		
Sunflower			
10.0	0.83	0.79	0.83
0.0	0.48	0.48	0.45
$LSD_{(P=0.05)}$			
Pi nutrition = 0.32	Temperature (NS)[a]		
Maize			
10.0	0.42	0.40	0.46
0.0	0.21	0.19	0.16
$LSD_{(P=0.05)}$			
Pi nutrition = 0.19	Temperature (NS)		

[a] Not significant at P = 0.05.

Table V. *Initial Specific Activity of Rubisco in Crude Extracts from Leaves of Sunflower and Maize Plants Grown with 10, 0.5, or 0 mM Pi and the Effect on the Total Activity of Incubation with Mg^{2+}, HCO_3^-, and Pi*

[Pi] in the Nutrient Solution	Initial Specific Activity	Total Activity 4 min after Preincubating at 25°C with	
		10 mM Pi	0 mM Pi
mM	μmol CO_2 mg^{-1} Rubisco min^{-1}		
Sunflower			
10.0	0.93	1.12 (+20)[a]	1.06 (+14)
0.5	0.80	0.74 (−8)	0.64 (−20)
0.0	0.42	0.39 (−7)	0.39 (−7)
$LSD_{(P=0.05)}$ (Pi nutrition) = 0.26. Presence of Pi in the activation mixture (NS)[b]			
Maize			
10.0	1.08	1.06 (−2)	1.39 (+29)
0.5	1.16	1.09 (−6)	1.42 (+22)
0.0	1.00	0.79 (−22)	0.88 (−12)
$LSD_{(P=0.05)}$ (Pi nutrition) = 0.24. Presence of Pi in the extract = 0.24			

[a] Figures in parentheses indicate percentage of decrease (−) or increase (+) from the initial activity. [b] Not significant at P = 0.05.

DISCUSSION

The $A \cdot C_i$-response curve has an initial linear phase when C_i is small and A is considered to be limited by the activity of Rubisco. The slope at $A = 0$ has been called the carboxylation efficiency (10). This is followed by a curvilinear phase approaching an asymptote at higher C_i, when the CO_2 supply exceeds the capacity of the photosynthetic system to provide RuBP which limits carboxylation (5). Phosphate-deficient sunflower and maize leaves had low photosynthetic rates and small carboxylation efficiency (Table I). Similar observations were reported in different species (4, 11, 19), yet the reason for the decrease is not clear; it may be related to the smaller

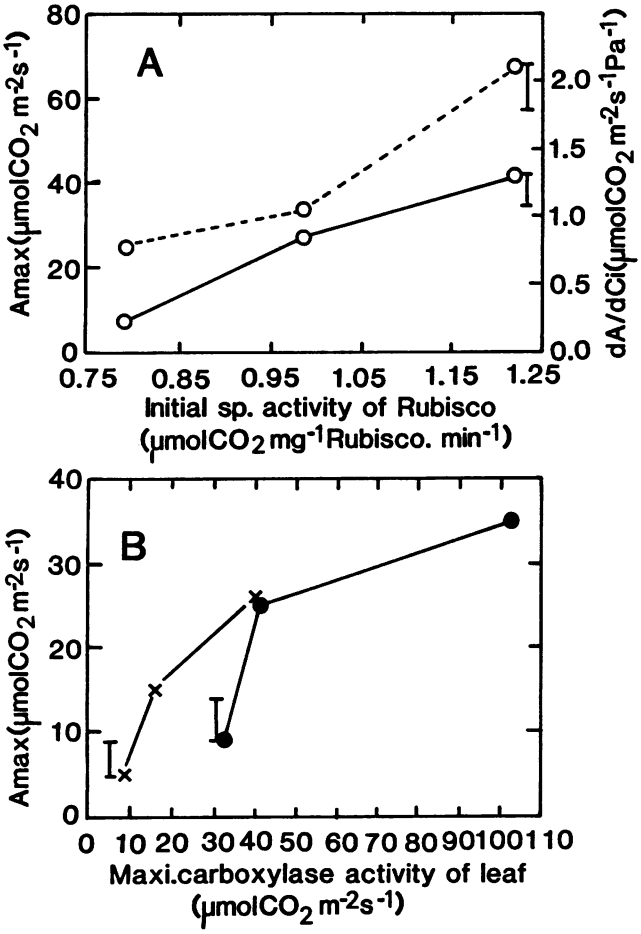

Figure 2. A, A_{max} (O——O) and dA/dC_i (O- - - - -O) related to the initial specific activity of Rubisco in sunflower. B, The relation between A_{max} and the maximum carboxylase activity of leaves of sunflower (●) and maize (X). Bars, LSD between mean values for each species at P = 0.05.

amount and specific activity of Rubisco (11), to the decreased rate of synthesis or pool size of RuBP (18), or to slower transport of assimilates out of, or decreased Pi flux into, chloroplasts (13).

The amount and specific activity of Rubisco and the availability of RuBP affect carboxylation efficiency and thus the photosynthetic rate, and all were strongly decreased by inadequate Pi nutrition. There was less soluble protein and Rubisco in the leaves of the plants grown in low Pi (Fig. 1, A and B), but their proportion remained unaltered in both sunflower and maize (Fig. 1C), suggesting that similar control mechanisms operated during growth despite differences in Pi supply. A decrease in the leaf soluble proteins and amount and specific activity of Rubisco in leaves of soybean grown with inadequate Pi was reported by Lauer *et al.* (11). They found the ratio of Rubisco to soluble protein to be constant (34%) during different Pi treatments, as we have found in our studies (Fig. 1C). Similar observations were made by Brooks (4), but Rao and Terry (19) reported an increase in the leaf protein content. The initial specific activity of Rubisco was significantly lower in Pi-deficient sunflower but not in maize plants (Table II). Hence, there was a strong relation among carboxylation efficiency, A_{max}, and the initial specific activity of Rubisco in sunflower (Fig. 2A), suggesting that reduced Rubisco activity was a cause of the reduction in photosynthetic rate with Pi deficiency in sunflower. However, Rao and Terry (19) found only a slight decrease in the activity of Rubisco in sugar beet plants grown in low Pi.

The relation between A_{max} and the calculated maximum carboxylase activity of the leaf (calculated from the amount and initial specific activity of Rubisco under optimum concentrations of RuBP and CO_2) reveals interesting trends. In both species, there was a strong correlation between these two parameters (Fig. 2B). However, the calculated maximum carboxylase activity of the leaf was always higher than A_{max}, the difference being more pronounced in sunflower than in maize. The difference between A_{max} and calculated maximum carboxylase activity of leaf is an estimate of mesophyll constraints for photosynthesis; it is independent of photorespiration because A_{max} and carboxylase activity are measured at saturating CO_2. A possible reason for this large difference in the Pi-sufficient plants, especially in sunflower, is that a fraction of the Rubisco protein was not functional *in vivo*. It

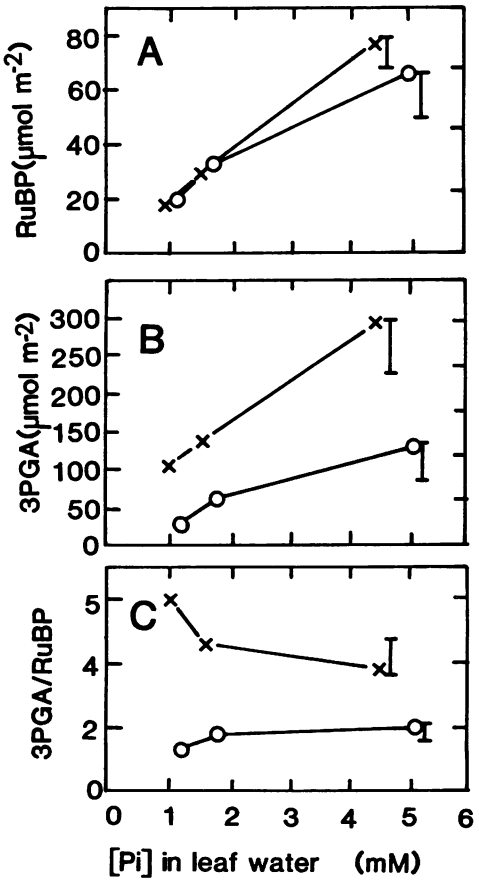

Figure 3. The relation between [Pi] in leaf water and (A) RuBP and (B) 3PGA contents of leaf and (C) the ratio of 3PGA to RuBP in sunflower (O) and maize (X). Bars, LSD between mean values for each species at P = 0.05.

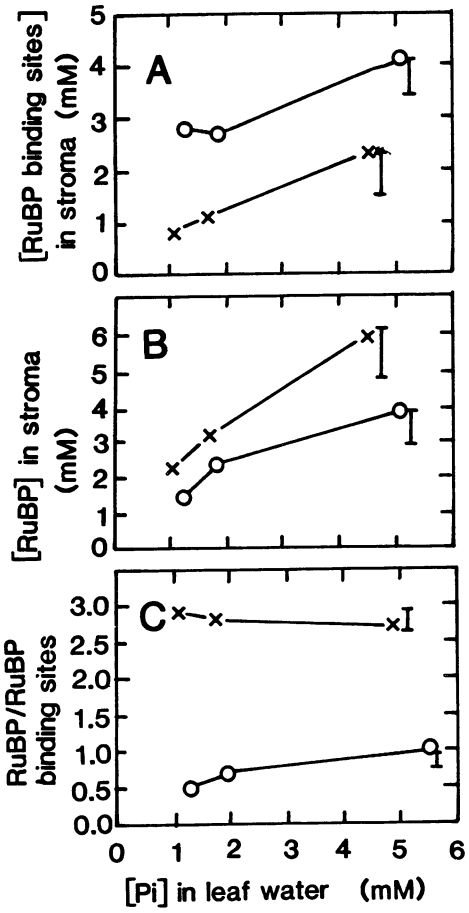

Figure 4. The relation between [Pi] in leaf water and the calculated stromal concentrations of (A) RuBP-binding sites and (B) RuBP and the ratio of the stromal concentration of (C) RuBP to RuBP-binding sites in sunflower (O) and maize (X). Bars, LSD between mean values for each species at P = 0.05.

should be recalled that sunflower leaves had much more Rubisco than maize (Fig. 1B), a consequence of the differences between the C_3 and C_4 modes of photosynthesis. Probably not all of the enzyme functioned, as suggested for plants grown with much nitrogen fertilizer (12). The number of active carboxylating (RuBP-binding) sites on Rubisco per unit leaf area is crucial in determining the rate of carboxylation and, therefore, *A*. The number of active sites may be related to the concentration of RuBP, and deficiency of RuBP observed in phosphate-deficient sunflower leaves may allow open sites to become blocked by other metabolites. It is generally considered that the ratio of RuBP to RuBP-binding sites should be greater than unity for the maximum rate of carboxylation (22). For example, during the life of the rice leaf, RuBP concentrations were always greater than the concentration of RuBP-binding sites (15). We found this ratio to be unity or more in Pi-sufficient sunflower and maize leaves, but it was always higher in maize than in sunflower. The ratio decreased in sunflower with Pi deficiency but was little affected by Pi deficiency in maize (Fig. 4C). This may explain the drastic reduction in the initial specific activity of Rubisco in sunflower plants grown with low Pi. The fact that in sunflower the total activity of Rubisco decreased more, the greater the duration (Table II) and the higher the temperature of preincubation (Table III), also suggests that the RuBP-binding sites were more vulnerable to blockage or inactivation in sunflower than maize. Rubisco is regulated by other constituents of the chloroplast stroma, *e.g.* Pi. It is possible that the effects of low Pi on Rubisco activity in our experiments are due to low stromal [Pi]. However, preincubating the extract with 10 mM Pi decreased the total activity of Rubisco in maize, especially in Pi-deficient plants (Table V). In sunflower, Pi increased the total activity of Rubisco in the Pi-sufficient plants but not in the Pi-deficient plants. We are unsure of the reasons for these effects. Rintamäki *et al.* (20) found that Pi increased the specific activity of Rubisco extracted from wheat and pumpkin but not that of maize.

Our observations of the activation state of Rubisco from leaves with different Pi status do not agree with those reported by Brooks (4), who showed that the Rubisco activation was low in Pi-deficient leaves. From our experiments no such conclusion can be drawn, because the Rubisco specific activity did not increase with preincubation, especially with the enzyme from Pi-deficient leaves. More experiments with modified preincubation medium are needed to study the activation status of the enzyme in these two species.

The decrease in RuBP content of the leaf (69%) and Rubisco activity (35–55%) observed with Pi deficiency in sunflower shows that the effects of Pi deficiency were greatest on the content of the CO_2 acceptor. Similarly, in maize, Pi deficiency decreased the RuBP content by >70%, but Rubisco activity was little affected. Under saturating light conditions, the stromal concentration of RuBP was 8 to 10 mM in wheat seedlings (17). In our studies, this concentration ranged from 1.5 to 4 mM in sunflower and 2.2 to 5.9 mM in maize.

From the above discussion it is evident that the decrease in photosynthesis in the sunflower plants grown with low Pi could be related to both decreased Rubisco activity and RuBP pool size. However, the data show that low Pi decreased *A* largely by decreasing RuBP because the ratio of RuBP to RuBP-binding sites decreased. This could also be a reason for the reduced specific activity of Rubisco in Pi-deficient sunflower. In maize grown in low Pi, *A* was clearly dependent on RuBP pool size and not on Rubisco because the Rubisco activity was not affected. The pool size of a metabolite is determined not only by the rates of its regeneration and consumption which, at steady-state are in equilibrium, but also the maximum size of the pool is determined by the capacity for its regeneration and consumption. In Pi-deficient leaves, a decrease in RuBP pool size suggests a new equilibrium between synthesis and consumption (*i.e.* a parallel decrease in the absolute rates of the regeneration and consumption of RuBP due to the decreased maximum carboxylase activity of the leaf and small *A*). Such a decrease in the above rates would have resulted in an increased pool size of RuBP if the capacity to regenerate RuBP were large in comparison with consumption, *i.e.* if RuBP regeneration capacity was not decreased in the Pi-deficient leaves. Hence, the observed decrease in the RuBP pool size in the Pi-deficient leaves is due more to decrease in the capacity to regenerate RuBP than the capacity to utilize it. Theoretically, the transition from

RuBP carboxylation limitation to RuBP regeneration limitation of A (5) should occur at a lower C_i when the RuBP pool size is small. It is possible to calculate this transition C_i in the C_3 species, sunflower, according to equation A18 of von Caemmerer and Farquhar (24). The calculations show that this occurs in Pi-sufficient sunflower leaves at a C_i of 30.7 Pa which is much above the C_i (21.5 Pa) corresponding to the normal ambient CO_2 of 35 Pa. In phosphate-deficient sunflower leaves, the transition from RuBP carboxylation limitation to RuBP regeneration limitation occurred at a C_i of 20 Pa which is below their normal C_i (24 Pa). This suggests that RuBP regeneration is a limiting factor for CO_2 assimilation in Pi-deficient sunflower leaves at normal ambient CO_2 concentration of 35 Pa.

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