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Extreme Phosphate Deficiency Decreases the *In Vivo* CO_2/O_2 Specificity Factor of Ribulose 1,5-*Bis*phosphate Carboxylase-Oxygenase in Intact Leaves of Sunflower

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ABSTRACT

Sunflower plants were grown under controlled environmental conditions with either 0 or 10 mol m^{-3} phosphate (Pi). From steady-state measurements of gas exchange and chlorophyll fluorescence made on intact leaves, the *in vivo* CO_2/O_2 specificity factor (*in vivo* K_{sp}) of ribulose 1,5-*bis*phosphate carboxylase-oxygenase (Rubisco) was determined following two methods based on models of C_3 photosynthesis by Brooks and Farquhar (1985) and Peterson (1989). The two methods gave *in vivo* K_{sp} values for control sunflower leaves which were similar to published values for higher plants. Extreme Pi deficiency decreased *in vivo* K_{sp} in sunflower leaves compared to adequate Pi. This suggests that Pi deficiency affected photorespiration less than photosynthesis. The decrease in *in vivo* K_{sp} may be due to a real change in the enzyme kinetics favouring oxygenation more than carboxylation or due to an increase in the number of CO_2 molecules released per oxygenation; in which case the observed decrease in the *in vivo* K_{sp} determined on intact leaves will not agree numerically with the true K_{sp} of Rubisco determined *in vitro* using purified enzyme from the same leaf. We discuss the implications of the relatively large photorespiration in Pi-deficient sunflower leaves with respect to the increased dissipation of photosynthetic electrons and photorespiratory recycling of Pi in the chloroplast stroma. Although our results on *in vivo* K_{sp} suggested a relatively larger photorespiratory potential in Pi-deficient than control sunflower leaves, photosynthesis was insensitive to O_2 in Pi-deficient leaves; the possible reasons for this phenomenon are discussed. Under extreme Pi deficiency, O_2 sensitivity of photosynthesis is not a reflection of the *in vivo* photorespiratory rates. Determination of *in vivo* K_{sp} of Rubisco is a useful approach to study the photorespiratory potential of intact leaves.

Key words: Chlorophyll fluorescence, phosphate deficiency, photorespiration, photosynthesis, PSII quantum yield, Rubisco specificity factor.

INTRODUCTION

Many studies have shown that phosphate (Pi) deficiency decreases the rate of net CO_2 assimilation (A) by intact leaves of plants (Brooks, 1986; Lauer *et al.*, 1989; Jacob and Lawlor, 1991; Rao and Terry, 1991). Reduction in A due to Pi deficiency was related to reduced pool size and presumably regeneration of RuBP and also to decreased specific activity of ribulose, 1-5, *bis*phosphate carboxylase-oxygenase (Rubisco) in soybean (Lauer *et al.*, 1989) and sunflower (Jacob and Lawlor, 1992). This enzyme determines the relative flux of carbon through the photosynthetic carbon reduction and photorespiratory cycles, thus affecting the net carbon gain of the leaves. Sunflower leaves, which have the C_3 photosynthetic pathway, generally have large rates of photosynthesis and photorespiration; however, given that Pi deficiency decreases the pool sizes of RuBP, NADPH and ATP (Rao *et al.*, 1989; Jacob, 1992; Jacob and Lawlor, 1993) and the specific activity of Rubisco, it is not possible to maintain such large rates of photorespiration in Pi-deficient sunflower leaves. However, it is unclear if Pi deficiency will have similar effects on photorespiration and photosynthesis. In water-stressed wheat leaves, Lawlor (1976) showed a decrease in photosynthesis and an increase in CO_2 release as a consequence of increased photorespiration and dark

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respiration, and Renou *et al.* (1990) reported a relative increase in photorespiration with respect to photosynthesis that was related to a decrease in the partial pressure of CO_2 in the chloroplast stroma. With Pi deficiency, which increased the leaf internal partial pressure of CO_2 (Lauer *et al.*, 1989; Jacob and Lawlor, 1991), a relative increase in photorespiration with respect to photosynthesis (i.e. an increase in the proportion between the *in vivo* oxygenase and carboxylase activities of Rubisco) would mean a decrease in the *in vivo* CO_2/O_2 specificity factor (*in vivo* K_{sp}) of Rubisco; such a possibility has been suggested by Lauer *et al.* (1989) for Pi-deficient soybean leaves.

It has been postulated that the CO_2/O_2 specificity factor of Rubisco, determined *in vitro* by biochemical techniques (*in vitro* K_{sp}) increased during the course of evolution of higher plants. Photosynthetic bacteria, for example, have much smaller *in vitro* K_{sp} than higher plants. Among C_3 plants there is only small variation in *in vitro* K_{sp} (Jordan and Ogren, 1983; Parry *et al.*, 1989). Both *in vitro* and *in vivo* K_{sp} decrease at high temperatures and extreme light intensities (Badger and Andrews, 1974; Jordan and Ogren, 1984; Brooks and Farquhar, 1985; Peterson, 1990*a*, *b*). It is not known if environmental stresses, including nutritional stress, would affect *in vivo* K_{sp}. Studies on the *in vivo* K_{sp} of Rubisco would help to understand the physiological behaviour of this enzyme and the photorespiratory potential of intact leaves under given conditions.

In a recent study (Jacob and Lawlor, 1993), using modulated chlorophyll *a* (chl *a*) fluorescence techniques, we showed that Pi deficiency decreased the photosynthetic electron transport rate, but increased the fraction of the excited electrons used for processes other than CO_2 reduction in sunflower and maize. It is highly likely that most of these photosynthetic electrons that are not used for CO_2 reduction are diverted to photorespiration leading to proportionately more photorespiration and less CO_2 assimilation in Pi-deficient leaves. This indicates a decrease in the *in vivo* K_{sp} of Rubisco with Pi deficiency. The objective of the present investigation was to determine the effect of Pi supply to sunflower plants on the *in vivo* K_{sp} of Rubisco based on models of C_3 photosynthesis (von Caemmerer and Farquhar, 1981; Brooks and Farquhar, 1985; Peterson, 1989).

MATERIALS AND METHODS

Plant material

Sunflower plants were grown in a controlled environment: photon flux density (*PFD*) 350–400 μmol $m^{-2} s^{-1}$ for 16 h a day, day/night temperature 22/20 °C and relative humidity 75–85%. Groups of plants grown in plastic pots containing washed sand as described previously (Jacob and Lawlor, 1991) were irrigated with modified Hoagland solution containing either 10 or 0 mol m^{-3} Pi (control and Pi-deficient, respectively) and these concentrations were maintained throughout the growth of the plants. Measurements were made on the third leaves when fully expanded.

Gas exchange measurements

Measurements were made with a six chamber computerized infra-red gas analysis system described previously (Jacob and Lawlor, 1991). The response of A to leaf intercellular partial pressure of CO_2 (C_i) was studied at different light intensities by increasing the partial pressure of CO_2 in the ambient air (C_a). Ambient partial pressure of O_2 was either 2 or 21 kPa. Carboxylation efficiency of the leaf, defined as the slope of the response of A to C_i (dA/dC_i) when $A=0$, was determined at saturating light intensity. The response of A to *PFD* was studied by varying light intensity from 0 to 1040 μmol $m^{-2} s^{-1}$ as described in Jacob and Lawlor (1991).

Determination of in vivo K_{sp}

From gas exchange and chlorophyll fluorescence measurements made on intact sunflower leaves, *in vivo* K_{sp} was determined by the following two methods. A brief description of the theory and procedure is given below.

Method 1: If v_c is the rate of carboxylation and v_o that of oxygenation of RuBP catalysed by Rubisco and R_d is the rate of non-photorespiratory release of CO_2 in the presence of light (day respiration), A can be expressed by the following equation:

$$A = v_c - t v_o - R_d \quad (1)$$

where t is the stoichiometry of CO_2 production by photorespiration (i.e. the number of molecules of CO_2 evolved by photorespiration for every molecule of RuBP oxygenated). The ratio v_c/v_o can be described by the following function (Laing *et al.*, 1974):

$$\frac{v_c}{v_o} = \frac{k_c K_o}{k_o K_c} \frac{[CO_2]}{[O_2]} \quad (2)$$

where $[CO_2]$ and $[O_2]$ are the respective concentrations of CO_2 and O_2 at the Rubisco site, k_c and k_o are, respectively, the turnover rate-constants of carboxylation and oxygenation and K_c and K_o are the Michaelis constants for CO_2 and O_2, respectively. The term $k_c K_o/k_o K_c$ is defined as the CO_2/O_2 specificity factor of Rubisco. Assuming that conversion of glycine to serine is the only site of CO_2 production during photorespiration, $t=0{\cdot}5$. Substituting $t=0{\cdot}5$ in equation 1, when $v_c=0{\cdot}5v_o$, $A=-R_d$ and the C_i corresponding to this point is termed Γ^*, the CO_2-photocompensation point (Laisk, 1977). Therefore, substituting $v_c=0{\cdot}5v_o$ and $[CO_2]=\Gamma^*$ in equation 2, we get the following expression for *in vivo* K_{sp} of Rubisco.

$$K_{sp} = \frac{0{\cdot}5[O_2]}{\Gamma^*}. \quad (3)$$

From gas exchange measurements, Γ^* was determined as the C_i corresponding to the intersection of the linear phases of six A versus C_i functions determined at low light intensities as described by Brooks and Farquhar (1985).

Method 2: This method depends upon the determination of the proportion of electron flow to photorespiration relative to the total linear photosynthetic electron transport (P_{dis}). By definition,

$$P_{dis} = \frac{m v_o}{m v_o + 4 v_c} \quad (4)$$

where m represents the theoretical number of electrons required per oxygenation during photorespiration and 4 is that per carboxylation during CO_2 assimilation. When t is set at 0·5, $m=6$ (Peterson, 1990*b*) and at this point, the relationship

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between P_{dis} and *in vivo* K_{sp} of Rubisco can be expressed by the following equation given by Peterson (1989).

$$P_{dis}=\frac{(t+1)[O_2]/[CO_2]}{K_{sp}+([O_2]/[CO_2])}. \quad (5)$$

Rearranging equation 5, we get

$$K_{sp}=\frac{([O_2]/[CO_2])(t+1-P_{dis})}{P_{dis}}. \quad (6)$$

Concentration of O_2 (corresponding to 21 kPa) and CO_2 (determined from gas exchange measurements) in the chloroplast stroma were calculated by taking into account their solubilities in water and assuming that the gaseous and aqueous phases were in equilibrium at 21 °C (Hodgman *et al.*, 1958), the temperature at which gas exchange and chl *a* fluorescence measurements were made. From equation 6, setting t at 0·5, *in vivo* K_{sp} can be calculated if P_{dis} is known. We used modulated chl *a* fluorescence techniques and gas exchange measurements to determine P_{dis}.

Chlorophyll a *fluorescence measurements*: After measuring gas exchange, chl *a* fluorescence was measured at room temperature on the same leaf and at the same *PFD* at which *A* was measured using a modulated fluorescence measuring system (Hansatech, UK) as described earlier (Jacob and Lawlor, 1993) following the method of Weis and Berry (1987). The efficiency of excitation capture by the PSII reaction centre ($\phi_e=(F_{m'}-F_{o'})/F_{m'}$), the coefficient of photochemical quenching ($q_p=(F_{m'}-F_s)/(F_{m'}-F_{o'})$) and the quantum yield of PSII activity ($\phi_{PSII}=\phi_e q_p$) were computed according to Schreiber *et al.* (1986) and Genty *et al.* (1989); $F_{m'}$ is the maximum and $F_{o'}$ is the minimum fluorescence yield from the dark adapted leaf and F_s is the steady-state fluorescence in the light-adapted state. The ambient CO_2 and O_2 partial pressures during the gas exchange and fluorescence measurements were 35 Pa and 21 kPa, respectively, and the ambient temperature was 21 °C.

ϕ_{PSII} represents the number of electrons transported across PSII reaction centre per mol of quantum absorbed by PSII. Theoretically (Genty *et al.*, 1989; Cornic and Briantais, 1991; Krall *et al.*, 1991), the product of ϕ_{PSII} and the amount of light absorbed by PSII should give the rate of total electron transport across PSII (J_T). Assuming 50% of the light absorbed by the leaf is allocated to PSII (Krall *et al.*, 1991), and the proportion of energy allocation between the two photosystems was not significantly altered by Pi treatment, we estimated J_T. Using the net CO_2 uptake data, as described by Cornic and Briantais (1991), it is possible to calculate the rate of electron transport supporting net CO_2 assimilation (4 electrons per absorbed CO_2). Thus, P_{dis} can be expressed as $(J_T-4A)/J_T$ where J_T and A are determined by independent techniques.

Throughout this paper, we use the terms true or *in vitro* K_{sp} to denote the CO_2/O_2 specificity factor of pure Rubisco determined biochemically to distinguish it from *in vivo* K_{sp} which is calculated from gas exchange and chl *a* fluorescence data derived from measurements on intact leaves.

RESULTS AND DISCUSSION

Phosphate deficiency increased Γ^* from 4·1 Pa to 5·5 Pa when measured at 21 °C (Fig. 1). In terms of molar concentration of CO_2, this corresponded to an increase from 1·56 to 2·10 mol m^{-3}. At the above temperature, an ambient O_2 partial pressure of 21 kPa is equivalent to 285 mol m^{-3} O_2 in the stroma. Substituting these values in equation 3 gives an *in vivo* K_{sp} of 91 for control and 68 for Pi-deficient sunflower leaves. The control value obtained according to this method is within the range of published values of K_{sp} based on *in vitro* studies with isolated Rubisco (Jordan and Ogren, 1984; Parry *et al.*, 1989) and *in vivo* studies on intact leaves based on Rubisco models (Brooks and Farquhar, 1985; Peterson, 1990*a*, *b*).

Phosphate deficiency significantly decreased *A* and increased C_i at a wide range of light intensities (Table 1). Analysis of chl *a* fluorescence data showed that Pi deficiency decreased the efficiency of excitation capture by the PSII reaction centre and the coefficient of photochemical quenching of variable fluorescence (data not shown). This resulted in a decrease in the *in vivo* quantum yield of PSII activity in Pi-deficient leaves (Table 1). However, the slope of the linear relationship between *in vivo* quantum yield of PSII activity and apparent quantum yield for CO_2 assimilation determined under photorespiratory conditions was greater in Pi-deficient than control leaves (Fig. 2). This suggests that there was proportionately more PSII activity per net CO_2 assimilated by Pi-deficient leaves. In other words, as further calculations show, Pi deficiency increased the allocation of electron flow to photorespiration relative to the total photosynthetic electron flow (P_{dis}) at a wide range of light intensities studied (Table 1). A large P_{dis} suggests increased proportion between photorespiration and photosynthesis. Assuming that $t=0·5$, substituting P_{dis} in equation 6 gives a smaller *in vivo* K_{sp} in Pi-deficient leaves at all the light intensities (Table 1). The mean (±s.e.) *in vivo* K_{sp} values averaged over the seven *PFD* levels was 102 (5) in control and 56 (5) in Pi-deficient leaves.

Our results on the *in vivo* K_{sp} can be explained in two ways. First, if the observed reduction in *in vivo* K_{sp} in the Pi-deficient leaf is real, it is suggestive of a direct effect of extreme Pi deficiency, by some unknown mechanism(s), on the structural configuration of the enzyme, probably at the active site, favouring more oxygenation than carboxylation. Chemical modification, amino acid substitution or mutation of the enzyme at certain site are known to affect the specificity factor of Rubisco (Smith *et al.*, 1990; Chen and Spreitzer, 1992; Madgwick *et al.*, 1992). It is not known whether the small concentration of Pi in the chloroplast stroma and the decreased adenylate energy charge observed in Pi-deficient sunflower leaves (Jacob, 1992; Jacob and Lawlor, 1993) could cause such effects. If this is the case, the true K_{sp} of purified Rubisco determined *in vitro* using biochemical techniques will also be small and numerically equal to the *in vivo* K_{sp} determined by gas exchange methods in intact Pi-deficient leaves. A decrease in the *in vitro* K_{sp} of Rubisco in Pi-deficient leaves will result in a large ratio of v_o to v_c leading to proportionately large photorespiration.

A second possibility, which may be more likely than the first one, is that the *in vivo* K_{sp} does not always reflect the *in vitro* K_{sp} of the enzyme and the two may not be

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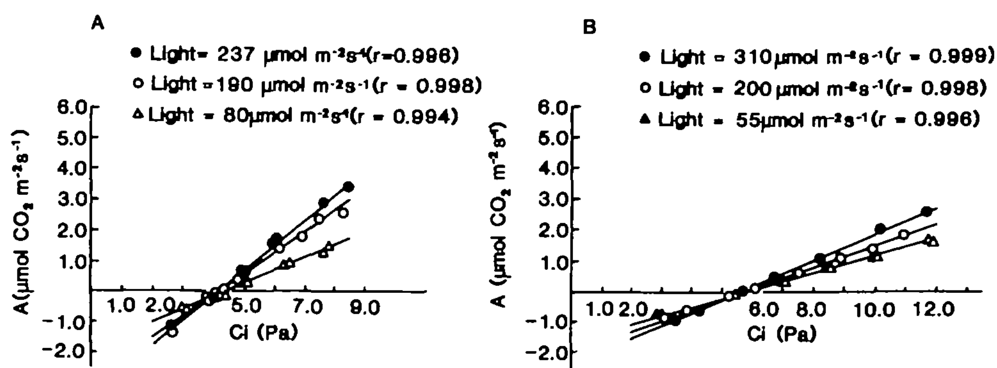

FIG. 1. Linear phases of the $A \times C_i$ response functions of control (A) and Pi-deficient (B) sunflower leaves. Data were obtained at three irradiances and each point is an average of 4–6 readings. Equations for the three straight lines in panel A are: $-3{\cdot}6+0{\cdot}867C_i$ (light=237 μmol m^{-2} s^{-1}), $-3{\cdot}2+0{\cdot}756C_i$ (light=190 μmol m^{-2} s^{-1}) and $-1{\cdot}8+0{\cdot}444C_i$ (light=80 μmol m^{-2} s^{-1}) and in panel B are: $-2{\cdot}37+0{\cdot}41C_i$ (light=310 μmol m^{-2} s^{-1}), $-2{\cdot}0+0{\cdot}381C_i$ (light=200 μmol m^{-2} s^{-1}) and $-1{\cdot}365+0{\cdot}235C_i$ (light=55 μmol m^{-2} s^{-1}). For each treatment Γ^* was estimated as the X-coordinate corresponding to the point of intersection of any two lines. Γ^* was taken as the mean of six such values (three from the above equations and three from a second set of experiments) and was 4·12 (0·16) Pa in control and 5·46 (0·42) Pa in Pi-deficient leaves. Figures in parentheses indicate ±s.e.

TABLE 1. *Effect of Pi nutrition on* in vivo K_{sp} *of Rubisco calculated from measurements of gas exchange and chl* a *fluorescence from intact leaves of sunflower*

Photosynthetic response to *PFD* was studied on 4–6 leaves at a C_a of 35 Pa and 21 kPa O_2 partial pressure in the ambient air. An asymptotic curve ($A=a-br^{PFD}$) was fitted for the data points (n=54–60; $R^2>99\%$). The values of A were directly estimated from the function. See Materials and Methods for details of calculations of ϕ_{PSII}, J_T, P_{dis} and *in vivo* K_{sp}.

PFD (μmol m^{-2} s^{-1})	*A* (μmol m^{-2} s^{-1})	C_i (Pa)	ϕ_{PSII}	J_T (μmol m^{-2} s^{-1})	P_{dis}	*in vivo* K_{sp}
Control Leaf						
170	11·1	30·5	0·773	66	0·324	89
208	13·2	29·5	0·737	77	0·311	97
416	21·5	25·1	0·648	135	0·362	94
520	24·2	23·5	0·603	157	0·383	93
650	26·8	22·1	0·475	154	0·306	132
820	29·2	20·7	0·487	200	0·415	94
1040	31·1	19·6	0·387	201	0·381	112
Pi-deficient leaf						
170	5·1	31·6	0·502	43	0·522	44
208	6·2	30·8	0·512	53	0·534	44
416	10·6	28·0	0·402	84	0·493	55
520	12·1	27·2	0·378	98	0·506	54
650	13·4	26·5	0·372	121	0·557	48
820	15·2	26·0	0·270	111	0·451	67
1040	16·4	25·7	0·213	111	0·408	78

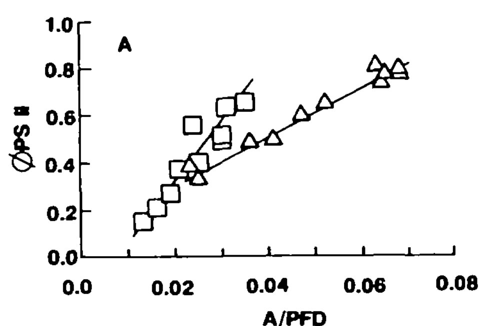

FIG. 2. Relation between *in vivo* quantum yield of PSII activity (ϕ_{PSII}) and apparent quantum yield for CO_2 assimilation (A/PFD) determined under photorespiratory conditions in control (△) and Pi-deficient (□) sunflower leaves.

numerically equal. This is because the *in vivo* K_{sp} can vary independent of the true K_{sp} of the enzyme if t is not a constant. There are biochemical (Hanson and Peterson, 1985) and genetic (Zelitch, 1989) evidence showing that t is not universally constant. Clearly, *in vivo* K_{sp} is inversely related to Γ^* at a given ambient concentration of O_2 (see equation 3), but it is unclear if the increase in Γ^* and the resulting decrease in the *in vivo* K_{sp} observed in the present investigation was actually due to a real decrease in the true K_{sp} of Rubisco, or from an increase in t. If the latter is the case (i.e. the decrease in *in vivo* K_{sp} observed with Pi deficiency is a consequence of the altered stoichiometry of CO_2 production by photorespiratory carbon metabolism in the cell and is not the result of a real change in the kinetic property of Rubisco or the true K_{sp}) the *in*

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vivo and *in vitro* measurements of K_{sp} will not agree numerically and the proportionately large photorespiration as suggested by the decreased *in vivo* K_{sp} in Pi-deficient leaves was not the result of the increased ratio of v_o to v_c, (decreased *in vivo* K_{sp} due to large t does not affect v_o/v_c whereas a real decrease in the true K_{sp} of Rubisco would increase this ratio without affecting t).

Although the models of C_3 photosynthesis used in this study are widely accepted and followed, few studies with whole leaves have concentrated on the extent of constancy of *in vivo* K_{sp} or t implied in them. It has been shown that t can be greater than 0·5 under certain situations (Hanson and Peterson, 1985; Peterson 1990*a*, *b*). Variation in t was found to be related to catalase activity in tobacco leaves (Zelitch, 1989). Chen and Spreitzer (1992) reported that increase in photorespiration at high temperatures was due to a decrease in the *in vitro* K_{sp} of Rubisco which was attributed to the difference between the free energies of activation for the oxygenation and carboxylation reactions. Large photorespiration in high O_2 environments is due to increased ratio of v_o to v_c and can be explained by the Warburg effect. However, Hanson and Peterson (1985) show that large photorespiration at high temperatures and high O_2 levels are associated with an increase in t above 0·5 resulting from possible peroxidation of hydroxypyruvate. Therefore, it may be inferred that more than one factor could be responsible for large photorespiration under a given condition.

Assuming that the true K_{sp} of Rubisco was the same in Pi-deficient and control sunflower leaves, and that the decrease in *in vivo* K_{sp} observed with Pi deficiency was due to an increase in t above 0·5, we calculated, using the data given in Table 1, the new stoichiometry of CO_2 release by photorespiration (t^*) for the Pi-deficient leaf that would give an *in vivo* K_{sp} value as large as in the control leaves. Our calculations show that the mean (±s.e.) value of t^* (over the range of *PFD*, 170–1040 μmol $m^{-2}\ s^{-1}$) must be as large as 1·38 (0·163) in Pi-deficient leaves (compared to 0·5 in control leaves) in order to maintain the *in vivo* K_{sp} of Pi-deficient leaves the same as the control leaves (Table 2). Since the relationship between m and t can be expressed as $m = 4t + 4$ (Peterson, 1990*b*), when t increases from 0·5 to 1·38, m increases from 6 to 9·5. It is evident from equations 4 and 6 that an increase in m (due to a large t) will increase P_{dis} (and thus decrease *in vivo* K_{sp}) even with constant values of v_o and v_c.

Although *in vivo* K_{sp} was small and, therefore, there was relatively more photorespiration, A and carboxylation efficiency were less sensitive to change in O_2 partial pressure in Pi-deficient leaves than in control leaves (Table 3). This is contrary to what one would expect when there is relatively large photorespiration, either because of an increase in the ratio of v_o to v_c (due to decrease in the true K_{sp} of Rubisco) or because of an increase in t. Although the above result appears to contradict our results on *in vivo* K_{sp}, closer analysis of the working of the chloroplast indicates that it is not so. Oxygen-insensitive photosynthesis has been reported in many C_3 plants and, according to Sharkey (1985), occurs when Pi concentration in the chloroplast is not high enough to allow photophosphorylation and not low enough to allow starch synthesis. Under optimum conditions the Pi concentration in the chloroplast is about 4 mol m^{-3} (Santarius and Heber, 1965). However, sometimes this concentration can be as low as 1 mol m^{-3} or even less (Sharkey, 1985) and under conditions of high light and saturating CO_2, which favour large A, photosynthesis by chloroplasts *in vivo*, like chloroplasts *in vitro*, can suffer from Pi limitation (Cockburn *et al.*, 1967; Sivak and Walker, 1986). Based on the compartmentation of Pi in the various pools in the cell, in a separate experiment identical to the one described in the present study, the

TABLE 2. *Using the data given in Table 1, we calculated what might be the new stoichiometry of CO_2 release by photorespiration (t^*) in Pi-deficient leaves, if the* in vivo *K_{sp} in Pi-deficient leaves was the same as in the control leaves*

This was calculated from the equation $t^* = \{K_{sp}P_{dis}[CO_2]/[O_2]\} + P_{dis} - 1$ derived from equation 6 in Material and Methods.

PFD (μmol $m^{-2}\ s^{-1}$)	t^* (Pi-deficient leaf)
170	1·49
208	1·67
416	1·22
520	1·22
650	2·16
820	0·93
1040	0·98
Mean (±s.e.)	1·38 (0·163)

TABLE 3. *Oxygen sensitivity of net CO_2 assimilation rate (A) and carboxylation efficiency (dA/dC_i) in control and Pi-deficient sunflower leaves*

Photosynthetic response to C_i was determined at saturating *PFD* on 4–6 intact leaves by changing C_a. Measurements were made at 2 and 21 kPa partial pressure of O_2 in the ambient air. An asymptotic function ($A = a - br^{C_i}$) was fitted for the data points (n = 32–48; $R^2 > 99\%$). The values of A given below correspond to C_a = 35 Pa. Carboxylation efficiency, defined as the initial slope of the A versus C_i response function at $A = 0$ was calculated as the differential quotient, $dA/dC_i = -a\ln r$. Figures in parenthesis indicate per cent increase (+) or decrease (−) from the value of 21 kPa O_2.

Ambient partial pressure of O_2 (kPa)	A (μmol $m^{-2}\ s^{-1}$)	dA/dC_i (μmol $m^{-2}\ s^{-1}\ Pa^{-1}$)
Control		
21	19·2	1·76
2	32·1	3·99
	(+67)	(+127)
Pi-deficient		
21	7·2	0·72
2	8·2	0·66
	(+14)	(−8)

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concentration of Pi in the chloroplast was calculated to be about 1·4 mol m^{-3} in Pi-deficient sunflower leaves as compared to 7·3 mol m^{-3} in control leaves (Jacob, 1992). At such a low concentration of Pi in the chloroplast, we believe that photosynthesis is more dependent on the photorespiratory release of Pi in Pi-deficient leaves than in control leaves. By decreasing the ambient partial pressure of O_2 from 21 to 2 kPa, photorespiration can be inhibited. By inhibiting photorespiration, the internal recycling of Pi due to the photorespiratory release of Pi in the chloroplast stroma is also inhibited. Since this recycling of Pi is important for photosynthesis, inhibiting photorespiration does not stimulate the Calvin cycle and, therefore, the expected increase in net photosynthesis by decreasing the ambient partial pressure of O_2 is not realized in the Pi-deficient leaf. Contrary to this, in control leaves, where rate of photosynthesis is high, but likely to be less dependent upon the photorespiratory release of Pi since there is a high concentration of Pi in the chloroplast stroma, preventing photorespiration stimulated net photosynthesis as expected. Therefore, under conditions of internal deficiency of Pi either due to poor nutrition as in the present case or due to environmental factors such as low temperature (Mächler *et al.*, 1984) oxygen sensitivity of photosynthesis is not an appropriate indicator of relative rates of photorespiration and hence the observed O_2 insensitivity of photosynthesis in Pi-deficient sunflower leaves does not invalidate our results on *in vivo* K_{sp} in those leaves.

In conclusion, growing sunflower plants under extreme Pi deficiency decreased net photosynthesis, although the leaf internal CO_2 partial pressure was large in Pi-deficient leaves. Calculations based on models of C_3 photosynthesis and chl *a* fluorescence data showed that the proportion of photosynthetic electrons used for photorespiration increased with Pi deficiency. This suggests that Pi deficiency affected photorespiration less than photosynthesis. We found that the *in vivo* K_{sp} of Rubisco was low in a Pi-deficient leaf which indicates the relatively large photorespiration in Pi-deficient leaves, but we are unsure of the reasons for the decrease in *in vivo* K_{sp}. We further demonstrate the occurrence of O_2-insensitive photosynthesis in Pi-deficient sunflower leaves and explain this phenomenon in the light of dependence of photosynthesis on photorespiratory recycling of Pi in the chloroplast stroma. Increased recycling of Pi and dissipation of excess energy are possible favourable effects of increased photorespiration in Pi-deficient leaves.

This article throws light on two interesting possibilities. First, if the observed decrease in *in vivo* K_{sp} of Rubisco was due to a real decrease in the true K_{sp} of the enzyme, then it can be assumed that the kinetic properties of Rubisco had been affected by extreme Pi deficiency in such a way that v_o was decreased less than v_c. Second, if the decrease in *in vivo* K_{sp} was not the result of a decrease in the true K_{sp} of Rubisco, the generally accepted view that glycine→serine conversion is the only site where CO_2 is released by photorespiration and only 0·5 mol of CO_2 is released per oxygenation is questioned; the reduction in *in vivo* K_{sp} can be the consequence of an increased number of CO_2 produced per oxygenation. Whatever the case, *in vivo* K_{sp} is a reflection of the photorespiratory potential of the leaf and a relatively large photorespiration in the Pi-deficient leaves may be a channel to dissipate the photosynthetic electrons in excess of the requirement for net CO_2 assimilation; the capacity for net CO_2 assimilation itself being determined by the overall metabolic state of the cell. Mechanism(s) regulating the *in vivo* K_{sp} of Rubisco and the extent of its constancy in intact leaves under various environmental conditions offer an important and interesting area for further studies.

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