The kinetics of ribulose-1,5-bisphosphate carboxylase/oxygenase in vivo inferred from measurements of photosynthesis in leaves of transgenic tobacco

ARTICLE *in* PLANTA · JANUARY 1994

Impact Factor: 3.26 · DOI: 10.1007/BF00206296

4 AUTHORS, INCLUDING:

Susanne von Caemmerer von Caemmerer
Australian National University
131 PUBLICATIONS **13,531** CITATIONS
SEE PROFILE

John R Evans
Australian National University
131 PUBLICATIONS **9,470** CITATIONS
SEE PROFILE

Planta (1994) 195:88–97

Planta

The kinetics of ribulose-1,5-bisphosphate carboxylase/oxygenase in vivo inferred from measurements of photosynthesis in leaves of transgenic tobacco

Susanne von Caemmerer[1,3], **John R. Evans**[1], **Graham S. Hudson**[2], **T. John Andrews**[2,3]

[1] Environmental Biology Group, Research School of Biological Sciences, Australian National University, G.P.O. Box 475, Canberra City, ACT 2601, Australia
[2] Cooperative Research Centre for Plant Science, Research School of Biological Sciences, Australian National University, G.P.O. Box 475, Canberra City, ACT 2601, Australia
[3] Molecular Plant Physiology Group, Research School of Biological Sciences, Australian National University, G.P.O. Box 475, Canberra City, ACT 2601, Australia

Received: 28 February 1994 / Accepted: 7 April 1994

Abstract. Transgenic tobacco (*Nicotiana tabacum* L. cv. W38) with an antisense gene directed against the mRNA of the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) small subunit was used to determine the kinetic properties of Rubisco in vivo. The leaves of these plants contained only 34% as much Rubisco as those of the wild type, but other photosynthetic components were not significantly affected. Consequently, the rate of CO_2 assimilation by the antisense plants was limited by Rubisco activity over a wide range of CO_2 partial pressures. Unlike in the wild-type leaves, where the rate of regeneration of ribulose bisphosphate limited CO_2 assimilation at intercellular partial pressures above 400 μbar, photosynthesis in the leaves of the antisense plants responded hyperbolically to CO_2, allowing the kinetic parameters of Rubisco in vivo to be inferred. We calculated a maximal catalytic turnover rate, k_{cat}, of 3.5 ± 0.2 mol $CO_2 \cdot$(mol sites)$^{-1} \cdot s^{-1}$ at 25° C in vivo. By comparison, we measured a value of 2.9 mol $CO_2 \cdot$(mol sites)$^{-1} \cdot s^{-1}$ in vitro with leaf extracts. To estimate the Michaelis-Menten constants for CO_2 and O_2, the rate of CO_2 assimilation was measured at 25° C at different intercellular partial pressures of CO_2 and O_2. These measurements were combined with carbon-isotope analysis ($^{13}C/^{12}C$) of CO_2 in the air passing over the leaf to estimate the conductance for transfer of CO_2 from the substomatal cavities to the sites of carboxylation (0.3 mol $\cdot m^{-2} \cdot s^{-1} \cdot bar^{-1}$) and thus the partial pressure of CO_2 at the sites of carboxylation. The calculated Michaelis-Menten constants for CO_2 and O_2 were 259 ± 57 μbar (8.6 ± 1.9 μM) and 179 mbar (226 μM), respectively, and the effective Michaelis-Menten constant for CO_2 in 200 mbar O_2 was 549 μbar (18.3 μM). From measurements of the photocompensation point ($\Gamma_* = 38.6$ μbar) we estimated Rubisco's relative specificity for CO_2, as opposed to O_2 to be 97.5 in vivo. These values were dependent on the size of the estimated CO_2-transfer conductance.

Key words: Gas exchange – *Nicotiana* – Photosynthesis (C_3) – Ribulose-1,5-bisphosphate carboxylase/oxygenase kinetics – Transgenic tobacco

Introduction

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) catalyses competing reactions between ribulose bisphosphate (RuBP) and CO_2 and O_2. The carboxylation of RuBP is the first step of the photosynthetic carbon-reduction cycle and leads to CO_2 uptake of leaves, whereas the oxygenation of RuBP necessitates recycling of phosphoglycolate by the photorespiratory carbon-oxidation cycle with a concomitant CO_2 loss. Laing et al. (1974) first derived rate equations which described the influence of Rubisco's properties on CO_2 assimilation and demonstrated that the kinetic properties of Rubisco could explain the O_2 sensitivity of CO_2 assimilation in soybean leaves. Mechanistic models of CO_2 assimilation in leaves of C_3 species based on these equations are now widely used in a variety of applications. They require values for Rubisco's Michaelis-Menten constants for CO_2 and O_2 and for its relative specificity for these two substrates (Hall 1979; Farquhar et al. 1980; Collatz et al. 1991; Harley and Sharkey 1991; Sellers

Abbreviations and symbols: $A = CO_2$-assimilation rate; g_w = conductance for CO_2 transfer from the substomatal cavities to the sites of carboxylation; K_c, K_o = Michaelis-Menten constants for carboxylation, oxygenation of Rubisco; $k_{cat} = V_{cmax}/$[active site]; O = partial pressure of O_2 at the site of carboxylation; p_c = partial pressure of CO_2 at the site of carboxylation; p_i = intercellular CO_2 partial pressure; R_d = 'day' respiration (non-photorespiratory CO_2 evolution); Rubisco = ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP = ribulose-1,5-bisphosphate; $S_{c/o}$ = relative specificity factor for Rubisco; SSu = small subunit of Rubisco; V_{cmax}, V_{omax} = maximum rates of Rubisco carboxylation, oxygenation; Γ_* = partial pressure of CO_2 in the chloroplast at which photorespiratory CO_2 evolution equals the rate of carboxylation

Correspondence to: S. von Caemmerer; FAX: 61 (6) 249 4919; Tel.: 61 (6) 249 5053; E-mail: susanne@rsbs-central.anu.edu.au

et al. 1992). Values determined using isolated Rubisco, such as those reported by Badger and Collatz (1977), Jordan and Ogren (1984) and Makino et al. (1988), are often used; but these estimates vary widely.

Enzyme assays are conducted in vitro under dilute conditions which do not reflect Rubisco's situation in vivo where the active-site concentration in the chloroplast can be of the order of 1–4 mM (Jensen and Bahr 1977). Therefore, it is of interest to infer the kinetics of Rubisco in vivo from measurements of CO_2 assimilation by leaves. The CO_2/O_2 specificities of Rubiscos from spinach and wheat have been inferred in this way and there is substantial agreement between the in-vivo and in-vitro estimates (Jordan and Ogren 1984; Brooks and Farquhar 1985). However, the Michaelis-Menten constants are more difficult to estimate from leaf gas-exchange data because the CO_2 dependence of CO_2 assimilation is determined by the Rubisco activity only at low CO_2 partial pressures (Ku and Edwards 1977; Harley et al. 1985).

In transgenic tobacco plants with an antisense gene directed against the mRNA for the small subunit (SSu) of Rubisco, this problem is overcome. The amount of Rubisco is reduced while the capacity for RuBP regeneration remains unaffected and the CO_2-assimilation rate remains limited by Rubisco activity even at high CO_2 partial pressures (Hudson et al. 1992). We have exploited these plants to estimate the kinetic constants of tobacco Rubisco in vivo from gas-exchange measurements on leaves. These estimated kinetic constants provide a data set that can be used in all models of photosynthesis but which should be particularly useful in the analysis of photosynthesis in tobacco. Some of these data have been presented elsewhere in preliminary form (von Caemmerer et al. 1992).

Materials and methods

Plant culture

R1 tobacco (*Nicotiana tabacum* L.) plants, resulting from self-fertilization of primary transformants, or control tobacco plants were grown in either an air-conditioned glasshouse or a growth cabinet. Seedlings were screened by measuring leaf fluorescence with a fluorometer (PAM-101; H. Walz, Effeltrich, Germany) (Hudson et al. 1992). The plants were grown in 5-l pots in sterilized garden soil and given a complete nutrient solution containing 12 mM nitrate (Hewitt and Smith 1975) three times a week. Midday irradiance in the glasshouse was approximately 1200 μmol quanta $\cdot m^{-2} \cdot s^{-1}$ and mean day and night temperatures were 28 and 16° C, respectively. In the growth cabinet, irradiance was 1000 μmol quanta $\cdot m^{-2} \cdot s^{-1}$ with a 12-h day; day and night temperatures were 25° C and 18° C, respectively, and the air humidity was 60%. *Spinacea oleracea* L. (Henderson hybrid 102) was grown in the glasshouse with the same culture conditions as tobacco.

The segregation of kanamycin resistance among surface-sterilised tobacco seeds was observed on agar plates as described earlier (Mate et al. 1993), except at atmospheric CO_2 partial pressure.

Gas-exchange measurements

Leaf gas exchange was measured using an open gas-exchange system described by Hudson et al. (1992) except that part of a leaf (30–40 cm^2) was enclosed in a large leaf chamber in which the air was stirred with a tangential fan. Oxygen concentration was varied by mixing CO_2-free air with either N_2 or O_2 using two mass-flow controllers. Gas-exchange parameters were calculated according to von Caemmerer and Farquhar (1981).

Measurement of CO_2-assimilation rate at different CO_2 and O_2 partial pressures. The irradiance was 1500 μmol quanta $\cdot m^{-2} \cdot s^{-1}$ (unless indicated otherwise), the leaf temperature was 25° C and leaf-to-air vapour-pressure difference was 12 mbar. The response of CO_2-assimilation rate, A, to varying CO_2 concentration was measured at four or five different O_2 concentrations on a single leaf during the course of one day. At any one O_2 concentration, CO_2 was varied either from high to low CO_2 partial pressure or vice versa. O_2 partial pressures were selected in a random order.

Measurement of g_w. The conductance for CO_2 transfer from the substomatal cavities to the sites of carboxylation, g_w, was calculated from simultaneous measurements of gas exchange and carbon-isotope discrimination as described by von Caemmerer and Evans (1991). Gas exchange and the carbon-isotope composition of the air passing over the leaf were measured several times at a CO_2 partial pressure of 350 μbar and an O_2 concentration of 2%. The irradiance was 1000 μmol quanta $\cdot m^{-2} \cdot s^{-1}$; other conditions were as described above. The conductance was then calculated from the following equation (Evans et al. 1986):

$$g_w = \frac{(b - a_i)}{((a + (b-a)p_i/p_a) - \Delta)} \cdot \frac{A}{p_a}, \quad \text{(Eq. 1)}$$

where b, a, and a_i are, respectively, the $^{13}C/^{12}C$ isotope fractionations for RuBP carboxylation (including a small amount of phosphoenolpyruvate carboxylation, 29‰), the fractionation during diffusion of CO_2 through air (4.4‰) and the fractionation occurring during the the dissolution and diffusion of CO_2 in water (1.8‰). The CO_2-assimilation rate, A, and the intercellular and external CO_2 partial pressures, p_i and p_a, were measured by gas exchange and the carbon-isotope discrimination, Δ, was measured by isotopic analysis.

Measurement of Γ_* and day respiration. The partial pressure of CO_2 in the chloroplast at which photorespiratory CO_2 evolution equals the rate of carboxylation, Γ_*, was measured as described by Brooks and Farquhar (1985) using CO_2-response curves obtained at low CO_2 partial pressures, 25° C, 21% O_2 and four different irradiances below 550 μmol quanta $\cdot m^{-2} \cdot s^{-1}$. The absolute value of the y coordinate of the point where all of these lines intersect revealed the CO_2 and light-independent component of leaf gas exchange, R_d (defined as all CO_2 evolution not associated with photorespiratory CO_2 evolution). The value of Γ_* was then calculated from the intercellular CO_2 partial pressure at this intersection point, p_i^*, as follows:

$$\Gamma_* = p_i^* + R_d/g_w. \quad \text{(Eq. 2)}$$

Γ_* at O_2 concentrations different from 21% was calculated from the value measured at 21% O_2 assuming a linear relationship between Γ_* and O_2 concentration (Brooks and Farquhar 1985).

Biochemical assays The carbamylation level and amount of Rubisco per unit leaf area was measured from the binding of [^{14}C]carboxyarabinitol bisphosphate to a polyethylene-glycol-precipitable species according to Butz and Sharkey (1989). The extraction of tobacco leaves, the measurements of Rubisco activity after full carbamylation in vitro and assay of soluble protein were performed as described by Mate et al. (1993). Nitrogen content was measured on oven-dried leaf material with a Carlo Erba (model 1108) elemental analyzer.

Theory

Calculation of Rubisco kinetic parameters from gas-exchange measurements. The photosynthetic carbon reduction and the photosynthetic carbon oxidation (PCO) cycles are linked by Rubisco. In the PCO cycle the oxygenation of one mole of RuBP with one mole of O_2 by Rubisco leads to the release of 0.5 mole of CO_2. Following Farquhar and von Caemmerer (1982) the net rate of CO_2 assimilation can thus be given by

$$A = V_c - 0.5V_o - R_d \qquad \text{(Eq. 3)}$$

Where V_c and V_o are the rates of Rubisco carboxylation and oxygenation respectively and R_d denotes mitochondrial respiration in the light other than that associated with the PCO cycle. Phosphoenolpyruvate carboxylations have not been explicitly included in Eq. 3. Since CO_2 and O_2 are competitive alternative substrates of Rubisco, it follows that (Laing et al. 1974)

$$S_{c/o} = \frac{V_{cmax}K_o}{K_cV_{omax}} = \frac{V_cO}{V_op_c} \qquad \text{(Eq. 4)}$$

where $S_{c/o}$ is the relative specificity of Rubisco for CO_2 as opposed to O_2, V_{cmax} and V_{omax} are the substrate saturated rates, K_c and K_o are the Michaelis Menten constants for carboxylation and oxygenation, respectively, and p_c and O are the partial pressures of CO_2 and O_2, respectively, at the the site of carboxylation. The p_c at which the carboxylation rate equals the rate of photorespiratory CO_2 release has been denoted by Γ_*. When $p_c = \Gamma_*$, $A = -R_d$ and $V_c/V_o = 0.5$. Therefore

$$S_{c/o} = 0.5\, O/\Gamma_* \qquad \text{(Eq. 5)}$$

and thus, at any p_c, $V_o = \Gamma_* V_c/(0.5\, p_c)$. Substituting for V_o in Eq. 3 yields

$$A + R_d = V_c(1 - \Gamma_*/p_c). \qquad \text{(Eq. 6)}$$

Since O_2 inhibits RuBP carboxylation competitively with respect to CO_2, the RuBP saturated carboxylation rate is

$$V_c = \frac{p_cV_{cmax}}{p_c + K_c(1 + O/K_o)}. \qquad \text{(Eq. 7)}$$

Substituting for V_c in Eq. 6

$$A + R_d = \frac{(p_c - \Gamma_*)V_{cmax}}{p_c + K_c(1 + O/K_o)} \qquad \text{(Eq. 8)}$$

which can be rewritten in a form analogous to the Michaelis-Menten equation:

$$A + R_d = \frac{(p_c - \Gamma_*)V_{cmax}}{(p_c - \Gamma_*) + (\Gamma_* + K_c(1 + O/K_o))} \qquad \text{(Eq. 9)}$$

Therefore a plot of $A + R_d$ versus $p_c - \Gamma_*$ should be hyperbolic. The CO_2 partial pressure at the carboxylation site, p_c may be calculated from the equation

$$p_c = p_i - A/g_w. \qquad \text{(Eq. 10)}$$

In some of the data shown in Fig. 6, V_c was estimated from the initial slopes, g_m, of CO_2-response curves. Von Caemmerer and Evans (1991) derived a quadratic equation relating A to p_i by combining Eqs. 8 and 10 and showed that to a first approximation assuming $V_{cmax} >> R_d$,

$$g_m = \frac{dA}{dp_i} = \frac{g_wV_{cmax}}{V_{cmax} + g_w(\Gamma_* + K_c(1 + O/K_o))} \qquad \text{(Eq.11)}$$

By rearranging the above equation, an expression for V_{cmax} may be obtained,

$$V_{cmax} = \frac{g_mg_w(\Gamma_* + K_c(1 + O/K_o))}{g_w - g_m} \qquad \text{(Eq. 12)}$$

which allows estimation of V_{cmax} from g_m using the constants given in Table 1 for $g_w = 0.3\ \text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\cdot\text{bar}^{-1}$.

The dependence of the estimate of K_c and K_o on g_w: The reciprocal of Eq. 11 was derived by Peisker and Apel (1975) and shows that the mesophyll resistance ($1/g_m$), which is the sum of the CO_2-transfer resistance ($1/g_w$) and a residual carboxylation resistance, is linearly dependant on O_2

$$\frac{1}{g_m} = \frac{1}{g_w} + \frac{K_c}{V_{cmax}} + \left(\frac{0.5}{S_{c/o}} + \frac{K_c}{K_o}\right)\cdot\frac{O}{V_{cmax}}. \qquad \text{(Eq. 13)}$$

The intercept of this equation is determined by g_w, K_c, and V_{cmax} and from it we can derive the following relationship between our estimate of K_c at a particular g_w and $K_{c(\infty)}$, the K_c estimated with the assumption of an infinite g_w:

$$K_{c(\infty)} = K_c + V_{cmax}/g_w. \qquad \text{(Eq. 14)}$$

The value of K_o is obtained from the slope of Eq. 13.

Results

Characterization of the R1 anti-SSu plants. The Rubisco content of the leaves of the primary anti-SSu transformants produced by Hudson et al. (1992) ranged from 2 to 34% of the mean value for control transformants and wild-type plants. We selected three individual anti-SSu transformants, S2, S4 and S10, with, respectively, 28, 29 and 32% of the Rubisco content of wild-type plants, and allowed them to self-fertilize. The R1 seeds were analysed for segregation of the kanamycin-resistance gene which was linked to the antisense gene in the T-DNA. The results were 65:21, 70:28 and 72:23 (resistant to sensitive), respectively, for the S2, S4 and S10 progeny, showing that each primary transformant had a single kanamycin-resistance locus (and by implication a single antisense gene) which segregated in a Mendelian fashion.

When germinated and grown in soil, the R1 progeny of each primary transformant could be grouped into three phenotypes, with a 1:2:1 segregation ratio, by assaying for Rubisco content, chlorophyll fluorescence and

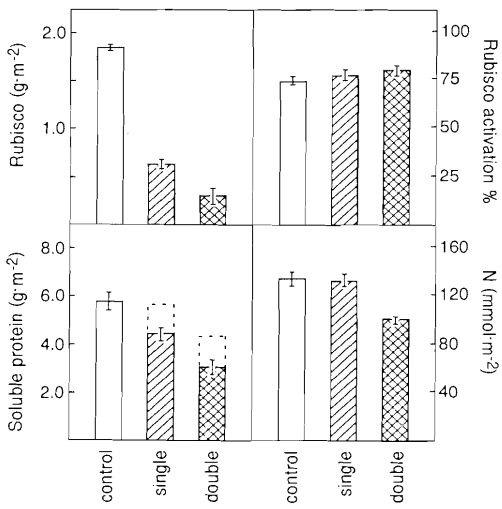

Fig. 1. Rubisco content and activation state, soluble protein and total leaf nitrogen in tobacco leaves of wild-type ($n = 7$), single-dose ($n = 7$), and double-dose ($n = 5$) R1 anti-SSu plants grown in the growth cabinet. The *dashed lines* give the amount of soluble protein there would be if Rubisco content were unaltered. Bars give standard errors

CO_2-assimilation rate. These presumably corresponded to the zero (null or wild-type), single-copy (hemizygous) and double-copy (homozygous) progeny with respect to the antisense gene. Within each phenotype, the Rubisco content varied little between the progeny of each primary transformant; therefore, the data for each phenotype were pooled. The mean Rubisco contents of the three phenotypes were 100, 34 and 15% of the wild-type content for the null, hemizygous and homozygous plants, respectively. The loss of soluble protein in the hemizygous plants corresponded to the reduction in Rubisco content, indicating that there was little decrease in other leaf proteins (Fig. 1). However, the deficiency in soluble protein in the homozygous plants exceeded the loss of Rubisco protein. These results were reflected in the nitrogen content of the leaves (Fig. 1). There was little difference in leaf nitrogen between the wild-type and hemizygous plants but, in the homozygous plants, leaf nitrogen was reduced. Masle et al. (1993) made similar observations with progeny of anti-SSu transformants.

There was little difference in Rubisco's activation state between wild-type, hemizygous and homozygous plants when measured at the growth irradiance of 1000 µmol quanta $\cdot m^{-2} \cdot s^{-1}$ (Fig. 1). The mean percentage activation measured at this irradiance was 77%. At 1500 µmol quanta $\cdot m^{-2} \cdot s^{-1}$, the value was 80% (data not shown).

Because of the general reduction in leaf protein observed in the homozygous anti-SSu plants, we used only the hemizygous R1 progeny for our study of Rubisco's kinetic parameters in vivo. Under strong illumination, the response of the CO_2-assimilation rate to intercellular CO_2 partial pressure, p_i, for wild-type and hemizygous anti-SSu plants, showed that CO_2 assimilation by the anti-SSu plant was limited only by Rubisco activity, even at high p_i (Fig. 2). The wild-type plant showed the usual limitation of photosynthesis at high p_i by the capacity for RuBP regeneration.

The CO_2-transfer conductance, g_w: To estimate the kinetic constants of Rubisco, the partial pressure of CO_2 at the sites of carboxylation, p_c, must be known. Because the fractionation of carbon isotopes which occurs during dissolution and diffusion of CO_2 through liquid differs from that which occurs during carboxylation by Rubisco, carbon-isotope discrimination during CO_2 assimilation can be used to estimate p_c and to calculate the conductance for transfer or CO_2 from the substomatal cavities to the sites of carboxylation, g_w (Evans et al. 1986). We expect g_w to be constant for a leaf at full expansion for several days because it depends on the anatomy of the leaf. We made simultaneous measurements of carbon-isotope discrimination and CO_2-assimilation rates with anti-SSu plants and obtained values of g_w of 0.27 and 0.38, respectively, for anti-SSu plants grown in the glasshouse and in the growth cabinet. These values were between 20 to 27% lower than those of similarly grown wild-type plants. A detailed study relating g_w to leaf anatomy is given elsewhere (Evans et al. 1994). Glasshouse-grown plants were used for estimating K_c, K_o and Γ_* from gas-exchange measurements and we used a mean value of g_w of 0.3 mol $\cdot m^{-2} \cdot s^{-1}$ bar^{-1} for these calculations.

Γ_* and day respiration. The value of p_c at which photorespiratory CO_2 evolution equals carboxylation is defined as Γ_*. We estimated Γ_* from the intersection of CO_2-response curves measured at various low irradiances as described by Laisk (1977) and Brooks and Farquhar (1985) (Fig. 3). Laisk (1977) showed, without at the time considering a drop in CO_2 pressure from the air spaces to the chloroplast, that the (negative) CO_2-assimilation rate at Γ_* does not vary with changes in irradiance, and equals $-R_d$, the "day" respiration other than photorespiratory CO_2 release. When a CO_2-transfer conductance

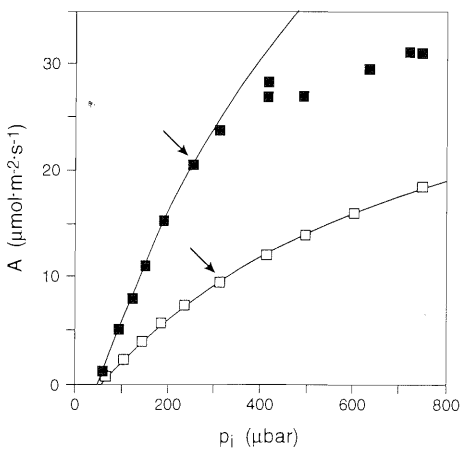

Fig. 2. Rate of CO_2 assimilation (A) as a function of intercellular CO_2 partial pressure (p_i) for a leaf of a control (■) and an anti-SSu (□) plant. Measurements were made at an irradiance of 1000 µmol quanta $\cdot m^{-2} \cdot s^{-1}$ and a leaf temperature of 25° C. The lines are the predicted A from Eqs. 8 and 10 with the kinetic constants from Table 1. The *arrows* indicate the points obtained at an external CO_2 partial pressure of 350 µbar

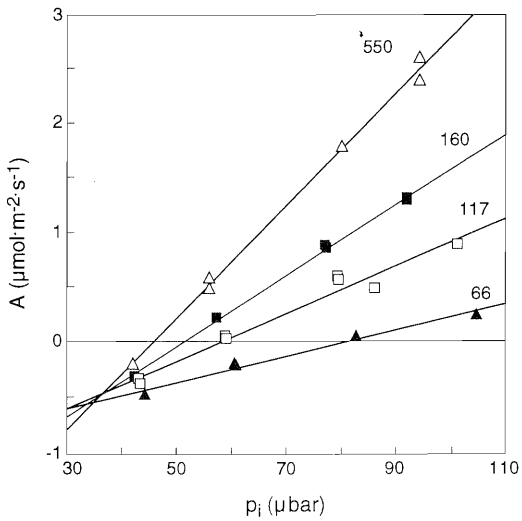

Fig. 3. Rate of CO_2 assimilation (A) of an anti-SSu tobacco leaf as a function of intercellular CO_2 partial pressure (p_i). Measurements were made at four irradiances (µmol quanta $\cdot m^{-2} \cdot s^{-1}$) as indicated in the figure, and at a leaf temperature of 25° C. Lines were fitted by least-squares regression and the mean of the co-ordinates of their intersections were taken as estimates of p_i^* and $-R_d$

Table 1. Rubisco kinetic constants for tobacco calculated from gas-exchange analysis

	$g_w = 0.3$ ($mol \cdot m^{-2} \cdot s^{-1} \cdot bar^{-1}$)	$g_w = \infty$ ($mol \cdot m^{-2} \cdot s^{-1} \cdot bar^{-1}$)
K_c	259 ± 57 μbar, (8.6 ± 1.7 μM)	404 ± 51 μbar, (13.5 ± 1.7 μM)
K_o	179 mbar, (226 μM)	248 mbar, (313 μM)
$K_c(1 + O/K_o)$ at 200 mbar O_2	549 μbar, (18.3 μM)	730 μbar, (24.4 μM)
Γ_*[a,b]	38.6 μbar	36.9 ± 0.5 μbar
$S_{c/o}$[a,b]	97.5	102
V_{omax}/V_{cmax}	0.255	0.226
k_{cat} (mol CO_2·(mol sites)$^{-1}$·s^{-1}	3.53 ± 0.18	3.64 ± 0.22
k_{cat} (mol CO_2·(mol sites)$^{-1}$·s^{-1} (in vitro)	2.87 ± 0.08	

[a] To convert values from partial pressures to concentrations, solubilities for CO_2 of 0.0334 mol·(1 bar)$^{-1}$ and for O_2 of 0.00126 mol·(1 bar)$^{-1}$ were used. Atmospheric pressure in Canberra averages 953 mbar

[b] Measurements were also made of *Spinacea oleracea* for comparison, assuming $g_w = \infty$, $\Gamma_* = 33.6 \pm 0.4$ μbar and $S_{c/o} = 112$

is incorporated, the equation relating A to p_i is quadratic but it is readily shown that the intersection of such curves measured at different irradiances still occurs at $-R_d$. However, the intersection does not occur at a p_i of Γ_*. Rather, it occurs when $p_i = \Gamma_* + R_d/g_w$ (von Caemmerer and Evans 1991). We estimated Γ_* at 200 mbar O_2 (21% O_2 in Canberra) on three individual wild-type tobacco leaves and the mean value of the intersection was 36.9 ± 0.5 μbar and R_d was 0.54 ± 0.02 μmol·m^{-2}·s^{-1}. Thus the mean Γ_* was 38.6 μbar. This gives an $S_{c/o}$ of 2590 when expressed in terms of the CO_2/O_2 partial pressure ratio of the gas phase, or 97.5 when expressed in terms of the ratio of the CO_2 and O_2 concentrations in solution (Table 1). If the CO_2-transfer conductance is assumed to be infinite, the analogous values of $S_{c/o}$ are 2710 or 102. The two values differ by 4.5%. As a comparison, we also measured $\Gamma_* + R_d/g_w$ for three leaves of spinach and obtained a mean value of 33.6 ± 0.4 μbar and a $R_d = 0.32 \pm 0.03$ μmol·m^{-2}·s^{-1}. This gives an $S_{c/o}$ of 2975 or 112 if $g_w = \infty$. We did not measure g_w for spinach.

Rate of CO_2 assimilation at different CO_2 and O_2 concentrations. To estimate the kinetic parameters of Rubisco in the anti-SSu leaves, CO_2 assimilation was measured at varying partial pressures of CO_2 and O_2 (Fig. 4A). The data in terms of A and p_i were then transformed to be in terms of $A + R_d$ and $p_c - \Gamma_*$ as described in *Materials and methods*, using a value of 0.3 mol·m^{-2}·s^{-1}·bar^{-1} for g_w. The hyperbolic nature of the curves becomes readily apparent (Fig. 4B). The data for each O_2 partial pressure were fitted to the Michaelis-Menten equation to estimate the parameters V_{cmax} and $K_c(1 + O/K_o)$. These parameters were then plotted as a function of O_2 partial pressure. Five complete data sets for measurements with different leaves are summarised (Fig. 5). As expected, O_2 behaved as a classic competitive inhibitor of carboxylation. This is shown by the linear dependence of $K_c(1 + O/K_o)$ on O (Fig. 5A) and the independence of V_{cmax} on O (Fig. 5B). The value of K_c can be calculated from the y-intercept of the fitted line in Fig. 5A. Similarly, K_o can be calculated from the slope. Because the analysis is dependent on the

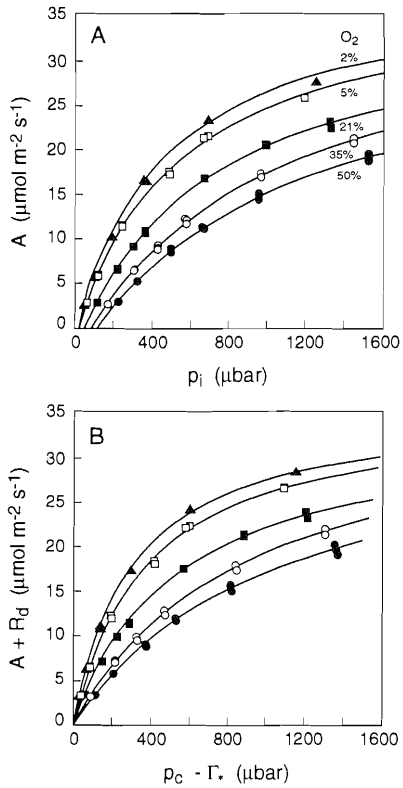

Fig. 4. A Rate of CO_2-assimilation (A) of an anti-SSu tobacco leaf as a function of intercellular CO_2 partial pressure (p_i) at five different O_2 concentrations. Measurements were made at an irradiance of 1500 μmol quanta·m^{-2}·s^{-1} and a leaf temperature of 25° C. **B** The data are redrawn as gross CO_2 assimilation ($A + R_d$) versus the CO_2 partial pressure at the carboxylation sites minus Γ_* ($p_c - \Gamma_*$) to show the hyperbolic nature of the curves. The lines in **A** and **B** are fitted using Eqs. 9 and 10 with $g_w = 0.3$ mol·m^{-2}·s^{-1}·bar^{-1}. $R_d = 0.5$ μmol·m^{-2}·s^{-1}, 21% O_2 is equivalent to 200 mbar in Canberra

value of g_w used, we analysed the data in two ways. The first analysis was made with the inclusion of the measured g_w (Figs. 4, 5) and the second analysis with the extreme assumption that there is no drop in CO_2 partial

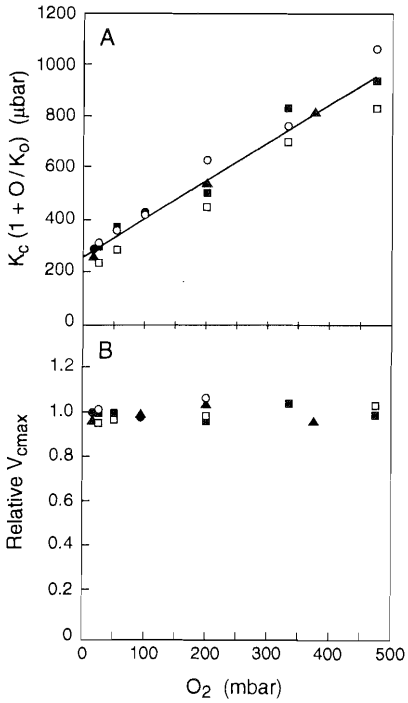

Fig. 5. A The apparent K_m (CO_2) as a function of the partial pressure of O_2. Estimates were obtained by subtracting Γ_* from the $\Gamma_*+K_c(1+O/K_o)$ parameter derived from fitted CO_2-response curves similar to those shown in Fig. 4B. Calculations assumed $g_w = 0.3\ mol \cdot m^{-2} \cdot s^{-1} \cdot bar^{-1}$. Different symbols denote different leaves. **B** V_{cmax} as a function of the partial pressure of O_2. The V_{cmax} parameter estimates from fitted CO_2-response curves similar to those shown in Fig. 4B were normalized by dividing by the mean values for each leaf (ranging from 35 to 42 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Calculations assumed $g_w = 0.3\ mol \cdot m^{-2} \cdot s^{-1} \cdot bar^{-1}$. Different symbols denote different leaves

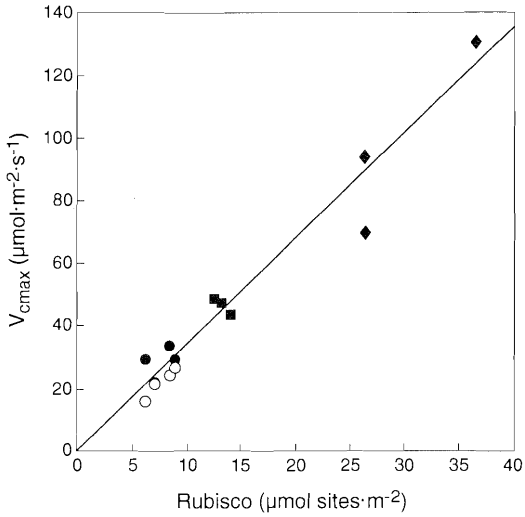

Fig. 6. In-vivo (●, ■, ◆) and in-vitro (○) maximum carboxylation rate (V_{cmax}) as a function of leaf Rubisco content. The line was fitted by least-square regression through the origin, $y = 3.341\ x$. (The unconstrained regression was, $y = -3.55 + 3.18\ x$, $r^2 = 0.944$.) V_{cmax} was estimated from the initial slopes of CO_2-response curves on control (◆) or anti-SSu plants (■) and these plants were grown in the growth cabinet. Otherwise, V_{cmax} was derived as shown in Fig. 4 (●) and from in-vitro (○) measurements on the same leaves. These plants were grown in the glasshouse

pressure between intercellular airspace and the sites of carboxylation, i.e., $g_w = \infty$ and $p_c = p_i$ (not shown). The results of both analyses are given in Table 1. The estimate of K_c is inversely related to the value assumed for g_w, (Eq. 14). The inclusion of a finite g_w lowered the estimated K_c and K_o but had only a marginal effect on the estimate of V_{omax}/V_{cmax} which was calculated from the Γ_* and K_c and K_o values.

Measurements of k_{cat} in vivo and in vitro. We calculated the in-vivo k_{cat} (V_{cmax}/[active sites]) in two ways. In the first instance, V_{cmax} was derived from gas-exchange measurements as shown in Figs. 4 and 5; in the second, V_{cmax} was estimated from initial slopes of A versus p_i curves (Eq. 12). In both instances, the amount of Rubisco in leaf extracts was measured by the stoichiometric binding of [^{19}C]carboxyarabinitol bisphosphate and the activity in vitro with saturating CO_2 and RuBP was determined on the same leaf extracts. The second type of measurement was made on anti-SSu and wild-type plants grown in the growth cabinet. For both methods of measurement, V_{cmax} correlated linearly with Rubisco content (Fig. 6). However, the in-vitro activity measurements lay on a line with a 20% smaller slope (k_{cat}) than the in-vivo measurements. Both of these values are reported in Table 1. For the in-vivo measurements, we have assumed that Rubisco was fully activated. We are unsure whether the experimentally observed activation level (80% activation at 1500 µmol quanta $\cdot m^{-2} \cdot s^{-1}$) could reflect some loss of carbamylation during extraction (Sage et al. 1993). If the activation state is included in the calculation, the in-vivo k_{cat} increases by 20% to $4.24 \cdot s^{-1}$.

Discussion

Catalytic parameters of Rubisco inferred from whole-leaf studies. It is attractive to study Rubisco in its natural environment since extraction and purification of enzymes may cause loss of activity. Furthermore, Rubisco exists in the chloroplast stroma at high concentrations which are difficult to mimic in vitro. The success of studies on the intact leaf, however, depend on how well we can describe and manipulate the chloroplast environment and the extent to which other processes interfere with our measurements of Rubisco activity. Measurements on whole leaves average over a large population of chloroplasts. Because the tobacco leaf is relatively thin, we felt that the problem of light and CO_2 gradients across the leaf would be small and that these leaves were, therefore, well suited to this analysis.

It is clear that, during CO_2 uptake, a CO_2 concentration gradient must exist from the air surrounding the leaf to the sites of carboxylation. As is common practice, we calculated the CO_2 concentration in the substomatal cavities from external CO_2 partial pressures and measurements of water-vapour exchange. Measurements of carbon-isotope discrimination were used to estimate the CO_2 gradient from the substomatal cavities to the sites of carboxylation (Evans et al. 1986; von Caemmerer and Evans 1991). The measurements of the CO_2-transfer conductance, which is constant for a given leaf and depends

on its anatomy, are similar to our previous measurements and those obtained by other reseachers who have used a variety of techniques (Lloyd et al. 1992; Loreto et al. 1992). However, because the calculation of the conductance, g_w, unavoidably rests on a number of assumptions, we have also analysed our data with the extreme assumption that no significant CO_2 drawdown from substomatal cavities to the sites of carboxylation occurred.

In the anti-SSu plants, where V_{cmax} can be obtained from data similar to those shown in Fig. 4B, the CO_2-transfer conductance, g_w, can also be estimated from the y-axis intercept of the plot of $1/g_m$ versus O, $((1/g_w)+(K_c/V_{cmax}))$ (Eq. 13) with the assumption of a K_c value. Using the K_c value of Makino et al. (1988) (Table 2), we calculated a value of g_w that was similar to that obtained from the carbon-isotope method (data not shown).

An in-vivo estimate of Γ_* and $S_{c/o}$ for tobacco Rubisco. The CO_2 uptake of leaves is the net result of carboxylation of RuBP by Rubisco, CO_2 loss through the photorespiratory cycle and a small amount of CO_2 loss through other respiratory processes. We measured Γ_* in order to distinguish between the photorespiratory and non-photorespiratory components of CO_2 release and, thus, to calculate Rubisco's relative specificity factor, $S_{c/o}$, in vivo. Our estimate for tobacco of 97.5 or 102 (depending on the assumption about g_w) is similar to other values of $S_{c/o}$ derived from gas-exchange measurements. Brooks and Farquhar (1985) obtained a value of 94.5 for spinach and of 101.6 for wheat assuming an infinite g_w. However, our spinach value of 112 ($g_w = \infty$) is greater than the spinach value obtained by Brooks and Farquhar (1985) and we cannot offer an explanation for this discrepancy. Similar procedures were used in both instances and the gas-exchange systems were carefully calibrated for accurate measurements at low CO_2 partial pressures.

In-vitro measurements of $S_{c/o}$ have shown that it varies only slightly between C_3 species. Jordan and Ogren (1981) observed values of $S_{c/o}$ in the range 77–82, Parry et al. (1987) obtained values in the range 77.5–103.7 and Kane et al. (1994) measured values in the range 78.6–89.9. Values for tobacco Rubisco have ranged from 77 to 94.1 in these studies. Gas-exchange values of $S_{c/o}$ are consistently 10–20% greater than in-vitro values (Jordan and Ogren 1981; Parry et al. 1987; Kane et al. 1994). Several explanations for the difference between the in-vitro and in-vivo estimates may be imagined. First, it is possible that other carboxylations, such as anapleurotic phosphoenolpyruvate carboxylations for example, could enhance carboxylation in vivo relative to oxygenation. This requires that the other carboxylations co-vary with Rubisco carboxylation as irradiance and CO_2 partial pressure vary; otherwise, the common intersection of CO_2-response curves measured in differing illumination would not be observed (Fig. 3). Second, discrepancies can arise because of different inter- and intracellular diffusion patterns. Brooks and Farquhar (1985), who give a detailed discussion on the complexities associated with the measurements of Γ_*, cautioned that their observed differences in $S_{c/o}$ between spinach and wheat need not reflect differences in Rubisco properties for these reasons. In the model used here, the CO_2-transfer conductance, g_w, considers a CO_2 gradient between intercellular airspaces and the chloroplast and mitochondria, but no gradient is assumed between the organelles. Because the flux of CO_2 is low near Γ_*, inclusion of g_w has only a small effect, increasing the estimated Γ_* (and thus decreasing $S_{c/o}$) by approximately 4%. Indeed, to explain higher in-vivo $S_{c/o}$ values, carboxylation needs to be enhanced relative to oxygenation, compared to the in-vitro situation. This could occur if there was a CO_2-concentrating mechanism at the chloroplast membrane; however, there is, at present, no convincing evidence to suggest that this is the case. Third, it is possible that photorespiratory CO_2 release may be less than 0.5 CO_2 per oxygenation, in which case $S_{c/o}$ would be overestimated by Eq. 5. This would require that some of the glycolate formed in the photorespiratory pathway is used in other synthetic processes (see also Harley and Sharkey 1991 for discussion). However, as implied by the work of Hanson and Peterson (1986) and Zelitch (1989), the CO_2 release could be greater, making the discrepancy between in-vivo and in-vitro estimates of $S_{c/o}$ even larger. Last, it is possible that $S_{c/o}$ of Rubisco is indeed greater in-vivo where Rubisco is present at high concentrations. The above discussion emphasises that it may be difficult to resolve small differences in $S_{c/o}$ amongst C_3 species from in-vivo measurements of Γ_*.

Estimation of K_c and K_o from gas-exchange measurements. Lilley and Walker (1975) and Ku and Edwards (1977) showed that double-reciprocal plots of CO_2-assimilation rate versus CO_2 partial pressure in C_3 leaves deviate drastically from linearity at high CO_2. This indicates that CO_2-assimilation rate and carboxylation are limited by other processes, such as the regeneration of RuBP, at high CO_2 partial pressures. However, with anti-SSu tobacco, our results (Fig. 4) confirm that the capacity for RuBP regeneration is high relative to Rubisco activity such that CO_2 assimilation rate is Rubisco-limited at all measured CO_2 partial pressures (Hudson et al. 1992). As a result, the CO_2-assimilation rate of these plants was stimulated by a reduction in O_2 concentration from 21% to 2% even at CO_2 partial pressures above 1000 µbar. This is usually not observed in leaves of C_3 species, where other limitations, such as the rate of resupply of inorganic phosphate to the chloroplast following conversion of triose phosphate to sucrose or starch, often negates the stimulation of CO_2 assimilation by low O_2 pressures (Sharkey 1990).

The estimates of K_c and K_o were dependent on the value of g_w chosen for the analysis, whereas the estimate of V_{cmax} (k_{cat}) was almost independent of this assumption (Table 1). In our analysis, the estimate of K_c ranged from 259 µbar at our measured g_w of 0.3 $mol \cdot m^{-2} \cdot s^{-1} \cdot bar^{-1}$ to 404 µbar at $g_w = \infty$, and K_o varied between 179 mbar and 248 mbar. Equation 14 shows how K_c at $g_w = \infty$ is related to the true K_c and gives the opportunity to interpolate K_c and K_o for different values of g_w.

There have been only a few in-vivo determinations of Rubisco kinetic parameters. The most comprehensive set is by Harley et al. (1985), who restricted their analysis to

low CO_2 partial pressures. However they did not measure Γ_* or day respiration and their estimates vary according to whether they assume that mitochondrial respiration takes place to the same extent in the light as in the dark or not at all. Their values of K_c and K_o were 478 μbar and 210 mbar, respectively, when respiration was assumed to be zero in the light or 318 μbar and 185 mbar, respectively, when they assumed that respiration proceeded at the same rate in the light as in the dark. Thus they probably overestimated the respiration occurring in the light (Brooks and Farquhar 1985) and they did not consider a drop in CO_2 partial pressure from the intercellular airspace to the site of carboxylation.

In-vitro measurements of K_c vary widely. Yeoh et al. (1981) measured K_c in 28 different C_3 species and reported values from 359 to 778 μbar (12–26 μM). Jordan and Ogren (1981) measured a range from 269 to 479 μbar (9–16 μM) for five C_3 species and their value for tobacco was 329 μbar (11 μM). Measurements by Seemann et al. (1981) and Seemann and Berry (1982) were 310 μbar (10.4 μM). Makino et al. (1988) measured K_c by a rapid assay with crude leaf extracts in several C_3 species and found values from 203 to 326 μbar (6.8–10.9 μM). Their value for tobacco was 293 μbar (9.8 μM). Our measurements suggest that the in-vivo K_c is at the low end of the in-vitro measurements reported.

The oxygenase activity of Rubisco has not been measured as frequently as carboxylation and fewer in-vitro values of K_o exist in the literature. In Table 2, in-vitro kinetic constants of Rubisco from C_3 species are collated from experiments where both carboxylase and oxygenase activity have been measured. The measured values of K_o range from 156 mbar (196 μM) to 516 mbar (650 μM). Jordan and Ogren (1981) measured a value of 516 mbar for tobacco. Again our value of 179 mbar is within the range of in-vitro measurements although on the low side. The K_o of Rubisco is an important parameter determining the CO_2-assimilation rate. In conjunction with K_c, it defines the apparent $K_m(CO_2)$ of Rubisco at the ambient O_2 concentration of 21%. We estimate that, at the measured g_w, the apparent $K_m(CO_2)$ at 21% O_2 is 549 μbar. This is approximately twice the intercellular CO_2 partial pressure observed under normal ambient CO_2 conditions (Table 1).

The greatest uncertainty in the kinetic constants surrounds the ratio of V_{omax}/V_{cmax}. Badger and co-workers determined both carboxylase and oxygenase activity and obtained a value of 0.22 (Table 2). Makino et al. (1988) calculated values of 0.29 and 0.33 in wheat and rice from measurements of carboxylase and oxygenase activity. However, Jordan and Ogren (1981, 1984) calculated this value from their measurements of $S_{c/o}$, K_c and K_o and arrived at a higher value of 0.54. Our calculation of this ratio from Γ_*, K_c and K_o agrees with the low estimates of Badger and Andrews (1974) and Badger and Collatz (1977).

Measurements of in-vivo and in-vitro k_{cat}. These transgenic anti-SSu plants have provided the first opportunity to calculate the k_{cat} of Rubisco's carboxylase activity in vivo from measurements of CO_2-assimilation rates at high CO_2 partial pressure (Fig. 4). This is not possible in wild-type plants because the CO_2-assimilation rate at high CO_2 partial pressures reflects limitations to CO_2 uptake other than Rubisco. Alternatively, k_{cat} may be estimated with wild-type leaves from the initial slope of the response of CO_2-assimilation rate to CO_2 partial pressure, provided that the values of K_c and K_o are known (see *Materials and methods*). We found a good agreement between the two methods, using our measured kinetic con-

Table 2. In-vitro kinetic constants[a] of Rubisco from several C_3 species

Species	k_{ccat} (mol·(mol sites)$^{-1}$·s^{-1})	K_c (μM)	K_c (μbar)	k_{ocat} (mol·(mol sites)$^{-1}$·s^{-1})	K_o (μM)	K_o (mbar)	V_{omax}/V_{cmax}	$S_{c/o}$[e]	Reference[b]
Atriplex glabriuscula	1.7	21	629	0.31	328	260	0.18	85	Badger and Collatz (1977)
Spinacea oleracea		13.6	407		354	280[c]	0.22	120	Badger and Andrews (1974)
		15.2[d]	455		196	156	0.22	60	
Glycine max		9	269		430	341[c]	0.58	82	Jordan and Ogren (1981)
Tetragonium expansa		13	389		600	476[c]	0.55	81	
S. oleracea		14	419		480	381[c]	0.43	80	
Lolium perenne		16	479		500	397[c]	0.38	80	
Nicotiana tabacum		11	329		650	516[c]	0.77	77	
S. oleracea	1.7	11	329	0.88	500	397[c]	0.52	88	Jordan and Ogren (1984)
Triticum aestivum	3.0	11.2	335	0.86	383	304	0.29	120	Makino et al. (1988)
Oryza sativa	2.0	8.0	239	0.66	335	266	0.33	128	

[a] All values were measured at 25° C. To convert K_c and K_o values from concentration to partial pressures the solubilities for CO_2 of 0.0334 mol·(1·bar)$^{-1}$ and for O_2 of 0.00126 mol·(1·bar)$^{-1}$ were used

[b] K_c values by Badger and Collatz (1977) and Badger and Andrews (1974) were measured in 100 mM Hepes pH 8.3. The values had been calculated with a pKa = 6.37 and were recalculated here with a pKa = 6.12. Measurements by Jordan and Ogren (1981), (1984) were made with 50 mM Bicine buffer pH 8.3 and a pKa = 6.23 used. Measurements by Makino et al. (1988) were made in 100 mM Bicine pH 8.15 and a pKa = 6.12 was used

[c] Measured as $Ki(O_2)$

[d] Measured as $Ki(CO_2)$

[e] $S_{c/o}$ values by Jordan and Ogren (1981, 1984) were derived from simultaneous measurements of carboxylation and oxygenation. Other $S_{c/o}$ values were calculated from the individual constants given in the Table

stants at $g_w = 0.3\,mol \cdot m^{-2} \cdot s^{-1}$ bar^{-1} (Fig. 6). Our in-vitro specific activity is higher than the one previously reported for tobacco (Hudson et al. 1992) but is similar to other recent in-vitro estimates for tobacco (Mate et al. 1993). The estimates are comparable to in-vitro estimates reported for wheat Rubisco of 3.0–3.8 mol $CO_2 \cdot$(mol sites)$^{-1} \cdot s^{-1}$ (Evans and Seemann 1984, Makino et al. 1988). The small observed difference between our in-vivo and in-vitro k_{cat} may be attributed to incomplete recovery of Rubisco activity in the crude leaf extracts.

References

Badger, M.R., Andrews, T.J. (1974) Effects of CO_2, O_2 and temperature on a high-affinity form of ribulose diphosphate carboxylase-oxygenase from spinach. Biochem. Biophys. Res. Commun. **60,** 204–210

Badger, M.R., Collatz, G.J. (1977) Studies on the kinetic mechanism of ribulose-1,5-bisphosphate carboxylase and oxygenase reactions, with particular reference to the effect of temperature on kinetic parameters. Carnegie Inst. Washington Yearb. **76,** 355–361

Brooks, A., Farquhar, G.D. (1985) Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Planta **165,** 397–406

Butz, N.D., Sharkey, T.D. (1989) Activity ratios of ribulose-1,5-bisphosphate carboxylase accurately reflect carbamylation ratios. Plant Physiol. **89,** 735–739

Collatz, G.J., Ball, J.T., Grivet, C., Berry, J.A. (1991) Physiological and environmental regulation of stomatal conductance, photosynthesis and transpiration: a model that includes a laminar boundary layer. Agric. For. Meteorol. **54,** 107–136

Evans, J.R., Seemann, J.R. (1984) Differences between wheat genotypes in specific activity of RuBP carboxylase and the relationship to photosynthesis. Plant Physiol. **74,** 759–765

Evans, J.R., Sharkey, T.D., Berry, J.A., Farquhar, G.D. (1986) Carbon-isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. Aust. J. Plant Physiol. **13,** 281–292

Evans, J.R., von Caemmerer, S., Setchell, B.A., Hudson, G.S. (1994) The relationship between CO_2 transfer conductance and leaf anatomy in transgenic tobacco with a reduced content of Rubisco. Aust. J. Plant Physiol. **21,** 475–495

Farquhar, G.D., von Caemmerer, S. (1982) Modelling of photosynthetic responses to environmental conditions. In: Encyclopedia of plant physiology, N.S., vol. 12B: Physiological plant ecology II. Water relations and carbon assimilation, pp. 549–587, Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H., eds. Springer-Verlag, Berlin

Farquhar, G.D., von Caemmerer, S., Berry, J.A. (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta **149,** 78–90

Hall, A.E. (1979) A model of leaf photosynthesis and respiration for predicting carbon dioxide assimilation in different environments. Oecologia **14,** 299–316

Hanson, R.K., Peterson, R.B. (1986) Regulation of photorespiration in leaves: evidence that the fraction of ribulose bisphosphate oxygenated is conserved and stoichiometry fluctuates. Arch Biochem Biophys **246,** 332–346

Harley, P.C., Sharkey, T.D. (1991) An improved model of C_3 photosynthesis at high CO_2: Reversed O_2 sensitivity explained by lack of glycerate reentry into the chloroplast. Photosynth. Res. **27,** 169–178

Harley, P.C., Weber, J.A., Gates, D.M. (1985) Interactive effects of light, leaf temperature, CO_2 and O_2 on photosynthesis in soybean. Planta **165,** 249–263

Hewitt, E.J., Smith, T.A. (1975) Plant Mineral Nutrition. English University Press, UK

Hudson, G.S., Evans, J.R., von Caemmerer, S., Arvidsson, Y.B.C., Andrews, T.J. (1992) Reduction of ribulose-bisphosphate carboxylase/oxygenase content by antisense RNA reduced photosynthesis in tobacco plants. Plant Physiol. **98,** 294–302

Jensen, R.G., Bahr, J.T. (1977) Ribulose 1,5-bisphosphate carboxylase-oxygenase. Annu. Rev. Plant Physiol. **29,** 379–400

Jordan, D.B., Ogren, W.L. (1981) Species variation in the specificity of ribulose carboxylase/oxygenase. Nature **291,** 513–515

Jordan, D.B., Ogren, W.L. (1984) The CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase. Planta **161,** 308–313

Kane, H.J., Viil, J., Entsch, B., Paul, K., Morell, M.K., Andrews, T.J. (1994) An improved method for measuring the CO_2/O_2 specificity of ribulosebisphosphate carboxylase-oxygenase. Aust. J. Plant. Physiol. **21,** 449–461

Ku, S.B., Edwards, G.E. (1977) Oxygen inhibition of photosynthesis. II. Kinetic characteristic as affected by temperature. Plant Physiol. **59,** 591–599

Laing, W.A., Ogren, W., Hageman, R. (1974) Regulation of soybean net photosynthetic CO_2 fixation by the interaction of CO_2, O_2 and ribulose-1,5, diphosphate carboxylase. Plant Physiol. **54,** 678–685

Laisk, A.K. (1977) Kinetics of photosynthesis and photorespiration in C_3 plants. (In Russ.) Nauka. Moscow

Lilley, R.McC., Walker, D.A. (1975) Carbon dioxide assimilation by leaves, isolated chloroplasts and ribulose bisphosphate carboxylase from spinach. Plant Physiol. **55,** 1087–1092

Lloyd, J., Syvertsen, P.J., Kriedemann, P.E., Farquhar, G.D. (1992) Low conductance for diffusion from stomata to the sites of carboxylation in leaves of woody species. Plant Cell Environ. **15,** 873–899

Loreto, F., Harley, P.C., Di Marco, G., Sharkey, T.D. (1992) Estimation of mesophyll conductance to CO_2 flux by three different methods. Plant Physiol. **98,** 1437–1443

Makino, A., Mae, T., Ohira, K. (1988) Differences between wheat and rice in the enzymic properties of ribulose-1,5-bisphosphate carboxylase/oxygenase and the relationship to photosynthetic gas exchange. Planta **174,** 30–38

Masle, J., Hudson, G.S., Badger, M.R. (1993) Effects of atmospheric CO_2 concentration on growth and nitrogen use in tobacco plants transformed with an antisense gene to the small subunit of ribulose-1,5,-bisphosphate carboxylase/oxygenase. Plant Physiol. **103,** 1075–1088

Mate, C.J., Hudson, G.S., von Caemmerer, S., Evans, J.R., Andrews, T.J. (1993) Reduction of ribulose-bisphosphate carboxylase activase levels in tobacco by antisense RNA reduces ribulose-bisphosphate carboxylase carbamylation and impairs photosynthesis. Plant Physiol. **102,** 1119–1128

Parry, M.A.J., Schmidt, C.N.G., Cornelius, M.J., Millard, B.N., Burton, S., Gutteridge, S., Dyer, T.A., Keys, A.J. (1987) Variations in properties of ribulose-1,5-bisphosphate carboxylase from various species related to different amino acid sequences. J. Exp. Bot. **38,** 1260–1271

Peisker, M., Apel, P. (1975) Influence of oxygen on photosynthesis and photorespiration in leaves of *Triticum aestivum* L. 1. Relationship between oxygen concentration, CO_2 compensation point, and intracellular resistance to CO_2 uptake. Photosynthetica **9,** (1) 16–23

Sage, R.S., Reid, C.D., Moore, B.D., Seemann, J.R. (1993) Long-term kinetics of the light-dependent regulation of ribulose-1,5-bisphosphate carboxylase/oxygenase activity in plants with and without 2-carboxyarabinotol 1-phosphate. Planta **191,** 220–230

Seemann, J.A., Berry, J.A. (1982) Interspecific differences in the kinetic properties of RuBP carboxylase protein. Carnegie Inst. Washington. Yearb. **81,** 78–83

Seemann, J.A., Tepperman, J.M., Berry, J.A. (1981) The relationship between photosynthetic performance and the levels and kinetic properties of RuBP carboxylase-oxygenase from desert winter annuals. Carnegie Inst. Washington Yearb. **80,** 67–72

Sellers, P.J., Berry, J.A., Collatz, G.J., Field, C.B., Hall, F.G. (1992) Canopy reflectance, photosynthesis and transpiration. III. A reanalysis using improved leaf models and improved canopy integration scheme. Remote Sensing Environ. **42,** 187–216

Sharkey, T.D. (1990) Feedback limitations of photosynthesis and the physiological role of ribulose-1,5-bisphosphate carboxylase carbamylation. Bot. Mag. Tokyo special issue **2,** 87–105

von Caemmerer, S., Farquhar, G.D. (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153,** 376–387

von Caemmerer, S., Evans, J.R. (1991) Determination of the average partial pressure of CO_2 in chloroplasts from leaves of several C_3 plants. Aust. J. Plant Physiol. **18,** 287–305

von Caemmerer, S., Evans, J.R., Hudson, G.S., Arvidsson, Y.B.C., Setchell, B.A., Andrews, T.J. (1992) Photosynthesis in transgenic tobacco plants with reduced amount of Rubisco contents. In: Research in photosynthesis, vol. IV, pp. 595–602, Murata, N., ed. Kluwer Academic Publishers, Dordrecht

Yeoh, H.-H., Badger, M.R., Watson, L. (1981) Variations in kinetic properties of ribulose-1,5-bisphosphate carboxylases among plants. Plant Physiol. **67,** 1151–1155

Zelitch, I. (1989) Selection and characterization of tobacco plants with novel O_2-resistant photosynthesis. Plant Physiol. **90,** 1457–1464