Journal of Experimental Botany, Vol. 36, No. 168, pp. 1117-1125, July 1985

Influence of Temperature on the Ratio of Ribulose Bisphosphate Carboxylase to Oxygenase Activities and on the Ratio of Photosynthesis to Photorespiration of Leaves

B. LEHNHERR, F. MÄCHLER AND J. NÖSBERGER

Institut für Pflanzenbau, Eidgenössische Technische Hochschule, Universitätstrasse 2, CH-8092 Zürich, Switzerland

Received 16 October 1984

ABSTRACT

Lehnherr, B., Mächler, F. and Nösberger, J. 1985. Influence of temperature on the ratio of ribulose bisphosphate carboxylase to oxygenase activities and on the ratio of photosynthesis to photorespiration of leaves.—J. exp. Bot. 36: 1117-1125.

Rates of net and gross photosynthesis of intact white clover leaves were measured by infrared gas analysis and by short term uptake of $^{14}CO_2$ respectively. Ribulose bisphosphate carboxylase oxygenase (RuBPCO) was purified from young leaves and kinetic properties investigated in combined and separate assays. The ratio of carboxylase to oxygenase activities was compared with the ratio of photosynthesis to photorespiration at various temperatures and CO_2 concentrations.

The ratio of photosynthesis to photorespiration at 30 Pa $p(CO_2)$ was consistent with the ratio of carboxylase activity to oxygenase activity when each was measured above 20 °C. However, the ratio of photosynthesis to photorespiration increased with decreasing temperature, whereas the ratio of carboxylase to oxygenase activity was independent of temperature. This resulted in a disagreement between the measurements on the purified enzyme and intact leaf at low temperature. No disagreement between enzyme and leaf at low temperature occurred, when the ratio of photosynthesis to photorespiration was determined at increased CO_2 concentrations.

The results suggest an effect of low temperature and low CO_2 concentration on the ratio of photosynthesis to photorespiration independent of the enzyme.

Key words—Ribulose bisphosphate carboxylase oxygenase, photorespiration, temperature.

Correspondence to: Institut für Pflanzenbau, Eidgenössische Technische Hochschule, Universitätstrasse 2, CH-8092, Zürich, Switzerland.

INTRODUCTION

The temperature dependence of the ratio of photosynthesis to photorespiration has been attributed to the kinetic properties of ribulose bisphosphate carboxylase oxygenase (RuBPCO) (Laing, Ogren, and Hageman, 1974; Badger and Collatz, 1978) and to a change in the solubility ratio of CO_2 to O_2 (Ku and Edwards, 1977*a*). The ratio of carboxylase to oxygenase of RuBPCO has been characterized by the CO_2/O_2 specificity factor (S) (Laing *et al.*, 1974; Jordan and Ogren, 1981):

$$S = \frac{V_c \cdot K_o}{V_o \cdot K_c} = \frac{v_c \cdot O}{v_o \cdot C} \qquad (1)$$

where V_c and V_o are maximal velocities of carboxylation and oxygenation, v_c and v_o are velocities of both reactions, K_c and K_o are Michaelis constants for CO_2 and O_2 respectively, and O and C are concentrations of O_2 and CO_2. The kinetic properties of RuBPCO are difficult to determine, but the methodology has improved in the last decade, since the requirement of CO_2 and Mg^{++} for activation of RuBPCO was recognized (Lorimer, Badger, and Andrews, 1977), and the ratio of carboxylase to oxygenase was measured in combined assays (Jordan and Ogren, 1981). However, there is still controversy as to whether the temperature influence on the ratio of photosynthesis to photorespiration is due to RuBPCO kinetics (Hall and Keys, 1983; Jordan and Ogren, 1984).

The ratio is reflected in the CO_2 compensation concentration (Γ). Laing *et al.* (1974) related Γ to the kinetic properties of RuBPCO by equation (2):

$$S_{in\ vivo} = 1/2 \cdot \frac{O}{\Gamma} \tag{2}$$

where the factor 1/2 is the proportion of CO_2 released per O_2 fixed. This enabled a direct comparison between gas exchange of leaves and kinetic properties of RuBPCO, whereby dark respiration was not considered. Ku and Edwards (1977*b*) calculated kinetic constants of photosynthesis and its oxygen inhibition at various temperatures and compared the data with RuBPCO kinetics. However, no direct comparison of purified RuBPCO has yet been made with the ratios of photosynthesis to photorespiration determined from gas exchange at air level concentrations of CO_2.

For that purpose the specificity factor *in vivo* can be determined from the differential uptake of $^{14}CO_2$ and $^{12}CO_2$ by leaves according to equation (3)

$$S_{in\ vivo} = \frac{P}{2(P-F)} \cdot \frac{O}{C_i} \tag{3}$$

where P is gross photosynthesis corrected for photorespired CO_2 refixed due to gas phase resistance, F is net photosynthesis, and C_i is CO_2 concentration in the intercellular gas space. P corresponds to the carboxylation rate (v_c) and $2(P-F)$ to the oxygenation rate (v_o). The present paper compares the effects of temperature on specificity factors *in vitro* and *in vivo* at air concentration of CO_2 and increased CO_2. The role of dark respiration, which is neglected in (3), is discussed.

MATERIALS AND METHODS

Plants of white clover (*Trifolium repens* L., native ecotype) were propagated vegetatively and grown as described earlier (Schnyder, Mächler, and Nösberger, 1984). Day/night temperatures were 20 °C/16 °C and irradiance was 400 μmol quanta m^{-2} s^{-1} (400–700 nm).

Assays with purified RuBPCO

Purification and activation: RuBPCO from young, newly unfolded white clover leaves was purified and freeze-dried as described by Mächler and Nösberger (1984). Protein was estimated according to Bensadoun and Weinstein (1976). To activate the enzyme used in *separate assays*, weighed amounts of the freeze-dried powder were dissolved in distilled water and an equal volume of 200 mol m^{-3} Tris-HCl (pH 8·4) containing 40 mol m^{-3} $MgCl_2$ and 10 mol m^{-3} $NaHCO_3$ was added. The mixture was heated at 50 °C for 20 min and then kept at 0 °C. For *combined assays*, the freeze-dried enzyme was dissolved in 100 mol m^{-3} Bicine (pH 8·2), 20 mol m^{-3} $MgCl_2$, 5·0 mol m^{-3} $NaHCO_3$ and 10 mol m^{-3} orthophosphate, heated at 40 °C for 30 min and then kept at 0 °C.

Separate assays: Procedures were based on the method of Lorimer *et al.* (1977). Carboxylase was tested in small test tubes and oxygenase in an oxygen electrode vessel (Hansatech Limited, King's Lynn, Norfolk, U.K.), both at the same temperatures and with the same buffers. One cm^3 of test mixture contained 100 mol m^{-3} Tris-HCl, 20 mol m^{-3} $MgCl_2$ and 0·4 mol m^{-3} ribulose bisphosphate. The pH

was adjusted to 8·23 at 20 °C and pH changes due to temperature effects were taken into account. The buffer was freed of CO_2 as described by Lorimer *et al.* (1977). For the carboxylase assay, $NaH^{14}CO_3$ was diluted in 'CO_2-free' water and added to the mixture in various concentrations. The reaction was started by adding the enzyme (about 100 μg protein). The mixture was stirred continuously. The reaction was stopped after 1 min by adding 0·1 cm^3 2 N HCl. An aliquot was transferred to a scintillation vial and dried at 60 °C overnight. The acid stable activity was determined by liquid scintillation. For oxygenase assays, various concentrations of O_2 were established in the oxygen electrode vessel by purging the buffer with gas mixtures of pure N_2 and O_2 using Wösthoff pumps (H. Wösthoff, Bochum, F.R.G.). The reaction was started by adding the enzyme solution which had been pre-equilibrated to the O_2 partial pressure of the assay mixture. Oxygenase activity was calculated from the initial decrease of O_2.

Combined assays were made in an oxygen electrode vessel according to the procedure of Hall and Keys (1983). The reaction buffer was 'CO_2-free' and consisted of 100 mol m^{-3} Bicine and 20 mol m^{-3} $MgCl_2$. The pH was adjusted to 8·21 at 20 °C and pH changes due to temperature effects were taken into account. Different O_2 concentrations were established by purging the medium with O_2/N_2 gas mixtures. $NaH^{14}CO_3$ (2·0 mol m^{-3}, 0·094 Ci mol^{-1}) and carbonic anhydrase (50 U cm^{-3}) were added. Enzyme (100 μg protein) was added and equilibrated for about 5 min. The actual O_2 partial pressures were 20, 38, 66 and 99 kPa. The reaction was started by adding 0·4 mol m^{-3} ribulose bisphosphate which had been pre-equilibrated to the O_2 concentration in the assay mixture. The combined test was stopped after 2 min by adding 0·1 cm^3 2 N HCl.

Oxygenase activity was determined from the O_2 consumption during the assay period; carboxylase activity was estimated from the acid stable radioactivity in the assay mixture by liquid scintillation method.

Calculations: The CO_2 concentrations in the solutions were calculated by the Henderson-Hasselbalch equation and *p*K values were calculated using tables of Harned and Bonner (1945), assuming an ionic strength of 0·06 (*p*K values were 6·326, 6·281, 6·242, 6·210 and 6·184 at 10 °C, 15 °C, 20 °C, 25 °C and 30 °C respectively). The O_2 concentrations in solutions were calculated using the solubility coefficients of standard tables (Lange's handbook of chemistry 1979). The slopes and intercepts of double reciprocal plots were determined according to the statistical method of Wilkinson (1961). The substrate specificity factor was calculated either from K_m and V_{max} values (separate assays) or from carboxylation and oxygenation rates in the combined assay.

Gas exchange analysis with intact leaves

Net photosynthesis and transpiration of attached white clover leaves were determined in an open gas exchange system using an IRGA (Binos, Leybold-Heraeus, Hanau, F.R.G.) and a dew point hygrometer (General Eastern, Watertown, Mass. U.S.A.). Air was mixed from gas cylinders using Wösthoff pumps and humidified by passing through washing flasks at 0–5 °C. The flow rate was 1·0 dm^3 min^{-1}. Light was provided by a 34 W halogen mirror projection lamp (General Electric, Cleveland, Ohio, U.S.A.). Infrared radiation was reduced by a Calflex C filter (Balzers, Balzers, Principality Liechtenstein) and a 2·5 cm water layer. A ball shaped flask containing water was located between the cuvette and the light source and provided for an equal distribution of the light on the leaf surface. The cuvette (volume 0·6 dm^3) was made of stainless steel, copper and plexiglass and contained a fan (Micronel Electronic, Tagelswangen, Switzerland). Air temperature in the cuvette was controlled by water circulating through the walls and the top of the cuvette. Leaf temperature was measured by a chromel-alumel thermocouple (0·1 mm) attached to the lower surface of the leaf. Leaf area was determined before IRGA measurements were made. Leaves were pre-adapted to experimental conditions for 30–45 min, before measurements of net photosynthesis and transpiration were taken.

$^{14}CO_2$ uptake (gross photosynthesis) was measured after net photosynthesis. A cylinder (4·0 dm^3) containing compressed dry air and $^{14}CO_2$ was prepared as described by Ludwig and Canvin (1970). Ten cm^3 air with a known amount of $^{14}CO_2$ was taken from the cylinder using a syringe and, after the system was closed, injected into the cuvette where it was rapidly distributed by the fan. After 20 s the bottom of the cuvette, where the stem of the leaf was held, was removed and the leaf was killed within 1–2 s by immersion in liquid N_2 and then burnt in a carbon oxidizer (Packard Instruments Comp., Downers Grove, Ill. U.S.A.). The CO_2 was absorbed in Carbosorb and after addition of Permafluor radioactivity was determined by liquid scintillation. The concentration of radioactivity in the cuvette at the beginning of exposure was determined in the absence of leaves: A sample of 10 cm^3 air was removed from the cuvette, about 0·5 cm^3 of inactive CO_2 added, the CO_2 absorbed in Carbosorb, Permafluor added and the radioactivity determined by liquid scintillation. The same content of radioactivity was

found in samples of 10 cm^3 air taken 1 s and 20 s after injection of $^{14}CO_2$ into the cuvette indicating rapid distribution. $^{14}CO_2$ uptake by leaves was linear for 30 s indicating that an exposure time of 20 s was sufficiently short and determinations not affected by respired $^{14}CO_2$.

Calculations: Net photosynthesis (F) was calculated according to Čatský (1971). Stomatal conductance (k_g) was determined from transpiration measurements according to Jarvis (1971). Gross photosynthesis (P) was calculated from $^{14}CO_2$ short time uptake. The application of a non-steady state system during $^{14}CO_2$ exposure brought about a slight deviation of the CO_2 concentration from that during measurement of net photosynthesis and a decrease in specific radioactivity due to the evolution of $^{12}CO_2$ respired by the leaf. The change in CO_2 concentration at the beginning of exposure due to the injection of 10 cm^3 air containing $^{14}CO_2$ (103 Pa $p(CO_2)$) into the cuvette (600 cm^3) and its decrease during exposure (1–2 Pa $p(CO_2)$) due to photosynthesis were calculated. The decrease in specific radioactivity was calculated using estimates of ratios of photorespiration to net photosynthesis (0·0–0·3, depending on temperature and CO_2 concentration). Gross photosynthesis was corrected for the deviation of the CO_2 concentration in the closed system from the concentration in the open system by using CO_2 concentration curves of photosynthesis. These corrections were made even though the effects were small and did not affect the significance of the results. Effects of temperature and barometric pressure on CO_2 concentration were taken into account. $^{14}CO_2$ uptake was corrected for discrimination against $^{14}CO_2$ diffusion in the gas phase ($k_{12}/k_{14} = 1{\cdot}0088$) and $^{14}CO_2$ carboxylation ($k_{12}/k_{14} = 1{\cdot}0568$) (O'Leary, 1981). Intercellular concentrations of CO_2 including respired CO_2 (C_i) and excluding respired CO_2 ($^{14}C_i$) were calculated by applying Fick's first law according to equations (4a) and (4b)

$$C_i = C_a - F/k_g \tag{4a}$$

$$^{14}C_i = C_a - \varphi/k_g \tag{4b}$$

where C_a was CO_2 concentration in the cuvette during measurement of net photosynthesis and φ was $^{14}CO_2$ uptake. The underestimation of gross photosynthesis (P) by the $^{14}CO_2$ uptake (φ) due to refixation of respired $^{12}CO_2$ was corrected by equation (5)

$$P = \frac{C_i}{^{14}C_i} \cdot \varphi \tag{5}$$

since the fixation rate of intercellular CO_2 is independent of its origin (from ambient air ($^{14}C_i$) or from respiration ($C_i - {}^{14}C_i$)).

The CO_2/O_2 specificity factor *in vivo* was calculated according to equation (3).

RESULTS AND DISCUSSION

The ratio of oxygenase to carboxylase *in vitro* and the ratio of photorespiration to photosynthesis *in vivo* were plotted against concentration ratios of dissolved O_2 to CO_2 in the assay medium or in the intercellular space of intact leaves, respectively (Figs 1, 2). Plots from *in vitro* experiments (Fig. 1) and from *in vivo* experiments at a light intensity of 2000 μmol quanta m^{-2} s^{-1} (Fig. 2A) were straight lines passing through the origin of the axes. Straight lines were also obtained from *in vivo* experiments at 400 μmol quanta m^{-2} s^{-1} (Fig. 2B). However, they did not pass through the origin indicating interactions with dark respiration at this lower light intensity. Specificity factors are represented by the reciprocals of the slopes of the lines. No temperature response of the slopes was obtained from *in vitro* experiments (Fig. 1), whereas the slopes from *in vivo* experiments increased with temperature, independent of light intensity, implying a decrease in specificity factors (Fig. 2). This temperature effect was not due to a change in the solubility ratio of O_2 to CO_2 since concentrations of dissolved gases were plotted.

The temperature response of specificity factors *in vitro* was compared with specificity factors *in vivo* in Fig. 3. Both were of the same magnitude at high temperature. They diverged at low temperature. The specificity factors *in vivo* increased with decreasing temperature, whereas no temperature response was found *in vitro*, independent of the method of assay. It is not fully understood why a difference in size of specificity factors *in vitro* occurred in

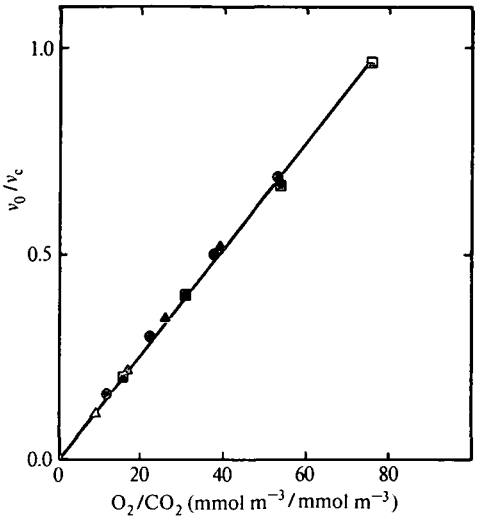

FIG. 1. The ratio of v_o to v_c of purified RuBPCO in relation to the ratio of dissolved O_2 to CO_2, determined in combined assays at 10 °C (■), 20 °C (●) and 30 °C (▲). $NaHCO_3$ concentration was kept constant (2·0 mol m^{-3}) and O_2 concentration was varied. The reciprocal of the slope represents the specificity factor *in vitro*. Means of two or three determinations are shown.

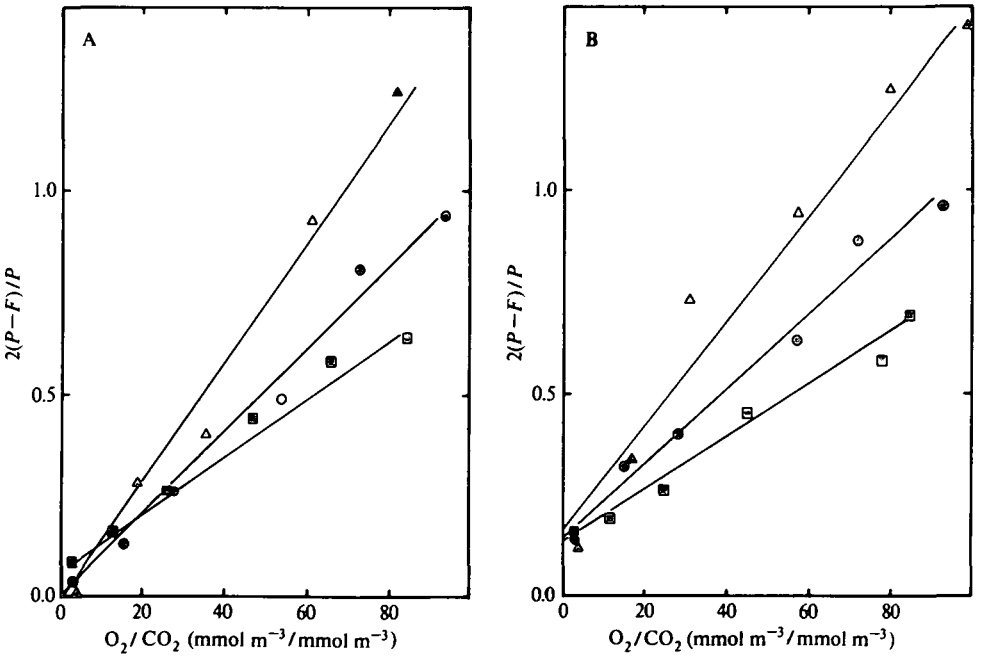

FIG. 2. The ratio of photorespiration to gross photosynthesis of leaves as a function of the ratio of dissolved O_2 to CO_2. Light intensity was 2000 μmol quanta m^{-2} s^{-1} (A) and 400 μmol quanta m^{-2} s^{-1} (B). Temperatures were 10 °C (▣), 20 °C (⊖) and 30 °C (▲). Concentration of dissolved CO_2 was in equilibrium with 30 Pa $p(CO_2)$ in the gas phase and O_2 concentration was varied. The reciprocals of slopes represent the specificity factors *in vivo*. Means of two determinations are shown.

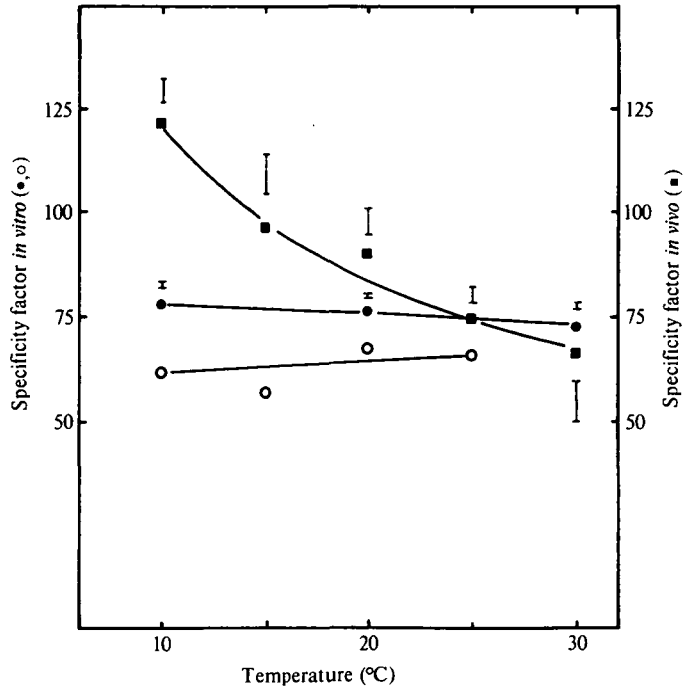

FIG. 3. Effect of temperature on the substrate specificity factors *in vivo* as calculated from gas exchange experiments at 30 Pa $p(CO_2)$ and 21 kPa $p(O_2)$ (■), and *in vitro* as calculated from combined (●) and separate (○) assays. Calculations of specificity factors are based on concentrations of dissolved gases. Means of two (■) or three (●) determinations are shown. Calculations from kinetic constants (○) are based on four determinations. Vertical bars indicate standard errors for combined assays and gas exchange experiments.

TABLE 1. *Effect of temperature on the kinetic constants of RuBPCO*

V_o and V_c are maximal velocities of oxygenase and carboxylase, K_o and K_c are Michaelis constants for O_2 and CO_2. Means of seven to nine estimations (two replications at four or five substrate concentrations) are presented and standard errors are indicated in parenthesis.

T (°C)	V_o (μmol O_2 min^{-1} mg^{-1} protein)	K_o (mmol m^{-3} O_2)	V_c (μmol CO_2 min^{-1} mg^{-1} protein)	K_c (mmol m^{-3} CO_2)
10	0·12	520	0·10	7·2
	(0·008)	(93·3)	(0·003)	(0·59)
15	0·15	521	0·17	10·3
	(0·003)	(26·7)	(0·004)	(0·55)
20	0·21	598	0·34	14·2
	(0·017)	(104·8)	(0·011)	(1·10)
25	0·27	619	0·60	20·8
	(0·023)	(104·9)	(0·016)	(1·16)

combined and separate assays. The independence of temperature of the specificity factor *in vitro* was characterized by an analysis of the kinetic constants (Table 1). The increase in the maximal velocity of the carboxylase reaction (V_c) with temperature was compensated for by an increase in the maximal velocity of the oxygenase reaction (V_o) and by an increase in the

Michaelis constant for CO_2 (K_c), whereas the Michaelis constant for O_2 (K_o) was only slightly dependent on temperature.

The temperature response of the specificity factors *in vivo*, as calculated from $^{14}CO_2$ and $^{12}CO_2$ uptake at 30 Pa $p(CO_2)$, was consistent with calculations based on CO_2 compensation concentrations (Jordan and Ogren, 1984). However, our finding that specificity factors *in vitro* were independent of temperature is in agreement only with some of the data as calculated from literature: independence of temperature, at least within a limited temperature range, could be shown when specificity factors were calculated from data by Badger and Collatz (1977) and Hall and Keys (1983). In contrast, Jordan and Ogren (1984) found an increase in specificity factors *in vitro* with decreasing temperature which agreed with our measurements of the temperature response *in vivo*.

The specificity factor *in vivo* at 30 Pa $p(CO_2)$ was low at 30 °C and high at 10 °C (Fig. 4). It was independent of CO_2 at 30 °C, whereas at 10 °C it decreased as CO_2 concentration was increased. At 120–150 Pa $p(CO_2)$, it was similar at 30 °C and at 10 °C. This CO_2 response is consistent with calculations from data on $^{14}CO_2/^{12}CO_2$ uptake by Fock, Klug, and Canvin (1979).

The disagreement between specificity factors *in vivo* and *in vitro* at low temperature and at a CO_2 partial pressure of about 30 Pa or less, suggests that the temperature dependence of the ratio of photosynthesis to photorespiration is not fully explained by the properties of RuBPCO and by effects on the solubility ratio of O_2 to CO_2.

Disagreement between gas exchange and RuBPCO kinetics cannot be explained by effects of dark respiration. (1) If dark respiration, which is saturated at 2·0 kPa $p(O_2)$ (Forrester,

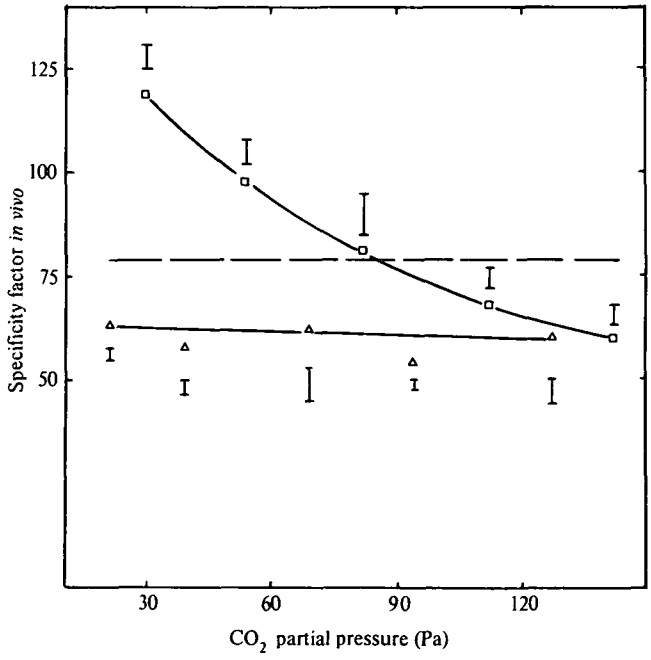

FIG. 4. Effect of intercellular CO_2 partial pressure on the substrate specificity factor *in vivo* at 10 °C (□) and 30 °C (△). The ratio of partial pressures of CO_2 to O_2 in the cuvette was held constant in the experiments (15×10^{-4}). Specificity factors were calculated using concentrations of dissolved O_2 and CO_2. The means of four or three determinations are shown; vertical bars indicate standard errors. The dashed line indicates the specificity factor *in vitro* at 10 °C as calculated from combined assays.

Krotkov, and Nelson, 1966), affected the data in Fig. 2A, then the lines would not pass through the origin. However, at 2000 μmol quanta m^{-2} s^{-1}, the lines pass through the origin. (2) Dark respiration would decrease the specificity factor *in vivo* as compared with *in vitro*. However, specificity factors *in vivo* tend to be higher than *in vitro* (Fig. 3). (3) The CO_2 dependence of the specificity factor in Fig. 4 cannot be attributed to dark respiration, since, as far as we know, dark respiration is not affected by CO_2.

A CO_2 concentrating system in the mesophyll cells could explain the discrepancy between gas exchange and RuBPCO kinetics. In the presence of a CO_2 concentrating system, the CO_2 concentration at the enzyme site would be increased and underestimated by the intercellular CO_2 concentration. The calculation of the specificity factor *in vivo* according to equation (3) would result in an overestimation. Assuming saturation kinetics, a CO_2 concentrating system is expected to be more efficient at low than at high CO_2 concentrations. This appears to be consistent with the CO_2 dependence of the specificity factor *in vivo* at low temperature in Fig. 4 which shows high specificity factors at low CO_2 concentrations.

However, further research is needed to substantiate this hypothesis; namely the effect of refixation of respired CO_2 due to intracellular resistances on the estimates of specificity factors *in vivo* should be elucidated.

ACKNOWLEDGEMENTS

The authors thank Mr Kiss and Mr Dörig for technical assistance, Ms Allenbach for growing the plants, and Ms Schoenberg for checking the English translation.

LITERATURE CITED

BADGER, M. R., and COLLATZ, G. J., 1978. 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 Institution of Washington, Yearbook*, **77**, 355-61.

BENSADOUN, A., and WEINSTEIN, D., 1976. Assay of proteins in the presence of interfering materials. *Analytical Biochemistry*, **70**, 241-50.

ČATSKÝ, J., 1971. General principles of using IRGA for measuring CO_2 exchange rates. In *Plant photosynthetic production. Manual of methods*. Eds Z. Šesták, J. Čatský and P. G. Jarvis. Dr. W. Junk, N.V. Publishers, The Hague. Pp. 162-6.

FOCK, H., KLUG, K., and CANVIN, D. T., 1979. Effect of carbon dioxide and temperature on photosynthetic CO_2 uptake and photorespiratory CO_2 evolution in sunflower leaves. *Planta*, **145**, 219-23.

FORRESTER, M. L., KROTKOV, G., and NELSON, C. D., 1966. Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves. I. Soybean. *Plant Physiology*, **41**, 422-7.

HALL, N. P., and KEYS, A. J., 1983. Temperature dependence of the enzymic carboxylation and oxygenation of ribulose-1,5-bisphosphate in relation to effects of temperature on photosynthesis. Ibid. **72**, 945-8.

HARNED, H. S., and BONNER, F. T., 1945. The first ionisation of carbonic acid in aqueous solutions of sodium chloride. *Journal of the American Chemical Society*, **67**, 1026-31.

JARVIS, P. G., 1971. The estimation of resistances to carbon dioxide transfer. In *Plant photosynthetic production. Manual of methods*. Eds Z. Šesták, J. Čatský and P. G. Jarvis. Dr. W. Junk, N.V. Publishers, The Hague. Pp. 566-631.

JORDAN, D. B., and OGREN, W. L., 1981. A sensitive assay procedure for simultaneous determination of ribulose-1,5-bisphosphate carboxylase and oxygenase activities. *Plant Physiology*, **67**, 237-45.

——— 1984. The CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase. Dependence on ribulose bisphosphate concentration, pH and temperature. *Planta*, **161**, 308-13.

KU, S. B., and EDWARDS, G. E., 1977*a*. Oxygen inhibition of photosynthesis. I. Temperature dependence and relation to O_2/CO_2 solubility ratio. *Plant Physiology*, **59**, 986-90.

——— 1977*b*. Oxygen inhibition of photosynthesis. II. Kinetic characteristics as affected by temperature. Ibid. **59**, 991-9.

LAING, W. A., OGREN, W. L., and HAGEMAN, R. H., 1974. Regulation of soybean net photosynthetic

CO_2 fixation by the interaction of CO_2, O_2 and ribulose-1,5-diphosphate carboxylase. Ibid. **54,** 678–85.

LANGE, 1979. *Handbook of chemistry.* J. A. Dean (Ed.). McGraw-Hill Book Company, New York.

LORIMER, G. H., BADGER, M. R., and ANDREWS, T. J., 1977. D-Ribulose-1,5-bisphosphate carboxylase oxygenase. Improved methods for the activation and assay of catalytic activities. *Analytical Biochemistry*, **78,** 66–75.

LUDWIG, L. J., and CANVIN, D. T., 1970. Gas-exchange system for simultaneous measurement of the CO_2 and $^{14}CO_2$ fluxes from leaves. *Canadian Journal of Botany*, **49,** 1299–1313.

MÄCHLER, F., and NÖSBERGER, J., 1984. Influence of inorganic phosphate on photosynthesis of wheat chloroplasts. II. RuBPCO activity. *Journal of Experimental Botany*, **35,** 488–94.

O'LEARY, M. H., 1981. Carbon isotope fractionation in plants, review. *Phytochemistry*, **20,** 553–67.

SCHNYDER, H., MÄCHLER, F., and NÖSBERGER, J., 1984. Influence of temperature and O_2 concentration on photosynthesis and light activation of RuBPCO in intact leaves of white clover. *Journal of Experimental Botany*, **35,** 147–56.

WILKINSON, G. N., 1961. Statistical estimation in enzyme kinetics. *Biochemical Journal*, **80,** 324–32.