A CO₂ Concentrating System in Leaves of Higher C₃-Plants Predicted by a Model Based on RuBP Carboxylase/Oxygenase Kinetics and ¹⁴CO₂/¹²CO₂ Exchange

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ABSTRACT
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A model is presented which compares the ratio of the two activities of the enzyme ribulose bisphosphate carboxylase/oxygenase as determined in vitro with the ratio of photosynthesis to photorespiration in leaves as determined from differential ¹⁴CO₂/¹²CO₂ uptake or from CO₂ compensation concentration. Discrepancies between measurements made in vitro and in vivo are attributed to the effect of a CO₂ concentrating system in the leaf cells. Interference from dark respiration is discussed. A CO₂ concentrating system is postulated which is efficient mainly at low temperature and low CO₂ concentration.

Key words—Photosynthesis, photorespiration, ribulose bisphosphate carboxylase/oxygenase.

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INTRODUCTION
Net uptake of CO₂ by illuminated leaves is the result of CO₂ fixation due to photosynthesis and of CO₂ release due to photorespiration and dark respiration. Photosynthetic CO₂ uptake and photorespiratory CO₂ release are related to the reactions catalysed by the enzyme ribulose bisphosphate carboxylase/oxygenase (RuBPCO), whereby photosynthetic CO₂ uptake corresponds to the carboxylase activity and photorespiratory CO₂ release to the oxygenase activity of the enzyme.

Agreement between gas exchange of leaves and RuBPCO kinetics was found by Jordan and Ogren (1984) who studied the influence of temperature on the properties of RuBPCO in vitro. They compared the ratio of carboxylase to oxygenase activity of the enzyme with the CO₂ compensation point (F) of leaves and suggested that gas exchange of leaves is fully explained by RuBPCO kinetics. Discrepancy between gas exchange and RuBPCO kinetics

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was found by Azcón-Bieto, Farquhar, and Caballero (1981), by Peisker, Tichá, and Čatský (1981), and by Fleck and di Marco (1984). These authors investigated the effect of leaf age on the oxygen dependence of $F$. Azcón-Bieto et al. (1981) attributed the deviation of their measurements from RuBPCO kinetics to dark respiration in the light. Peisker et al. (1981) found that the discrepancy of their results could only be related to dark respiration if the dark respiration was assumed to be partly inhibited in the light, and if the extent of inhibition decreased with age. Fleck and di Marco (1984) found a similar increase of the $O_2$ dependent part of $F$ with age which does not correspond with dark respiration effects as predicted by the model of Farquhar, von Caemmerer, and Berry (1980).

Lehnherr, Mächler, and Nosberger (1985) compared differential $^{14}\text{CO}_2/^{12}\text{CO}_2$ uptake of leaves with RuBPCO kinetics and found higher ratios of photosynthesis to photorespiration than of carboxylase to oxygenase activity at low temperature, and a $CO_2$ dependence of this difference which could not be explained by dark respiration. A $CO_2$ concentrating mechanism in leaf cells was proposed. However, some uncertainties in the interpretation of the results arose from the underestimation of gross photosynthesis in the $^{14}\text{CO}_2/^{12}\text{CO}_2$ method due to refixation of photorespired $CO_2$ and from the unknown concentration of $CO_2$ in the chloroplast stroma. The present paper describes a model which eliminates these uncertainties.

The specificity of the enzyme RuBPCO for the substrates $CO_2$ and $O_2$ is defined by the specificity factor $(S)$ (Laing, Ogren, and Hageman, 1974; Jordan and Ogren, 1981)

$$S = \frac{v_c}{v_o} \frac{O}{C} = \frac{V_c K_o}{V_o K_c}$$

(1)

where $v_c$ and $v_o$ are the velocities of the carboxylation and oxygenation, $V_c$ and $V_o$ the maximal velocities of the two reactions, $K_c$ and $K_o$ the Michaelis constants for $CO_2$ and $O_2$, and $C$ and $O$ the concentrations of the substrates $CO_2$ and $O_2$.

Oxygenation by RuBPCO is related to the release of photorespired $CO_2$ in leaves. The $CO_2$ release is due to glycine decarboxylation in the photorespiratory glycolate pathway. Half a $CO_2$ molecule is produced per $O_2$ fixed (Ogren and Chollet, 1982). Thus the specificity factor of RuBPCO can be expressed in terms of $CO_2$ exchange of leaves.

$$S = \frac{P}{2(P - F)} \frac{O}{C_e}$$

(2)

where $P$ is gross photosynthesis, $F$ is net photosynthesis, and $C_e$ is $CO_2$ concentration in the stroma. Oxygen concentration ($O$) is assumed to be the same in the stroma as in ambient air since it is known to penetrate well even across hydrophobic parts of leaves (Lendzian, 1982).

Gross photosynthesis and net photosynthesis can be estimated by measuring differential $^{14}\text{CO}_2/^{12}\text{CO}_2$ uptake immediately after exposing a leaf to $^{14}\text{CO}_2$ (Ludwig and Canvin, 1971). In previous experiments we exposed leaves to $^{14}\text{CO}_2$ for 20 s, killed them, and determined fixed $^{14}\text{C}$. $^{12}\text{CO}_2$ uptake was measured by IRGA. Discrimination against $^{14}\text{CO}_2$ was accounted for (Lehnerr et al., 1985). However, the short time uptake of $^{14}\text{CO}_2$ underestimates gross photosynthesis since the specific activity of $^{14}\text{CO}_2$ in the stroma is decreased due to photorespired $CO_2$ released by the mitochondria (Peisker, 1980). The decrease of specific activity of $^{14}\text{CO}_2$ within the leaf is due to the super-position of two $CO_2$-gas streams which are assumed to be independent (Fig. 1). One of them is labelled with $^{14}\text{CO}_2$ at a defined specific activity and diffuses from ambient air to the chloroplast stroma. The other $CO_2$-stream is unlabelled. It is formed in the mitochondria of the leaf cells due to photorespiration and diffuses partly to the ambient air, and partly to the chloroplast stroma. The $CO_2$ concentrations in ambient air ($C_a$), in the intercellular gas space ($C_i$), in the cytosol of the leaf cells ($C_c$), and in the stroma ($C_e$) are sums of the two $CO_2$ components with different origins as shown in Fig. 1. The two $CO_2$ components compete for the active sites of the RuBPCO with the same affinity. Discrimination against
Fluxes

Concentrations

Conductances

Fig. 1. Scheme of fluxes, concentrations, and conductances of $^{12}\text{CO}_2$ (bright areas) and $^{14}\text{CO}_2$ (shaded areas) during short time uptake by a leaf. Fluxes of $\text{CO}_2$: $F$: Net photosynthesis. $P$: Gross photosynthesis. $P'$: Gross uptake of intercellular $\text{CO}_2$. $P''$: Gross uptake of ambient $\text{CO}_2$. Concentrations of $\text{CO}_2$: $C_a$: Total $\text{CO}_2$ in ambient space. $C_{a}^{l}$: $^{14}$C-labelled component in ambient space. $C_i$: Total $\text{CO}_2$ in intercellular space. $C_{i}^{l}$: $^{14}$C-labelled component in intercellular space. $C_{c}^{l}$: $^{14}$C-labelled component in cytosol. $C_c$: Total $\text{CO}_2$ in cytosol. $C_{c}^{l}$: $^{14}$C-labelled component in cytosol. $C_{s}^{l}$: $^{14}$C-labelled component in stroma. $C_s$: Total $\text{CO}_2$ in stroma. Conductances of $\text{CO}_2$ and concentration ratio: $k_g$: Between ambient and intercellular space, gas phase. $k_l$: From intercellular space to cytosol, liquid phase. $k_{l-1}$: From cytosol to intercellular space, liquid phase. $f$: Ratio of concentrations in stroma and cytosol.
Mächler et al.—$^{14}$CO$_2$ Concentrating System

$^{14}$CO$_2$ has to be accounted for according to O'Leary (1981) and is not considered in the present model. The fixation rates of the two CO$_2$ components depend on their concentrations in the stroma:

$$k \cdot C_s = P$$  
$$k \cdot C'_s = \varphi$$  
$$k \cdot (C_s - C'_s) = P - \varphi$$

where $\varphi$ is the initial rate of $^{14}$CO$_2$ uptake and $k$ is a rate factor which is affected by the concentration of total CO$_2$ ($C_s$) and is the same for both gas components $C_s$ and $C_s - C'_s$. Gross photosynthesis $P$ can now be related to $\varphi$ by

$$P = \frac{k \cdot C_s}{k \cdot C'_s} \cdot \varphi = \frac{C_s}{C'_s} \cdot \varphi = \frac{C_i}{C_c} \cdot \varphi$$

$C_s/C'_s$ can be substituted by $C_s/C_c$ if it is assumed that the ratio of the two gas components is the same in the cytosol of the leaf cells as in the stroma.

$P$ is gross photosynthesis and consequently the rate of uptake of cytosolic and stromal CO$_2$. Auxiliary magnitudes can be introduced describing the gross rates of uptake of intercellular and ambient CO$_2$ ($P'$ and $P''$, Fig. 1). In analogy to equation 4 they can be expressed by

$$P' = \frac{C_i}{C_c} \cdot \varphi$$

$$P'' = \frac{C_s}{C'_s} \cdot \varphi$$

$P$ can now be expressed in terms of $P'$ and $P''$

$$P = \frac{C_s}{C_s} \cdot P' = \frac{C_i}{C_c} \cdot P''.$$

$P$ in equation 2 can be substituted by (6). $C_s$ and $C'_s$ can be measured. $C_s$, $C_c$, $C_i$ and $C'_c$ can be expressed in terms of parameters which can be determined by applying Fick's first law to data from $^{14}$CO$_2/^{12}$CO$_2$ experiments, and in terms of liquid phase conductance for CO$_2$ between intercellular space and cytosol of the cells (see appendix). CO$_2$ translocation between intercellular space and cytosol may be by diffusion or by an active process. The conductance from the intercellular space to the cytosol ($k_l$) is the same as the conductance in the opposite direction ($k_{il}$) if translocation is purely by diffusion. However, $k_l$ is greater than $k_{il}$ if an active process is involved.

CO$_2$ translocation between cytosol and stroma may be by diffusion or by an active process. The ratio of the concentrations in the two compartments can be characterized by

$$f = \frac{C_i}{C_c}$$

$f$ is smaller than one if this translocation step is by diffusion, and can be greater than one if it is by an active process. $C_i$ in equation 2 can be substituted by $f \cdot C_c$.

Transformations as described in the appendix result in

$$S \cdot \frac{k_l}{k_{il}} \cdot f = \frac{P''}{2(P'' - F)} \cdot \frac{O}{C_s} = \frac{P'}{2(P' - F)} \cdot \frac{O}{C_c}$$

$P''$ can be determined from the initial rate of $^{14}$CO$_2$ uptake if the decrease in specific activity of ambient CO$_2$ due to photorespiration is known. $P'$ and $C_i$ can be determined if the gas phase resistance to CO$_2$ diffusion is known from transpiration measurements (Lehnerr et al., 1985).

Equation 8 shows that substitution of the unknown parameters $P$ and $C_s$ in equation 2 by either $P''$ and $C'_s$ or $P'$ and $C_i$ enables the determination of $S$ times the factor $f \cdot k_l/k_{il}$. The factor $f \cdot k_l/k_{il}$ can be determined if $S$ is known from in vitro experiments. The factor $f \cdot k_l/k_{il}$ depends on the transport processes of CO$_2$ in the liquid phase. The factor is smaller than one if transport occurs purely by diffusion ($k_l/k_{il} = 1; f < 1$). However, the factor can be greater than one if an active process is involved either in the translocation of CO$_2$ from the intercellular space to the cytosol ($k_l/k_{il} > 1$) or in the translocation from the cytosol to the stroma ($f \geq 1$). Equation 8 shows that the factor is independent of the gas phase conductance and of the liquid phase conductance between intercellular space and cytosol, as long as no active processes are involved.
Table 1. Influence of temperature and CO₂ concentration on the concentrating factor $f \cdot k_i/k_{i-}$ and on parameters which are needed for its calculation. From data by Lehnherr et al. (1985)

| Temperature (°C) | 10  | 20  | 30  | 10  | 20  | 30  |
|------------------|-----|-----|-----|-----|-----|-----|
| $O_2$ (mole fraction) | 0.21 | 0.21 | 0.21 | 1.0 | 1.0 | 1.0 |
| $C_s$ (mole fractions · 10⁶) | 319 | 316 | 309 | 1471 | 1464 | 1459 |
| $k_i$ (nmol m⁻² s⁻¹) | 277 | 261 | 255 | 186 | 198 | 165 |
| $C_i$ (mole fraction · 10⁶) | 262 | 241 | 225 | 1390 | 1366 | 1327 |
| $F$ (µmol m⁻² s⁻¹) | 15.8 | 19.7 | 21.4 | 14.9 | 19.5 | 22.0 |
| $P$ (µmol m⁻² s⁻¹) | 17.7 | 23.8 | 28.8 | 18.1 | 24.2 | 28.4 |
| $P''$ (µmol m⁻² s⁻¹) | 17.3 | 22.7 | 26.3 | 17.9 | 23.8 | 27.6 |
| $S \cdot f \cdot k_i/k_{i-}$ (for mole fractions of O₂ and CO₂) | 3699 | 2494 | 1810 | 2032 | 1877 | 1670 |
| $S \cdot f \cdot k_i/k_{i-}$ (for dissolved O₂ and CO₂) | 118 | 88 | 71 | 65 | 66 | 66 |
| $S$ (in vitro, for dissolved O₂ and CO₂) | 78 | 78 | 78 | 78 | 78 | 78 |
| $f \cdot k_i/k_{i-}$ | 1.51 | 1.13 | 0.91 | 0.83 | 0.85 | 0.84 |

At the CO₂ compensation point the CO₂ concentration in the stroma can be determined by

$$C_s = \frac{k_{i-}}{k_i} \cdot f \cdot \Gamma$$

as shown in the appendix.

Calculations from Experimental Data

The concentrating factor $f \cdot k_i/k_{i-}$ was calculated from data by Lehnherr et al. (1985) for various temperatures and CO₂ concentrations (Table 1). The factor is smaller than one at high CO₂ concentration, independent of whether temperature is 10°C or 30°C, and at air level CO₂ concentration if temperature is high (30°C). A concentrating factor smaller than one is to be expected in the absence of a CO₂ concentrating mechanism when $k_i/k_{i-} = 1$ (same conductance from intercellular space to cytosol as vice versa) and $f < 1$ (decrease in CO₂ concentration between cytosol and chloroplast stroma due to diffusion resistance). At air level CO₂ concentration, the concentrating factor increases as temperature is decreased reaching a value of 1.51 at 10°C. This means either that the conductance from the intercellular space to the cytosol ($k_i$) is 1.82 (1.51/0.83) times higher than the conductance in the opposite direction ($k_{i-}$) or that the CO₂ concentration in the chloroplast stroma is 1.51 times higher than in the cytosol. The two possible sites for a CO₂ concentrating mechanism, plasmalemma and chloroplast envelope, cannot be distinguished.

Discussion

The model presented in this paper does not consider dark respiration. However, effects of dark respiration on gas exchange of leaves can be distinguished from effects of a CO₂ concentrating system. The CO₂ dependence of the ratio of CO₂ uptake to CO₂ release is linear in the presence of dark respiration. This is apparent in the linear relationship between O₂ concentration and $\Gamma$ as predicted by the model of Farquhar et al. (1980) and as shown by Azcón-Bieto et al. (1981). The CO₂ dependence of the ratio of CO₂ uptake to CO₂ release is
not linear in the presence of a CO₂ concentrating system with saturation kinetics. A nonlinear CO₂ response of the ratio of CO₂ uptake to CO₂ release is shown by Lehnherr et al. (1985) if the temperature is low. The ratio of CO₂ uptake to CO₂ release is decreased by dark respiration as compared with the ratio of carboxylase to oxygenase in vitro, whereas it is increased in the presence of a CO₂ concentrating system. Although dark respiration and the CO₂ concentrating system may occur in parallel, dark respiration may predominate at high temperatures and the CO₂ concentrating system at lower ones.

The validity of the diffusion resistance concept is usually a presupposition when RuBPCO kinetics are applied to photosynthetic gas exchange (Hall, 1971; Peisker, 1976; Tenhunen, Yocum, and Gates, 1976; Farquhar et al., 1980). Tenhunen, Weber, Yocum, and Gates (1979) applied RuBPCO kinetics and the diffusion resistance concept to data on photosynthetic gas exchange as influenced by CO₂ and O₂ from Ku and Edwards (1977). They calculated Michaelis constants for stromal CO₂ ($K_c$) of 0.4 mmol m⁻³. This is extremely low and does not compare with $K_c$ of purified enzyme (16 mmol m⁻³) (Mächler, Keys, and Cornelius, 1980). However, underestimation of $K_c$ in vivo seems to be inevitable if Michaelis–Menten kinetics are applied to data on the CO₂ dependence of photosynthesis since RuBP can be limiting and the activation state of RuBPCO decreases as CO₂ concentration is increased (Mächler, 1981; Schnyder, Mächler, and Nösberger, 1984). Saturating RuBP and a constant RuBPCO activity would be a prerequisite for the application of Michaelis–Menten kinetics. Underestimation of $K_c$ in the Michaelis–Menten equation results in an underestimation of the CO₂ concentration in the stroma and, therefore, in an overestimation of the intracellular diffusion resistance. The presence of a CO₂ concentrating system with saturation kinetics would further decrease $K_c$ as calculated from CO₂ concentration curves of photosynthesis. Although Michaelis–Menten kinetics are obviously not applicable directly to photosynthetic gas exchange, it seems that the specificity factor of RuBPCO is unchangeable (Ogren and Chollet, 1982) and that it is therefore the same in vivo as in vitro. The comparison of the ratio of carboxylase to oxygenase activity in vitro with the ratio of photosynthesis to photorespiration in vivo is the basis for our postulation of a CO₂ concentrating system in higher C₃-plants.

Active transport of inorganic carbon in the HCO⁻₃-form has been shown in aquatic plants (Badger, Kaplan, and Berry, 1980; Badger and Andrews, 1982; Spalding and Ogren, 1982). Inorganic carbon uptake in land plants has been investigated using isolated cells and protoplasts. They preferentially take up CO₂ by diffusion (Espie and Colman, 1982), although at least some uptake of HCO⁻₃ could be shown by Volokita, Kaplan, and Reinhold (1981). $^{14}$CO₂/$^{12}$CO₂ experiments with leaves by Lehnherr et al. (1985) showed that the suggested CO₂ concentrating system is only active at low temperature, and that its efficiency is much lower than in aquatic plants. A concentrating effect could only be shown at low CO₂ concentrations. It is suggested that protoplast experiments performed at low temperature and low CO₂ concentration may show a clear concentrating effect.

A decrease in the efficiency of a CO₂ concentrating mechanism with increasing temperature is surprising since it is certainly based on enzymatic processes. However, a concentrating effect is also dependent on the permeability of the membranes, and decreases as the permeability is increased. The permeability of the membranes is expected to increase with temperature. It is suggested that the temperature dependence of the CO₂ concentrating effect is related to the permeability of the membranes.

The discrepancy between the specificity factor of RuBPCO in vitro and the ratio of photosynthesis to photorespiration could be explained alternatively by incomplete metabolization of glycolate via the glycolate pathway at low temperature and low CO₂ concentration.
APPENDIX

Derivation of equation 8

$P$ in equation 2 can be substituted by (6). $C_s$ in equation 2 can be substituted by $f \cdot C_c$ according to (7). Equation 2 becomes

$$S = \frac{C_c C_i P'}{C_c C_i (P - F)} \cdot \frac{O}{2f} = \frac{C_i P'}{C_c C_i P' - C_c C_i F} \cdot \frac{O}{2f}$$  \hspace{1cm} (10a)

$$S = \frac{C_c C_s P''}{C_c C_s (P' - F)} \cdot \frac{O}{2f} = \frac{C_s P''}{C_c C_s P'' - C_c C_s F} \cdot \frac{O}{2f}$$  \hspace{1cm} (10b)

$C_i$ and $C_i'$ can be determined if gas phase conductance ($k_g$) is known by applying Fick’s first law according to Jarvis (1971).

$$C_i = C_s - \frac{F}{k_g}$$  \hspace{1cm} (11a)

$$C_i' = C_s' - \frac{\varphi}{k_g}$$  \hspace{1cm} (11b)

$F$ and $\varphi$ can be expressed in terms of $C_c$, $C_c'$ and liquid phase conductances $k_h$ and $k_l$:

$$F = C_i k_h - C_c k_{l_{-1}} = \left(C_s - \frac{F}{k_g}\right) k_h - C_c k_{l_{-1}},$$  \hspace{1cm} (12a)

$$\varphi = P \frac{C_i'}{C_i} = C_i k_h - C_c k_{l_{-1}},$$  \hspace{1cm} (12b)

$$\varphi = P' \frac{C_s'}{C_s} = \left(C_s - \frac{\varphi}{k_g}\right) k_h - C_c k_{l_{-1}},$$  \hspace{1cm} (12c)

$k_h$ and $k_{l_{-1}}$ are not purely diffusion parameters, but also rate factors of chemical reactions which depend on $CO_2$ concentration. $C_c$ can now be calculated from equation 12a and $C_c'$ either from (12b) or from (12c) and substituted in equations 10a and 10b.

$$S = \frac{C_i P'}{C_i P' \left(\frac{C_i k_h - F}{k_{l_{-1}}}\right) - C_i F \left(\frac{C_i k_h - P' C_i}{k_{l_{-1}}}\right)} \cdot \frac{O}{2f}$$  \hspace{1cm} (13a)

$$S = \frac{C_s P''}{C_s P'' \left(\frac{C_s - F}{k_h}\right) k_h - F) - C_s F \left(\frac{C_s - P''/C_s}{k_{l_{-1}}}\right) \left(\frac{C_s - C_s P''/C_s}{k_{l_{-1}}}\right)} \cdot \frac{O}{2f}$$  \hspace{1cm} (13b)

Transformation of equation 13a and 13b results in equation 8.
Derivation of equation 9

At the CO$_2$ compensation point the fluxes of CO$_2$ from the intercellular space to the cytosol and vice versa are the same

$$C_i k_i = C_e k_i . 	ag{14}$$

The CO$_2$ concentration in the intercellular space is the same as in ambient air. Substitution of $C_e$ by $C_i / f$ results in equation 9.

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