Regulation of Soybean Net Photosynthetic CO₂ Fixation by the Interaction of CO₂, O₂, and Ribulose 1,5-Diphosphate Carboxylase¹,²

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WILLIAM A. LAING³
Department of Agronomy, University of Illinois, Urbana, Illinois 61801

WILLIAM L. OGREN
United States Regional Soybean Laboratory, North Central Region, Agricultural Research Service, United States Department of Agriculture, Urbana, Illinois 61801

RICHARD H. HAGEMAN
Department of Agronomy, University of Illinois, Urbana, Illinois 61801

ABSTRACT

Kinetic properties of soybean net photosynthetic CO₂ fixation and of the carboxylase and oxygenase activities of purified soybean (Glycine max [L.] Merr.) ribulose 1,5-diphosphate carboxylase (EC 4.1.1.39) were examined as functions of temperature, CO₂ concentration, and O₂ concentration. With leaves, O₂ inhibition of net photosynthetic CO₂ fixation increased when the ambient leaf temperature was increased. The increased inhibition of CO₂ fixation at higher temperatures was caused by a reduced affinity of the leaf for CO₂ and an increased affinity of the leaf for O₂. With purified ribulose 1,5-diphosphate carboxylase and ribulose 1,5-diphosphate carboxylase for CO₂ and O₂; these results reported here and in the literature, a scheme outlining the stoichiometry between CO₂ and O₂ reaction in vitro is proposed.

Oxygen competitively inhibited carboxylase activity with respect to O₂ and CO₂ competitively inhibited oxygenase activity with respect to O₂. Within the limits of experimental error, the Michaelis constant (CO₂) in the carboxylase reaction was identical with the inhibition constant (CO₂) in the oxygenase reaction, and the Michaelis constant (O₂) in the oxygenase reaction was identical with the inhibition constant (O₂) in the carboxylase reaction. The Michaelis constant, (ribulose 1,5-diphosphate) was the same in both the carboxylase and oxygenase reactions. This equality of kinetic constants is consistent with the notion that the same enzyme catalyzes both reactions.

Oxygen inhibition of net photosynthetic CO₂ fixation in leaves of C₃ plants consists of two components, (a) a direct inhibition of photosynthesis by O₂ and (b) a stimulation of glycolate synthesis and subsequent photorespiratory oxidation of glycolate to glycine, serine, and CO₂ (10-12, 19, 20). This reversible inhibition is associated with the photosynthetic carbon reduction cycle (11), and the inhibition can be reduced by increasing the CO₂ concentration, decreasing the O₂ concentration, or by reducing the temperature (15, 16). Direct inhibition of photosynthetic CO₂ fixation has been explained by depletion of Calvin cycle intermediates, particularly the CO₂ acceptor RuDP, which is presumed to occur because of the O₂ stimulation of glycolate synthesis (21, 23), or by competitive inhibition of RuDP carboxylase by O₂ with respect to CO₂ (6, 20). Two theories have also been advanced to explain oxygen stimulation of glycolate synthesis in C₃ species. The dihydroxyethyllyamine pyrophosphate moiety produced in the transketolase reactions of the Calvin cycle may be oxidized to glycolate by H₂O₂, which can be formed in a Mehler-type reaction between reduced ferredoxin and O₂ (9, 21, 23, 27). This reaction has been shown to occur in the light with chloroplast fragments (23). Alternatively, Bowes et al. (7) showed that RuDP carboxylase can catalyze the oxidation of RuDP by O₂ to form P-glycolate, P-glycolate can then be hydrolyzed to glycolate by a specific chloroplast phosphatase (22).

The importance of RuDP carboxylase in regulating photosynthetic CO₂ fixation has been inferred from the correlation between photosynthesis rate and extractable RuDP carboxylase activity in leaves for a number of species (4, 8, 26). Ogren and Bowes (20) suggested, from an analysis of O₂ inhibition of net

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² Dedicated to the memory of Milton Zucker.
³ Present address: Plant Physiology Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand.

Abbreviations: RuDP: ribulose 1,5-diphosphate; PG: P-glycolate; Kᵢ: inhibition constant; CE: carboxylation efficiency.
photosynthesis and of RuDP carboxylase, that the carboxylase also regulates photorespiration. This suggestion was supported by the subsequent observations that the carboxylase could catalyze P-glycolate formation (2, 7, 18, 24), and by the experiments of Bassham and Kirk (3), which showed that subjecting photosynthesizing Chlorella cells to 100% O_2 induced sequential transients of RuDP and P-glycolate, followed by glycolate accumulation. If RuDP carboxylase regulates both photosynthetic CO_2 fixation and photorespiratory CO_2 evolution, then the kinetic properties of the carboxylase and oxygenase activities of this enzyme must be manifested in the characteristics of photosynthetic and photorespiratory CO_2 exchange of the leaf. In this paper, we compare the effect of temperature on the photosynthetic properties of intact soybean leaves and on the kinetic constants of purified soybean RuDP carboxylase.

**MATERIALS AND METHODS**

**Photosynthesis Measurements.** Soybean (*Glycine max [L.]* Merr. var. "Wayne") plants were grown in vermiculite in growth chambers (30 C day/20 C night, 14 hr photoperiod, 550 μeinstein m^-2 sec^-1) and subirrigated with modified Hoagland's solution (13). The youngest mature trifoliate leaf was used in all measurements. Photosynthesis rates were determined as described earlier (8). Leaves were cut from the plant under water at the base of the petiole, placed in an assimilation chamber, and maintained at the desired temperature by immersion in a water bath. The leaf was illuminated with 650 μeinstein m^-2 sec^-1. Air was circulated through the assimilation system at 7.0 liters min^-1. After the leaf had reached a constant photosynthesis rate in air, the desired gas mixture was made from tanks of N_2, O_2, and 1% CO_2 in N_2 and flushed over the leaf. The system was monitored for about 10 sec. The change in CO_2 concentration ranged from 5 to 20 μl/l. The volume of the system was 1.06 liters. At least four rate determinations were made for each gas mixture, and all experiments were done in triplicate. Photosynthesis rates were measured every 20 min in air to ascertain that the rate did not change over the course of the experiments. In photorespiration measurements, the flushing gas was free of CO_2, and the rate of CO_2 evolution was determined. The leaf was flushed with air for 5 min between photorespiration measurements. CO_2 concentration was monitored with an infrared gas analyzer, and O_2 with a Clark-type electrode modified for use with gases. Carboxylation efficiency (CE) of leaf photosynthetic CO_2 fixation was calculated according to Forrester et al. (12), where CE equals the measured photosynthesis rate divided by the difference between the ambient CO_2 concentration and the CO_2 compensation concentration. CE is an exponential function of O_2 concentration (20), so carboxylation efficiency in the absence of O_2 (CEO) was determined by extrapolating a logarithm plot of CE versus O_2 to zero O_2. The inhibition constant of O_2 on leaf photosynthesis equals the negative of the slope of this plot.

**Enzyme Isolation and Assay.** RuDP carboxylase (EC 4.1.1.39) was purified from field- or greenhouse-grown soybean plants (var. "Wayne") and assayed as described previously (6). RuDP oxygenase activity was assayed by converting one of the products of the reaction, P-glycolate, to the 1,5-diphosphoribonucleotide derivative of glycolate. The concentration of this derivative was determined spectrophotometrically (17). CO_2 and O_2 solubilities were calculated from standard tables (14), as were the dissociation constants of carbonic acid.

**RESULTS AND DISCUSSION**

**Photosynthesis Measurements.** The two components of total oxygen inhibition of soybeans photosynthesis, direct inhibition of photosynthetic CO_2 fixation and stimulation of photorespiratory CO_2 evolution, have been mathematically described by Ogren and Bowes (20) as shown in equation 1.

\[ Pn = (CE, e^{-0.5K} O) - (CE, e^{-0.5K} K) \]

(1)

where \( Pn \) is net photosynthesis, \( CE, \) is carboxylation efficiency of a leaf in the absence of O_2, \( K \) is the inhibition constant of direct O_2 inhibition of photosynthesis, \( k \) is the slope of a plot of CO_2 compensation concentration versus O_2 concentration, \( C \) is the CO_2 concentration, and \( O \) is the O_2 concentration. The quantity \( kO \) equals the CO_2 compensation concentration, \( I. \) This equation applies only where the rate of photosynthetic CO_2 fixation is a linear function of CO_2 concentration.

Since CE is a factor in both the photosynthesis and photorespiration terms of equation 1, Ogren and Bowes (20) concluded that CE regulated both processes, and further suggested that CE represented in vivo RuDP carboxylase activity. This suggestion is supported by the observations that O_2 competitively inhibits RuDP carboxylase with respect to CO_2 (6, 20) and by the discovery of Bowes et al. (7) that O_2 can substitute for CO_2 in the carboxylase reaction to yield P-glycolate. The P-glycolate formed in this reaction can be hydrolyzed to glycolate by P-glycolate phosphatase (22), and glycolate can then be oxidized to CO_2, glycine, and serine by the photorespiratory pathway (25). If CE represents RuDP carboxylase activity, then the effect of temperature on photosynthesis and photorespiration in the leaf should be similar to the effect of temperature on the interaction of CO_2, O_2, and RuDP carboxylase in vitro.

The rate of photosynthesis was determined at 186 μl/l CO_2 and several O_2 concentrations at three temperatures. Carboxylation efficiency was then calculated by dividing photosynthesis rate by 186 - \( I. \) as described by Forrester et al. (12). A linear relationship between log CE and O_2 concentration was found to occur (Fig. 1), as expected from equation 1. The intercept gives \( CEo, \) and the slope equals \(-K. \) The values of \( K. \) are listed in Table I. Equation 1 also dictates that the CO_2 compensation concentration is a linear function of the O_2 concentration. Experimental evidence of the linear relationship between O_2 concentration and the CO_2 compensation concentration is provided by the data in Figure 2 and by previous observation (5, 12, 16).

Carboxylation efficiency can also be determined by measuring the rate of CO_2 evolution into CO_2-free gases of various O_2 concentrations. In zero CO_2, true photosynthesis equals zero, so equation 1 can be rewritten,

\[ -Pn = CE, e^{-0.5K} K \]

(2)

CO_2 evolution (\(-Pn\)) into several mixtures of O_2/N_2 was measured at three temperatures. Carboxylation efficiency was then calculated by dividing the measured rate of CO_2 evolution by the CO_2 compensation concentration at the O_2 concentration and temperature. The calculated values of carboxylation efficiency are plotted in Figure 3. Again, a linear relationship between CE and O_2 concentration was obtained. The values for \( K. \) determined from Figure 3 are listed in Table I.

Photosynthesis rate of soybean leaves as a function of CO_2 concentration was determined at three O_2 concentrations and three temperatures (Fig. 4). CE, and \( K. \) were determined for each temperature (Table I). Equation 1 was then used to calculate photosynthesis rates at four CO_2 concentrations for each O_2 concentration and temperature (Fig. 4). Excellent agreement was found between measured and calculated photosynthesis rates. The agreements between CE, calculated from photosynthesis and photorespiration measurements, between \( K. \) calculated from these two types of measurements, and be-
Fig. 1. Carboxylation efficiency (logarithm scale) as a function of O₂ concentration at three temperatures. Carboxylation efficiency was calculated from photosynthesis measurements as described in the text. For clarity in presentation, CE values were multiplied by 2 at 35 °C and by 1/2 at 15 °C.

Table I. Kinetic Constants for CO₂ and O₂ Determined from Leaf Photosynthesis and Purified RuDP Carboxylase Measurements

<table>
<thead>
<tr>
<th>Temp</th>
<th>K⁺⁺</th>
<th>K⁺⁺</th>
<th>K⁺⁺</th>
<th>K⁺⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>15°</td>
<td>0.20</td>
<td>0.90</td>
<td>1.43</td>
<td>0.90</td>
</tr>
<tr>
<td>25°</td>
<td>0.71</td>
<td>0.79</td>
<td>0.98</td>
<td>0.79</td>
</tr>
<tr>
<td>35°</td>
<td>0.52</td>
<td>0.60</td>
<td>0.82</td>
<td>0.60</td>
</tr>
</tbody>
</table>

1 Determined by dividing relative photosynthesis rate by CEₚ.
2 Determined from the data in Fig. 1.
3 Determined from the data in Fig. 3.
4 Determined from the data in Fig. 4.
5 Determined from the data in Fig. 5.
6 Determined from the data in Fig. 6.

Fig. 2. CO₂ compensation concentration as a function of O₂ concentration at three temperatures.

Fig. 3. Carboxylation efficiency (logarithm scale) as a function of O₂ concentration at three temperatures. Carboxylation efficiency was calculated from photorespiration measurements as described in the text. For clarity in presentation, CE values were multiplied by 2 at 35 °C and by 1/2 at 15 °C.
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Figure 4: Net photosynthesis rate in soybean leaves as a function of CO₂ concentration at three O₂ concentrations at three temperatures. ○: measured values; ×: calculated values.

Table II: Kinetic Properties of Purified Soybean RuDP Carboxylase at 30°C

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Kₘ (M)</th>
<th>Kᵢ (M)</th>
<th>Kₘ (RuDP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂</td>
<td>34</td>
<td>0.38</td>
<td>0.19</td>
</tr>
<tr>
<td>O₂</td>
<td>0.39</td>
<td>0.39</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Ki(CO₂) in the oxygenase reaction (38 μM), and the Kₘ(O₂) in the oxygenase reaction (0.39 mM) was identical with the Kᵢ(O₂) in the carboxylase reaction (0.37 mM). The Kₘ(RuDP) was the same for the carboxylase (0.19 mM) and oxygenase (0.17 mM) reactions. This equality of kinetic constants in both reactions is consistent with the suggestions (2, 7, 20) that RuDP carboxylase catalyzes both reactions. The data in Table II were obtained with the same enzyme preparation. Absolute Kₘ and Kᵢ varied with different enzyme preparations, but relative values of Kₘ and Kᵢ were always similar for each substrate.

The velocity of an enzyme reaction in the presence of a competitive inhibitor is given in equation 3,

\[ v = \frac{V}{1 + \frac{K_m}{S} (1 + \frac{1}{K_i})} \]  

If both the carboxylation and oxygenation of RuDP are catalyzed by the same enzyme, the velocity of the two reactions in the presence of CO₂ and O₂ can be written as below (at saturating RuDP concentration),

\[ v_c = \frac{V_c K_c}{K_c K_s + K_c O + K_o} \]  

\[ v_o = \frac{V_o K_o}{K_o K_s + K_o C + K_o O} \]  

where \( v_c \) is the velocity of the carboxylase reaction, \( v_o \) is the velocity of the oxygenase reaction, \( V_c \) the maximal velocity of the carboxylase reaction, \( V_o \) the maximal velocity of the oxygenase reaction, \( K_s \) the Michaelis (or inhibition) constant for CO₂, \( K_o \) the Michaelis (or inhibition) constant for O₂, \( C \) the CO₂ concentration, and \( O \) the O₂ concentration.

At low CO₂ concentration the quantity \( K_c C \) becomes negligible and equations 4 and 5 simplify to,

\[ v_c = \frac{V_c}{K_c K_s + O} \]  

\[ v_o = \frac{V_o}{K_o K_s + O} \]

\[ V_o / K_o \] in equation 7 can be expanded as \( (V_o / K_o) V_c (K_c / V_c) \), so that

\[ v_o = \frac{V_o}{K_o K_s + O} \frac{V_c}{V_c} \frac{K_c}{K_c} \]
Net photosynthesis is the difference between \( v_r \) and \( v_o \), when \( v_o \) is corrected for the proportion \( t \) of glycolate carbon that is released in photorespiration. According to the scheme of Tlblert (25), \( t = 0.25 \).

By combining equations 6 and 8, net photosynthesis can thus be expressed,

\[
P_{ni} = v_r - tv_o = \frac{V_r}{K_r + C} = \frac{v_o}{K_o + C} \quad (9)
\]

Equation 9 is an expression of net photosynthesis in terms of the kinetic parameters of RuDP carboxylase. From equation 1,

\[
P_{ni} = CE e^{-K_r / K_o} (C - kO) \quad (10)
\]

By defining \( CE \) as \( V_r / K_r \) and \( k \) as \( V_r K_r / V_o K_o \), and since \( e^{-K_r / K_o} \) is approximated by \( K_r / (K_r + O) \), it is apparent that the empirical equation describing the effect of \( O_2 \) on photosynthesis developed by Ogren and Bowes (20) is equivalent to the description of photosynthesis derived from the kinetic properties of RuDP carboxylase. The equivalence of equations 1 and 9 further supports the concept that \( CE \) is an expression of RuDP carboxylase activity in vivo, and that RuDP carboxylase regulates both soybean photosynthesis and photorespiration.

To further examine this concept, kinetic constants of the carboxylase and oxygenase activities of RuDP carboxylase were determined at three temperatures for comparison with the analogous constants derived from leaf photosynthesis measurements. From the data in Figure 5, the \( Km(CO_2) \) and \( Ki(O_2) \) were determined at 15, 25, and 35°C for the carboxylase reaction. As reported previously (6, 20), \( O_2 \) is a competitive inhibitor of RuDP carboxylase with respect to \( CO_2 \). The \( Km(O_2) \) and \( Ki(CO_2) \) for the oxygenase reaction at the same three temperatures were determined from the data in Figure 6. Carbon dioxide was found to inhibit competitively the oxygenase reaction with respect to \( O_2 \). The inhibition of oxygenase activity by \( CO_2 \) was not due to depletion of RuDP, for the concentration of RuDP was maintained at greater than saturating levels (0.4 mM, 6) for the duration of the assay.

The \( Km \) and \( Ki \) for the carboxylase and oxygenase activities of RuDP carboxylase at 15, 25, and 35°C are compared with analogous data obtained with intact leaves in Table I. The relative values of \( K_c \) were calculated from leaf photosynthetic data by dividing relative \( V_r \) by \( CE_{no} \), where relative \( V_r \) was calculated on the basis that the energy of activation for the RuDP carboxylase reaction was 16.7 kcal/mole (data not shown). With both leaves and purified enzyme, the affinity for \( CO_2 \) decreased with increasing temperature, while the affinity for \( O_2 \) increased with increasing temperature. The effect of temperature on \( K_c \) and \( K_o \) predicts that \( O_2 \) inhibition of photosynthesis should increase with increasing temperature. Increased \( O_2 \) inhibition of photosynthesis at higher temperature has been observed previously (15, 16), and this result is also apparent from the data in Figures 1 to 4.

Analysis of the \( CO_2 \) compensation concentration also permits comparison of kinetic constants of RuDP carboxylase with the physiology of leaf photosynthesis. The \( CO_2 \) compensation concentration is the \( CO_2 \) concentration where the rates of photosynthesis and photorespiration are equal. If photosynthesis and photorespiration rates are both regulated by RuDP carboxylase, the \( CO_2 \) compensation concentration should be a function of those kinetic properties of the carboxylase which determine the relative rates of fixation of \( CO_2 \) and \( O_2 \). At the \( CO_2 \) compensation concentration, net photosynthesis equals
Table III. Effect of Temperature on the Affinity of RuDP Carboxylase for CO₂ and O₂, and on the Relative CO₂ Compensation Concentration (Γ) Calculated from Enzyme Data and Observed with Leaves

<table>
<thead>
<tr>
<th>Temp</th>
<th>Kᵣ</th>
<th>Kᵣ</th>
<th>Relative Γ₀</th>
<th>Relative Γ³</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>μM CO₂</td>
<td>mM O₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>20</td>
<td>0.42</td>
<td>0.50</td>
<td>0.44</td>
</tr>
<tr>
<td>25</td>
<td>25</td>
<td>0.39</td>
<td>0.54</td>
<td>0.71</td>
</tr>
<tr>
<td>30</td>
<td>34</td>
<td>0.38</td>
<td>0.70</td>
<td>0.85</td>
</tr>
<tr>
<td>35</td>
<td>49</td>
<td>0.36</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

1 Data from Fig. 5, 30 C data from Table II.
2 Relative Γ calculated from equation 11.
3 Relative Γ calculated from direct measurement of Γ.

Therefore any effect of temperature on the kinetic constants V or K of the carboxylase should be reflected in changes in Γ. For the temperature range studied in these experiments, the energy of activation of the carboxylase reaction was 16.7 kcal/mole, and for the oxygenase reaction was 16 kcal/mole (data not shown). Thus the ratio Vᵣ/Vₑ is constant with temperature, and Γ should be determined by the ratio Kᵣ/Kₑ. Kᵣ and Kₑ were determined as the Km(CO₂) and Ki(O₂) for the RuDP carboxylase reaction at 15, 25, 30, and 35 C (Table III). From these constants, relative values of Γ were calculated and compared with the relative Γ obtained at these four temperatures with a soybean leaf. Because Kₑ increases and Kᵣ decreases with increasing temperature, the calculation predicts that Γ will increase with increasing temperature. Higher Γ at higher temperatures was observed in Figure 2 and Table III, and by other workers (5, 16).

The effect of temperature on the relative activities of the carboxylase and oxygenase reactions was also determined directly by simultaneous measurement of CO₂ and O₂ fixation in the same reaction vessel at pH 8.5 in the presence of 2.5 mM NaHCO₃ and 100% O₂. The experiment was done at four temperatures (Fig. 7). These direct measurements show that the ratio of oxygenase to carboxylase activity increases as the temperature increases them 15 to 35 C. This finding is consistent with predictions made from the effect of temperature on Kᵣ and Kₑ, and with the observations reported here and elsewhere that O₂ effects, including direct inhibition of photosynthesis and stimulation of glycolate synthesis and thereby photorespiration, increase with increasing temperature.

From Equations 6 and 7,

\[
\frac{Vₑ}{Vᵣ} = \frac{Vᵣ Kₑ O}{Vₑ Kᵣ C}
\]  

Thus \(\frac{Vₑ}{Vᵣ}\) is directly proportional to the ratio of O₂ to CO₂ concentrations at any given pH and temperature, so if \(\frac{Vₑ}{Vᵣ}\) is known for a given ratio of O₂ to CO₂ concentration, \(\frac{Vₑ}{Vᵣ}\) can be determined and \(\frac{Vᵣ}{Vₑ}\) can be calculated for any other O₂ and CO₂ concentration. The ratio \(\frac{Vᵣ}{Vₑ}\) equals 1.5 at pH 8.5 and 25 C in 2.5 mM NaHCO₃ (16.7 μM CO₂) and 100% O₂ (Fig. 7) and \(\frac{Vᵣ}{Vₑ}\) equals 2.2 at 1.0 mM NaHCO₃ (6.7 μM CO₂) and 100% O₂ at the same pH and temperature (17).
Calculation of \( v_r / v_a \) at atmospheric concentrations of substrates (10 \( \mu M \) CO\(_2\), 21% O\(_2\)) from the data above gives a ratio in the range of 0.3 to 0.5. Zelitch (29) has stated that any proposed pathway of glycylate synthesis must equal the rate of net photosynthesis in a leaf since he has observed (28), with excised tobacco leaves in the presence of an inhibitor of glycylate metabolism, that 50% of the radiocarbon fixed accumulated in glycylate. Under these conditions, glycylate is an end product of photosynthesis. Since most of the 3-P-glyceraldehyde formed in photosynthesis must be recycled to RuDP, glycylate accumulation will equal 50% of the net carbon fixed in photosynthesis when RuDP carboxylase metabolizes one O\(_2\) molecule for every four CO\(_2\) molecules (Fig. 8). Thus the ratio of oxygenase to carboxylase activity observed with purified RuDP carboxylase is sufficient to account for the observed rates of glycylate accumulation and photosynthesis.

Bassham and Kirk (3) calculated that up to 50% of the total glycylate synthesized in the light in Chlorella can be produced from RuDP via P-glycylate, but that the remainder must come from alternate sources. This calculation was based on two types of experiments. In one experiment, algae in O\(_2\) were flushed with N\(_2\) and the rate of P-glycylate hydrolysis compared with the rate of glycylate synthesis. Such changes in gas phase must be accomplished instantaneously, for any O\(_2\) remaining in solution after the N\(_2\) flushing begins will support P-glycylate synthesis and cause an underestimate of P-glycylate hydrolysis. The data (Fig. 4 and 6 in ref. 3) show that some O\(_2\) is present for at least 30 sec after flushing with N\(_2\) is begun, so the estimated rate of P-glycylate hydrolysis is too low. It is not possible to quantitate the magnitude of the error.

In the second experiment, rates of glycylate formation and P-glycylate hydrolysis were calculated at two points where the
rate of change of P-glycolate concentration was zero, and the rates of glycolate accumulation and P-glycolate hydrolysis were determined from the solution of two simultaneous equations (3). In these calculations, it was assumed that the rate of P-glycolate hydrolysis was proportional to the P-glycolate concentration (i.e. velocity of P-glycolate hydrolysis equals $K[PG]$). However, P-glycolate is hydrolyzed by P-glycolate phosphatase (22), and so it is more reasonable to expect that the rate of P-glycolate hydrolysis is governed by enzyme kinetics (i.e. velocity of P-glycolate hydrolysis equals $V[PG]/(K_m + [PG])$). The $K_m$ for P-glycolate in the phosphatase reaction in the presence of $Mg^{2+}$ is 0.08 mm (1). (Calculation of the in vivo $K_m$(PG) for P-glycolate phosphatase, from the data at times 21 and 34 min in Table II in ref. 3, gives a value of 0.06 mm).

Solving the simultaneous equations given by Bassham and Kirk (3) on the basis that P-glycolate hydrolysis follows enzyme kinetics shows that the rate of P-glycolate hydrolysis is greater than the rate of glycolate accumulation, indicating that some of the glycolate synthesis is subsequently metabolized, as was found to be the case. Thus the data of Bassham and Kirk (3) is also consistent with the concept that the RuDP oxygenase pathway is sufficiently active to account for the major portion, if not the total amount, of oxygen-dependent glycolate synthesis.

If one O$_2$ molecule is fixed for every four CO$_2$ molecules fixed in leaf photosynthesis in air, and if 0.5 mole of CO$_2$ is released for every mole of glycolate formed, as described by Tolbert (25), net photosynthesis will equal 3.5 C fixed, and the ratio of photorespiration to net photosynthesis will equal 0.5/3.5 or about 14% (Fig. 8). Ludwig and Canvin (19), measuring photorespiration in air as the difference between true photosynthesis and net photosynthesis, found that photorespiration was equal to about 18% of net photosynthesis. The stoichiometry depicted in Figure 8 is consistent with the observed rates of glycolate synthesis and photorespiration in leaf tissue, and with the measured rates of oxygenase and carboxylase activities of RuDP carboxylase.

The total oxygen inhibition of soybean photosynthesis in air equals 40 to 45% of net photosynthesis (12, 19). The stoichiometry presented in Figure 8 is consistent with this observation, and predicts that, in the absence of O$_2$, net photosynthesis will increase from 3.5 to 5.0 units, an increase of 43%. As indicated above, photorespiratory CO$_2$ evolution is about 15% of net photosynthesis, or one-third of the total inhibition. The remaining two-thirds of the total O$_2$ inhibition is expressed by the exponential term in equation 1 and by the term $K_o/(K_o + O)$ in equation 9, and represents a direct inhibition of photosynthesis by O$_2$. The values of $K_o$ are similar in both equations 1 and 9, a strong indication that the direct inhibition of photosynthesis by O$_2$ the major portion of the total inhibition, is due to competitive inhibition of RuDP carboxylase in vivo by O$_2$ with respect to CO$_2$.

LITERATURE CITED


