Regulation of Soybean Net Photosynthetic CO₂ Fixation by the Interaction of CO₂, O₂, and Ribulose 1,5-Diphosphate Carboxylase\textsuperscript{1, 2}

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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, O₂ inhibition of CO₂ incorporation and the ratio of oxygenase activity to carboxylase activity increased with increased temperature. The increased O₂ sensitivity of the enzyme at higher temperatures was caused by a reduced affinity of the enzyme for CO₂ and a slightly increased affinity of the enzyme for O₂. The similarity of the effect of temperature on the affinity of intact leaves and of purified ribulose 1,5-diphosphate carboxylase for CO₂ and O₂ provides further evidence that the carboxylase regulates the O₂ response of photosynthetic CO₂ fixation in soybean leaves. Based on results reported here and in the literature, a scheme outlining the stoichiometry between CO₂ and O₂ fixation in vivo is proposed.

Oxygen competitively inhibited carboxylase activity with respect to CO₂ 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 photosynthetic 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 the 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 dihydroxyethylthiamine pyrophosphate moiety produced in the transketolase reactions of the Calvin cycle may be oxidized to glycophosphate 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 glycophosphate 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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\textsuperscript{4} Abbreviations: RuDP: ribulose 1,5-diphosphate; PG: P-glycolate; \( K_i \): 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% O2 induced sequential transients of RuDP and P-glycolate, followed by glycolate accumulation. If RuDP carboxylase regulates both photosynthetic CO2 fixation and photorespiratory CO2 evolution, then the kinetic properties of the carboxylase and oxygenase activities of this enzyme must be manifested in the characteristics of photosynthetic and photorespiratory CO2 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 μeinsteins 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 μeinsteins 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 N2, O2, and 1% CO2 in N2, and flushed over the leaf. The system was monitored for about 10 sec. The change in CO2 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 CO2, and the rate of CO2 evolution was determined. The leaf was flushed with air for 5 min between photorespiration measurements. CO2 concentration was monitored with an infrared gas analyzer, and O2 with a Clark-type electrode modified for use with gases. Carboxylation efficiency (CE) of leaf photosynthetic CO2 fixation was calculated according to Forrester et al. (12), where CE equals the measured photosynthesis rate divided by the difference between the ambient CO2 concentration and the CO2 compensation concentration. CE is an exponential function of O2 concentration (20), so carboxylation efficiency in the absence of O2 (CEO2) was determined by extrapolating a logarithm plot of CE versus O2 to zero O2. The inhibition constant of O2 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-diphenylformazan derivative of glycolate. The concentration of this derivative was determined spectrophotometrically (17). CO2 and O2 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 CO2 fixation and stimulation of photorespiratory CO2 evolution, have been mathematically described by Ogren and Bowes (20) as shown in equation 1.

\[ Pn = (\text{Ce} e^{-0/K_0}) - (\text{Ce} e^{-0/K_0} kO) \]  

(true photosynthesis) - (photorespiration)

where \( Pn \) is net photosynthesis, \( \text{Ce} \), is carboxylation efficiency of a leaf in the absence of O2, \( K_0 \) is the inhibition constant of direct O2 inhibition of photosynthesis, \( k \) is the slope of a plot of CO2 compensation concentration versus O2 concentration, \( C \) is the CO2 concentration, and \( O \) is the O2 concentration. The quantity \( kO \) equals the CO2 compensation concentration, \( \Gamma \). This equation applies only where the rate of photosynthetic CO2 fixation is a linear function of CO2 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 O2 competitively inhibits RuDP carboxylase with respect to CO2 (6, 20) and by the discovery of Bowes et al. (7) that O2 can substitute for CO2 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 CO2, glycine, and serine by the photorespiratory pathway (23). 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 CO2, O2, and RuDP carboxylase in vitro.

The rate of photosynthesis was determined at 186 μl/l CO2 and several O2 concentrations at three temperatures. Carboxylation efficiency was then calculated by dividing photosynthesis rate by 186 - \( \Gamma \), as described by Forrester et al. (12). A linear relationship between log CE and O2 concentration was found to occur (Fig. 1), as expected from equation 1. The intercept gives \( CE_0 \), and the slope equals \( -K_0 \). The values of \( K_0 \) are listed in Table I. Equation 1 also dictates that the CO2 compensation concentration is a linear function of the O2 concentration. Experimental evidence of the linear relationship between O2 concentration and the CO2 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 CO2 evolution into CO2-free gases of various O2 concentrations. In zero CO2, true photosynthesis equals zero, so equation 1 can be rewritten,

\[ -Pn = CE_0 e^{-0/K_0} kO \]  

CO2 evolution (\(-Pn\)) into several mixtures of O2/N2 was measured at three temperatures. Carboxylation efficiency was then calculated by dividing the measured rate of CO2 evolution by the CO2 compensation concentration, and graphing the O2 concentration at the O2 compensation concentration and temperature. The calculated values of carboxylation efficiency are plotted in Figure 3. Again, a linear relationship between CE and O2 concentration was obtained. The values for \( K_0 \) determined from Figure 3 are listed in Table I.

Photosynthesis rate of soybean leaves as a function of CO2 concentration was determined at three O2 concentrations and three temperatures (Fig. 4). \( CE \), and \( K_0 \) were determined for each temperature (Table I). Equation 1 was then used to calculate photosynthesis rates at four CO2 concentrations for each O2 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 \( K_0 \), calculated from these two types of measurements, and
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 ½ 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>Kᵣ</th>
<th>Kᵢ</th>
<th>K₃</th>
<th>K₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>35°C</td>
<td>2.49</td>
<td>0.52</td>
<td>0.82</td>
<td>0.60</td>
</tr>
<tr>
<td>25°C</td>
<td>1.36</td>
<td>0.71</td>
<td>0.98</td>
<td>0.79</td>
</tr>
<tr>
<td>15°C</td>
<td>1.00</td>
<td>1.43</td>
<td>0.90</td>
<td>0.90</td>
</tr>
</tbody>
</table>

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

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 ½ at 15 °C.
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Standard reaction mixtures contained 50 mM tris (pH 8.5), 6 mM MgCl₂, 0.1 mM EDTA, and variable concentrations of NaHCO₃, RuDP, and O₂. Km(CO₂) and Ki(O₂) were determined at 0.4 mM RuDP at 5 NaHCO₃ concentrations, under N₂ or O₂. Km(O₂) and Ki(CO₂) were determined at 0.6 mM RuDP at four O₂ concentrations, in the presence of 0 and 5 mM NaHCO₃. Km(RuDP) was determined from 5 RuDP concentrations in either 20 mM NaHCO₃, under N₂ or under O₂. All experiments were initiated with enzyme, and the reactions were stopped after 5 min with 1 N HCl.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Km (µM)</th>
<th>Ki (µM)</th>
<th>Km (RuDP) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂</td>
<td>34.0 ± 0.5</td>
<td>38.0 ± 0.5</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>O₂</td>
<td>0.39 ± 0.02</td>
<td>0.37 ± 0.01</td>
<td>0.17 ± 0.01</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,

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

(3)

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),

\[ v_c = \frac{V K_c}{K_c K_o + K_c I + K_o} \]

(4)

\[ v_o = \frac{V K_o}{K_c K_o + K_c I + K_o} \]

(5)

where \( v_c \) is the velocity of the carboxylation reaction, \( v_o \) is the velocity of the oxygenase reaction, \( V_c \) the maximal velocity of the carboxylation reaction, \( K_c \) 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,

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

(6)

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

(7)

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

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

(8)

Fig. 4. Net photosynthesis rate in soybean leaves as a function of CO₂ concentration at three O₂ concentrations at three temperatures. O: measured values; X: calculated values.

Between the observed and calculated rates of photosynthesis indicates that equation 1 is an accurate description of the effect of O₂ on soybean photosynthesis when photosynthesis is proportional to CO₂ concentration.

Enzyme Measurements. The Km and Ki constants for CO₂ and O₂, and the Km (RuDP) were determined for the carboxylase and oxygenase activities of RuDP carboxylase at 30 C (Table II). Within the limits of experimental error, the Km (CO₂) in the carboxylase reaction (34 µM) was identical with the...
Net photosynthesis is the difference between \( v_s \) and \( v_s \), when \( v_s \) is corrected for the proportion \( t \) of glycolate carbon that is released in photorespiration. According to the scheme of Tobert (25), \( t = 0.25 \).

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

\[
P_n = v_s - t v_s = \frac{V_s}{K_s} \left( \frac{K_s}{K_s + O} \right) \left( C - t \frac{V_s K_s}{V_s K_s} \right)
\]

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

\[
P_n = CE_v e^{-K_t/K_r} (C - kO)
\]

By defining \( CE_v \) as \( V_s/K_s \), and \( k \) as \( V_s K_s/V_s K_s \), and since \( e^{-K_t/K_r} \) is approximated by \( K_s/(K_s + O) \), 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 \( CE_v \) 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 \( K_m(CO_2) \) and \( K_i(O_2) \) 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 \( K_m(O_2) \) and \( K_i(CO_2) \) 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 \( K_m \) and \( K_i \) 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 \( V_s \) by \( CE_v \), where relative \( V_s \) 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...
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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 (°C)</th>
<th>Kₐ (μM CO₂)</th>
<th>Kₐ (mM O₂)</th>
<th>Relative Γ⁺</th>
<th>Relative Γ⁻</th>
</tr>
</thead>
<tbody>
<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 Vₐ/Vₓ is constant with temperature, and Γ should be determined by the ratio Kₐ/Kₓ. Kₓ and K₁ 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 Kₓ increases and K₁ 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 K₁ and Kₓ, and with the observations reported here and elsewhere that O₂ effects, including direct inhibition of photosynthesis and stimulation of glycolate synthesis and thereby photosynthesis, increase with increasing temperature.

From Equations 6 and 7,

\[ \frac{v_x}{v_o} = \frac{V_x K_o}{V_o K_x} \]

Thus \( \frac{v_x}{v_o} \) is directly proportional to the ratio of O₂ to CO₂ concentrations at any given pH and temperature, so if \( \frac{v_x}{v_o} \) is known for a given ratio of O₂ to CO₂ concentration, \( \frac{V_x K_o}{V_o K_x} \) can be determined and \( \frac{v_x}{v_o} \) can be calculated for any other O₂ and CO₂ concentration. The ratio \( \frac{v_x}{v_o} \) 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 \( \frac{v_x}{v_o} \) equals 2.2 at 1.0 mM NaHCO₃ (6.7 μM CO₂) and 100% O₂ at the same pH and temperature (17).

![Fig. 6. Double reciprocal plots of RuDP oxygenase activity as a function of O₂ concentration in the presence or absence of NaHCO₃ at three temperatures. Reaction mixtures contained 50 mM tris (pH 8.5), 0.3, or 4 mM NaHCO₃, 0.6 mM RuDP, 6 mM MgCl₂ and 0.1 mM EDTA. The reaction mixtures were flushed with various O₂/N₂ mixtures for 5 min, water or NaHCO₃ added, and the reactions were initiated with enzyme. After 5 min, the reactions were stopped by the addition of 1 N HCl. P-glycolate produced was determined as described elsewhere (17).](https://www.plantphysiol.org)
FIG. 7. Ratio of P-glycolate formation to CO₂ fixation catalyzed by RuDP carboxylase at 15, 25, 30, and 35 C. Reaction mixtures contained 50 mM tris (pH 8.5), 2.5 mM NaHCO₃, 0.6 mM RuDP, 6 mM MgCl₂, and 0.1 mM EDTA. Reaction mixtures were flushed with O₂ for 5 min, NaHCO₃ added, and the reactions were initiated with enzyme. After 4 min, the reactions were stopped by the addition of 1 N HCl. P-glycolate formation and incorporation of radiocarbon were determined in aliquots of the same reaction mixture, as described elsewhere (17).

Calculation of vₐ / vₛ at atmospheric concentrations of substrates (10 μM CO₂, 21% O₂) 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 glycolate 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 glycolate metabolism, that 50% of the radiocarbon fixed accumulated in glycolate. Under these conditions, glycolate is an end product of photosynthesis. Since most of the 3-P-glycerate formed in photosynthesis must be recycled to RuDP, glycolate accumulation will equal 50% of the net carbon fixed in photosynthesis when RuDP carboxylase metabolizes one O₂ molecule for every four CO₂ 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 glycolate accumulation and photosynthesis.

Bassham and Kirk (3) calculated that up to 50% of the total glycolate synthesized in the light in Chlorella can be produced from RuDP via P-glycolate, but that the remainder must come from alternate sources. This calculation was based on two types of experiments. In one experiment, algae in O₂ were flushed with N₂, and the rate of P-glycolate hydrolysis compared with the rate of glycolate synthesis. Such changes in gas phase must be accomplished instantaneously, for any O₂ remaining in solution after the N₂ flushing begins will support P-glycolate synthesis and cause an underestimate of P-glycolate hydrolysis. The data (Fig. 4 and 6 in ref. 3) show that some O₂ is present for at least 30 sec after flushing with N₂ is begun, so the estimated rate of P-glycolate hydrolysis is too low. It is not possible to quantitate the magnitude of the error.

In the second experiment, rates of glycolate formation and P-glycolate 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 \((i.e. \text{velocity of P-glycolate hydrolysis equals } K[P]G)\). 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 \((i.e. \text{velocity of P-glycolate hydrolysis equals } V[PG]/(Km + [PG]))\). The \(Km\) for P-glycolate in the phosphatase reaction in the presence of Mg²⁺ is 0.08 mm \((1)\). (Calculation of the in vivo \(Km(PG)\) 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 43% 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 \(K_p/(K_p + O)\) in equation 9, and represents a direct inhibition of photo- synthesis by \(O_2\). The values of \(K_p\) 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


