Oxygen Inhibition and Other Properties of Soybean Ribulose 1,5-Diphosphate Carboxylase*

(Received for publication, September 10, 1971)

GEORGE BOWES† AND WILLIAM L. OGREN

From the Department of Agronomy, University of Illinois, Urbana, Illinois 61801, and the United States Regional Soybean Laboratory, Plant Science Research Division, Agricultural Research Service, United States Department of Agriculture, Urbana, Illinois 61801

SUMMARY

\(d\)-Ribulose-1,5-di-P carboxylase, purified from soybeans, had \(K_n\) values of 0.13 mM for \(\text{CO}_2\) and 0.19 mM for ribulose-1,5-di-P under a \(\text{N}_2\) atmosphere. \(\text{O}_2\) inhibited \(\text{CO}_2\) incorporation by the enzyme and this inhibition was rapidly reversed by \(\text{N}_2\). Inhibition was competitive with respect to \(\text{CO}_2\) and uncompetitive with respect to ribulose-1,5-di-P. The \(K_i\) for \(\text{O}_2\) was 0.8 mM. This \(\text{O}_2\) inhibition, together with the ribulose-1,5-di-P carboxylase-catalyzed oxidation of ribulose-1,5-di-P to P-glycolate observed previously (Bowes, G., Ogren, W. L., and Hageman, R. H. (1971) Biochem. Biophys. Res. Commun. 45, 716-722), explains the “Warburg effect”: the rapidly reversible \(\text{O}_2\) inhibition of photosynthesis and stimulation of glycolate production seen in plants and isolated chloroplasts. In corn and soybean extracts, ribulose-1,5-di-P carboxylase was inhibited by \(\text{O}_2\) but P-enolpyruvate carboxylase was unaffected. These data may explain the different response to \(\text{O}_2\) by plants which utilize ribulose-1,5-di-P carboxylase for the initial photosynthetic carboxylation and those utilizing P-enolpyruvate carboxylase. The optimum temperature for purified ribulose-1,5-di-P carboxylase was 55°C and activation energies, in kilocalories per mole, were 18.4 in \(\text{N}_2\) and 20.4 in \(\text{O}_2\).

Phosphorylated compounds inhibited \(14\text{CO}_2\) incorporation by the enzyme. Nonphosphorylated sugars, including ribulose, did not inhibit. Fructose-1,6-di-P was a competitive inhibitor with respect to ribulose-1,5-di-P, the \(K_i\) being 0.88 mM. Fructose-1,6-di-P was a more effective inhibitor than fructose-6-P, fructose-1-P, and ribulose-5-P suggesting that both phosphate groups of ribulose-1,5-di-P are involved in binding to the enzyme. \(\text{HgCl}_2\) was a noncompetitive inhibitor with respect to \(\text{CO}_2\) and a mixed inhibitor with respect to ribulose-1,5-di-P, suggesting that sulfhydryl groups are not involved in \(\text{CO}_2\) binding but may be close to the site where ribulose-1,5-di-P binds to the enzyme.

\(d\)-Ribulose-1,5-di-P carboxylase (EC 4.1.1.39), the predominant component of Fraction I protein, is associated with the chloroplast and comprises up to 50% of the soluble protein in leaves (1). This enzyme, purified from spinach (2), catalyzes the reaction

\[d\text{-ribulose-1,5-di-P} + \text{CO}_2 \rightarrow 2 3\text{-d-phosphoglycerate}\]

Evidence (3, 4) indicates this enzyme is the rate-limiting step in light-saturated photosynthesis of Calvin cycle plants (C\(_3\) species).

An important factor in the photosynthesis of C\(_4\) species is the reversible \(\text{O}_2\) inhibition of photosynthetic \(\text{CO}_2\) fixation, termed the Warburg effect. Many schemes have been proposed to account for this inhibition (5, 6), however, elucidation of the mechanism has been complicated by another phenomenon, photorespiration, which also occurs in C\(_3\) species. Photorespiration is characterized by a light- and \(\text{O}_2\)-dependent efflux of \(\text{CO}_2\), this efflux being inhibited by increasing \(\text{CO}_2\) concentrations (3, 7). These two phenomena are re-examined in view of the properties observed with purified soybean ribulose-1,5-di-P carboxylase.

In certain plants another \(\text{CO}_2\) fixation pathway occurs (8). These plants, termed C\(_4\) dicarboxylic acid cycle or C\(_4\) species, utilize P-enolpyruvate carboxylase for the initial photosynthetic carboxylation. However, ribulose-1,5-di-P carboxylase is also important in the photosynthesis of C\(_4\) species as carbon flows through this enzyme to carbohydrate (9). \(\text{O}_2\) levels below atmospheric do not inhibit the photosynthesis of C\(_4\) species and these species lack photorespiratory \(\text{CO}_2\) release (3, 7). The different response to \(\text{O}_2\) by C\(_3\) and C\(_4\) species was investigated with a C\(_4\) plant, corn, and a C\(_3\) plant, soybean.

Purified ribulose-1,5-di-P carboxylase has a reasonably high affinity for ribulose-1,5-di-P, the \(K_m\) being 0.12 mM (10). Until recently the enzyme was considered to possess a low affinity for \(\text{HCO}_3^-\) in vitro, with reports of \(K_m\) values ranging from 2.5 to 30 mM (1). However \(\text{CO}_2\), and not \(\text{HCO}_3^-\), is the reactive species (11). \(K_m\) calculations on this basis give values approximating 0.15 mM (4), but in vivo \(K_m\) values for leaves and intact chloroplasts appear lower by an order of magnitude (12, 13). Compounds such as \(p\)-CMB\(^3\) and IAA (14), 5,5'-dithiobis-(2-nitrobenzoic acid) (15), and \(\text{HgCl}_2\) (2) are effective inhibitors, indicating that the enzyme contains sulfhydryl groups (16). These groups

\(p\)-CMB, \(p\)-chloromercuribenzoic acid; IAA, iodoacetamide.
appear to have a closer relationship with the substrate ribulose-1,5-di-P than with CO$_2$ (15, 17), although whether they are an integral part of the catalytic site is still unresolved. Part of the work reported here attempts to clarify some problems associated with the role of sulfhydryl groups and the mechanism of substrate binding to the enzyme.

A preliminary report of part of this work has appeared (18).

**EXPERIMENTAL Procedure**

**Purification**—Ribulose-1,5-di-P carboxylase was purified from leaves of field- or greenhouse-grown soybeans (Glycine max (L.) Merrill var. Wayne) by a modification of a method described for spinach (10). Extraction difficulties with soybean necessitated two homogenization steps, with the filtrates being combined. Overnight storage at the ammonium sulfate I stage reduced activity, therefore purification was continued through to where the enzyme could be stored at -20℃.

The isolated enzyme was stored at 5℃ as a precipitate in 55% saturated ammonium sulfate, pH 6.5, with 0.1 mM EDTA and 5.0 mM 2-mercaptoethanol. Prior to assay, an aliquot of the suspension was centrifuged at 10,000 × g for 10 min, the supernatant discarded, and the precipitated enzyme dissolved in 120 mM Tris buffer, pH 8.0, containing 0.25 mM EDTA, and 10.0 mM MgCl$_2$. After dissolving, enzyme activity gradually increased by up to 60%. Thus to achieve maximum activity the enzyme solution was kept at 2℃ for 4 hours before use.

Stored enzyme lost activity over 2 months, so control values in different experiments varied. Although absolute values between experiments could not be compared, repetition of experiments with different preparations and enzyme ages showed that findings were comparable when the control values were taken into account. The specific activity was lower than that reported for spinach (10), being in the range of 10 to 100 nmoles of CO$_2$ fixed per min per mg of protein. This can be partially attributed to inactivation during isolation and to the use of lower assay concentrations of ribulose-1,5-di-P and NaH$_2$CO$_3$.

Soluble protein in extracts was determined by the procedure of Lowry et al. (19) and in purified enzyme solutions by the method of Warburg and Christian (20).

Assay Procedures—Ribulose-1,5-di-P carboxylase activity was assayed by $^{14}$CO$_2$ incorporation into acid stable products. At 5.0 mM MgCl$_2$, the pH optimum was 8.0. The reaction vessels contained 50.0 mM Tris at pH 8.0, 5.0 mM MgCl$_2$, 0.1 mM ribulose-1,5-di-P, and 0.05 mM EDTA in a final volume of 1.0 ml. The vessels were flushed with N$_2$, CO$_2$-free air or O$_2$ and shaken for 15 min, then were sealed and 20.0 mM NaH$_2$CO$_3$ (1.0 μCi) injected via a serum cap. CO$_2$-free air was used to avoid isotope dilution by atmospheric CO$_2$. The reaction was initiated by injection of the enzyme, or alternatively ribulose-1,5-di-P, and stopped with 0.1 ml of 6.0 mM acetic acid after 4 min at 25℃. Aliquots were then taken and dried at 90℃, dissolved in a modified Bray’s solution (21) and the disintegrations per min determined with a scintillation counter.

In experiments with crude extracts of soybean and corn (Zea mays L. var. WF9 × M14), approximately 500 mg (fresh weight) of leaf discs were homogenized under N$_2$ at 2℃ in a Ten Broeck homogenizer with 5.0 ml of 50.0 mM Tris at pH 8.0 containing 10.0 mM MgCl$_2$, 0.1 mM EDTA, and 5.0 mM d-isoascorbate. The extracts were centrifuged at 35,000 × g for 15 min in capped tubes under N$_2$, and then assayed immediately for P-enolpyruvate carboxylase activity (22). The P-enolpyruvate carboxylase assay contained, in a final volume of 1.0 ml, 50.0 mM Tris at pH 8.0, 10.0 mM MgCl$_2$, 0.1 mM EDTA, 5.0 mM sodium glutamate, 5.0 mM NaH$_2$CO$_3$ (2.0 μCi), and 2.0 mM P-enolpyruvate. The crude ribulose-1,5-di-P carboxylase assay mixture was similar with sodium glutamate was omitted and 0.2 mM ribulose-1,5-di-P substituted for P-enolpyruvate. Both the P-enolpyruvate and ribulose-1,5-di-P carboxylase assays were initiated with 0.1 ml of the extract and stopped with 0.1 ml of 6.0 mM acetic acid after 3 min at 25℃ under atmosphere of N$_2$ or O$_2$.

In crude and purified enzyme assays, reaction rates were linear over the assay period employed. No prior incubation of ribulose-1,5-di-P carboxylase with Mg$^{2+}$ and HCO$_3^-$ was required for linear rates.

**RESULTS**

**Effect of Ribulose-1,5-di-P on Carboxylase Activity in N$_2$, CO$_2$-free Air, and O$_2$**—As reported for spinach carboxylase (2), increasing concentrations of ribulose-1,5-di-P up to 0.5 mM increased the reaction velocity, while higher concentrations caused inhibition (Fig. 1). O$_2$ inhibited $^{14}$CO$_2$ incorporation at all ribulose-1,5-di-P concentrations, 100% O$_2$ being more effective than 21% O$_2$ (CO$_2$-free air).

**Kinetic Studies of O$_2$ Inhibition**—Further investigation showed the O$_2$ inhibition was fully and rapidly reversible (Table I). Prior to initiation of the reaction with ribulose-1,5 di P, flushing the reaction mixture and enzyme with N$_2$ for 6 min followed by O$_2$ for 6 min, or vice versa, produced inhibition only if the last gas treatment was O$_2$. Thus the inhibitory effect of O$_2$ was not due to permanent inactivation of the enzyme.

A double reciprocal plot of the inhibitory effect of O$_2$ showed that inhibition was competitive with respect to CO$_2$ (Fig. 2). The $K_m$ value was 0.13 mM in N$_2$, 0.19 mM in 21% O$_2$ (CO$_2$-free air), and 0.35 mM in 100% O$_2$. The $K_m$ in CO$_2$-free air is within the range of values reported for spinach carboxylase (1), when all values are calculated on the basis of CO$_2$ rather than HCO$_3^-$ concentration. The $K_i$ for O$_2$ was 0.8 mM, which is slightly lower than previously reported (4). The competitive nature of
Reversible effect of O$_2$ on rate of $^{14}$CO$_2$ incorporation by soybean ribulose-1,5-di-P carboxylase

Prior to addition of 5.0 mM NaH$^{14}$CO$_3$ and initiation of the reaction with 0.1 mM ribulose-1,5-di-P, reaction mixtures containing the enzyme were shaken at 25$^\circ$C and flushed with either N$_2$ or O$_2$ for 12 min. Alternatively, reaction mixtures were flushed with N$_2$ for 6 min followed by O$_2$ for 6 min or vice versa. The flasks were then sealed and enzyme activity assayed under the atmosphere of the last gas treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^{14}$CO$_2$ incorporation (nmoles CD/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N$_2$ 12 min/assay 3 min in N$_2$.</td>
<td>43.5</td>
</tr>
<tr>
<td>O$_2$ 12 min/assay 3 min in O$_2$.</td>
<td>30.0</td>
</tr>
<tr>
<td>N$_2$ 6 min/O$_2$ 6 min/assay 3 min in O$_2$.</td>
<td>32.0</td>
</tr>
<tr>
<td>O$_2$ 6 min/N$_2$ 6 min/assay 3 min in N$_2$.</td>
<td>46.7</td>
</tr>
</tbody>
</table>

O$_2$ inhibition suggests that O$_2$ binds to the site that CO$_2$ occupies on the enzyme.

With respect to ribulose-1,5-di-P, O$_2$ produced mixed inhibition of the uncompetitive type (Fig. 3). The $K_m$ for ribulose-1,5-di-P was 0.19 mM in N$_2$, which compares with reported spinach carboxylase values in air ranging from 0.12 to 0.7 mM (1). One enzyme preparation, stored in an intermediate stage of purification, exhibited variation from these $K_m$ values, although in other respects its properties were unaltered.

The reduced rate of $^{14}$CO$_2$ incorporation observed in CO$_2$-free air could be largely overcome by GSH. The rate of $^{14}$CO$_2$ incorporation at 20 mM HCO$_3^-$, measured as nanomoles per min per mg of protein, was reduced from 25.5 in N$_2$ to 21.9 in CO$_2$-free air, and the addition of 3.0 mM GSH increased the rate to 24.3. Measurements with an O$_2$ electrode showed that GSH reduced the O$_2$ concentration in a reaction mixture under CO$_2$-free air. Thus the protection afforded by GSH can be attributed to a decrease in the O$_2$ concentration of the reaction mixture, through the autoxidation of GSH, and not to an effect on enzyme sulfhydryl groups.

Effect of O$_2$ on Carboxylases in Corn and Soybean Leaf Extracts—$^{14}$CO$_2$ incorporation by P-enolpyruvate carboxylase in extracts of both corn and soybean was unaffected by O$_2$ (Table II). In contrast, ribulose-1,5-di-P carboxylase from both plants was inhibited by 100% O$_2$. On a protein basis, corn showed 29% and soybean 31% inhibition of ribulose-1,5-di-P carboxylase at 5.0 mM NaH$^{14}$CO$_3$ concentration. Repeating the experiment with 20.0 mM NaH$^{14}$CO$_3$ produced similar results, except the inhibition of ribulose-1,5-di-P carboxylase was less at the higher CO$_2$ level.

Temperature Response of Ribulose-1,5-di-P Carboxylase Activity in N$_2$ and O$_2$—Over a 3-min assay period purified ribulose-1,5-di-P carboxylase showed maximum activity in both N$_2$ and O$_2$ at 55$^\circ$C (Fig. 4). Activity was greatly reduced above 60$^\circ$C, due to rapid inactivation of the enzyme. The percentage inhibition by O$_2$ when calculated on a millimolar O$_2$ basis, to allow for lower O$_2$ solubility at higher temperatures, varied only 6% over the temperature range 15-60$^\circ$C (Fig. 4). Between 15 and 35$^\circ$C, energy of activation values calculated in kilocalories per mole from the Arrhenius equation were 18.4 in N$_2$, 18.5 in CO$_2$-free air, and 18.3 in O$_2$. The data are presented as a double reciprocal plot (Fig. 2) for each gas phase with NaH$^{14}$CO$_3$ concentration as the substrate (1). The slopes of the lines in each plot are equal and parallel, with the y-axis intercepts being determined by the maximal activity in the respective gas phase. The reciprocal of the y-axis intercepts for the data sets are similar, with the values observed in N$_2$ being slightly higher than in CO$_2$-free air.

Table II

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Gas phase</th>
<th>$^{14}$CO$_2$ incorporation (nmoles CD/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-Enolpyruvate carboxylase activity</td>
<td>Ribulose-1,5-di-P carboxylase activity</td>
</tr>
<tr>
<td>Corn</td>
<td>N$_2$</td>
<td>2670</td>
</tr>
<tr>
<td></td>
<td>O$_2$</td>
<td>2600</td>
</tr>
<tr>
<td>Soybean</td>
<td>N$_2$</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td>O$_2$</td>
<td>190</td>
</tr>
</tbody>
</table>

Fig. 2. Double reciprocal plot of the rate of $^{14}$CO$_2$ incorporation by soybean ribulose-1,5-di-P carboxylase as a function of CO$_2$ concentration in N$_2$, CO$_2$-free air, and O$_2$. The general assay conditions were as described under “Experimental Procedure” except the addition of 0.2 mM ribulose-1,5-di-P and 1.0 to 50.0 mM NaH$^{14}$CO$_3$ were added.
**Fig. 4.** Activity and O₂ inhibition of soybean ribulose-1,5-di-P carboxylase as a function of temperature. The general assay conditions were as described under "Experimental Procedure." The reaction flasks were incubated at temperatures ranging from 15 to 65°C while being shaken and flushed with N₂ or O₂ prior to sealing and initiation of the reaction with enzyme. To compensate for reduced O₂ solubility at higher temperatures, the percentage inhibition of activity in O₂, as compared to N₂, was plotted on the basis of the calculated O₂ concentration in the reaction mixture.

20.4 in O₂. The figure for CO₂-free air is comparable to 16.9 kcal per mole calculated for spinach carboxylase (2).

**Inhibitor Studies—**The product of the carboxylase reaction, 3-P-glycerate, is an inhibitor of spinach carboxylase (10). The results for soybean carboxylase, in Table III, confirm the inhibitory nature of 3-P-glycerate. 2-P-Glycolate was even more inhibitory. All phosphate compounds tested produced inhibition, but fructose-1,6-di-P was the most effective. A double reciprocal plot of inhibition by 1.0 mM fructose-1,6-di-P with respect to ribulose-1,5-di-P showed competitive inhibition (Fig. 3), with a Kᵣ of 0.88 mM in N₂. This supports the proposal that ribulose-1,5-di-P binds to the enzyme through one or both phosphate groups (10). An fructose-1,5 di-P inhibited to a greater extent than the monophosphates fructose-1-P, fructose-6-P, and ribulose-5-P (Table III) it is likely that both phosphate groups are involved in the binding. Sucrose, glucose, fructose, ribulose, glycolate, and glyoxylate had no appreciable inhibitory effect, thus it would seem that the phosphate groups of ribulose-1,5-di-P are the principal groups involved in its binding to the enzyme. None of the sugar phosphates tested for inhibition were able to substitute for ribulose-1,5-di-P as a CO₂ acceptor.

**NAD⁺, NADH, NADP⁺, NADPH, ADP, and ATP all caused inhibition of the reaction, probably due to the phosphate groups (Table III). The products of the photosynthetic light reactions, ATP and NADPH, were the most effective nucleotide inhibitors. Acetate, formate, and CS₂, substances resembling CO₂, had no inhibitory effect. The photosynthesis inhibitor α-hydroxy-2-pyridinemethanesulfonate inhibited soybean carboxylase by 33% at 10.0 mM.

Spinach ribulose-1,5-di-P carboxylase is inhibited by various compounds which are known to interact with sulfhydryl groups (2, 14, 15). Soybean ribulose-1,5-di-P carboxylase was effectively inhibited by HgCl₂ (Fig. 5). Incubation with HgCl₂ prior to assay increased inhibition. Incubation for 5 min with 0.01 mM HgCl₂ reduced the rate of ¹⁴CO₂ incorporation from 22.6 (without HgCl₂) to 10.9 nmol of CO₂ per min per mg of protein, while 15-min incubation further reduced the rate to 8.3. The presence of 5 mM GSH or 6 mM dithiothreitol during the 5-min incubation period overcame inhibition, the rates being 21.1 and 21.7, respectively. This suggests that the effect of the Hg²⁺ ions is on sulfhydryl groups of the enzyme.

With respect to CO₂, HgCl₂ was a noncompetitive inhibitor of

### Table III

<table>
<thead>
<tr>
<th>Compound added to the assay</th>
<th>Percentage inhibition of ¹⁴CO₂ incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 mM compound</td>
</tr>
<tr>
<td>Fructose-1,6-di-P</td>
<td>33 (a)</td>
</tr>
<tr>
<td>Fructose-6-P</td>
<td>2 (a)</td>
</tr>
<tr>
<td>Fructose-1-P</td>
<td>3 (a)</td>
</tr>
<tr>
<td>Ribulose-5-P</td>
<td>38 (d)</td>
</tr>
<tr>
<td>3-P-Glycerate</td>
<td>8 (a)</td>
</tr>
<tr>
<td>2-P-Glycolate</td>
<td>1 (a)</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
</tr>
<tr>
<td>Ribulose</td>
<td></td>
</tr>
<tr>
<td>Glycerate</td>
<td></td>
</tr>
<tr>
<td>Glycolate</td>
<td></td>
</tr>
<tr>
<td>Glyoxylate</td>
<td>4 (e)</td>
</tr>
<tr>
<td>NAD⁺</td>
<td>0 (a)</td>
</tr>
<tr>
<td>NADH</td>
<td></td>
</tr>
<tr>
<td>NADP⁺</td>
<td></td>
</tr>
<tr>
<td>NADPH</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>6 (a)</td>
</tr>
<tr>
<td>ADP</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 5.** Rate of ¹⁴CO₂ incorporation by soybean ribulose-1,5-di-P carboxylase as a function of the logarithm of HgCl₂ concentration. The assay conditions were as described under "Experimental Procedure." Assays were initiated with enzyme, without prior incubation in HgCl₂.
**Discussion**

**Oxygen Effects**—The Warburg effect is manifested by O₂ inhibition of photosynthesis in C₄ species and a concomitant O₂ stimulation of glycolate production. In leaves of higher plants, the glycolate produced is subsequently metabolized in part to CO₂ by the photorespiratory pathway (23). These O₂ effects are rapidly reversed by removing O₂ or by increasing the CO₂ level (5, 6, 24). Soybean ribulose-1,5-diphosphate carboxylase is reversibly and competitively inhibited by O₂ with respect to CO₂ (Table I, Fig. 2) and this enzyme catalyzes an oxygen-dependent oxidation of ribulose-1,5-diphosphate to 2-P-glycolate (25), a glycolate precursor (23). Thus the effects of O₂ on purified ribulose-1,5-diphosphate carboxylase mimic the effects of O₂ in the Warburg effect, strongly suggesting that ribulose-1,5-diphosphate carboxylase is the specific site of action of O₂ in the Warburg effect.

It has been suggested (5) that O₂ may reversibly inactive sulfhydryl groups in enzymes of the Calvin cycle, with reactivation being induced by natural reductants. In addition to ribulose-1,5-diphosphate carboxylase, two other enzymes of the Calvin cycle are sensitive to O₂: NADPH-glyceraldehyde-3-P dehydrogenase (5, 26), and ribulose-5-P kinase (27). The O₂ inactivation of these two enzymes is probably due to sulfhydryl oxidation. There is no evidence that the oxidation of these enzymes is reversible, and sulfhydryl oxidation cannot explain the competitive nature of O₂ and CO₂ in the Warburg effect. In contrast, O₂ inhibition of ribulose-1,5-diphosphate carboxylase is not an oxidation of sulfhydryl groups. This can be deduced from the observations that Hg²⁺, a sulfhydryl inhibitor, is a noncompetitive inhibitor of carboxylase with respect to CO₂, whereas O₂ is a competitive inhibitor with respect to CO₂. Unlike NADPH-glyceraldehyde-3-P dehydrogenase and ribulose-5-P kinase, extractable ribulose-1,5-diphosphate carboxylase activity is not in excess of the photosynthetic rate in the plant. Any contribution to the Warburg effect by the dehydrogenase and kinase appears to be small, especially if ribulose-1,5-diphosphate carboxylase limits photosynthetic rate (3, 4).

In contrast to the marked inhibition of C₄ photosynthesis by O₂ in air, C₃ photosynthesis is affected only by O₂ concentrations considerably higher than atmospheric (22, 28, 29). In species with C₃ photosynthesis, including corn, the initial photosynthetic carboxylation is catalyzed by P-enolpyruvate carboxylase in the mesophyll. The oxaloacetate produced is reduced to malate, transported to the bundle sheath, and decarboxylated. The CO₂ released is fixed by ribulose-1,5-diphosphate carboxylase and enters the C₃ cycle (8, 9). P-Enolpyruvate carboxylase may function as a CO₂ pump, increasing the CO₂ concentration in the bundle sheath to a level higher than atmospheric (30).

In corn extracts, P-enolpyruvate carboxylase is unaffected by oxygen while ribulose-1,5-diphosphate carboxylase is inhibited (Table II). Thus in C₄ photosynthesis, the initial carboxylation is insensitive to O₂ while the second carboxylation, transfer of CO₂ into the Calvin cycle, is inhibited by O₂. At atmospheric O₂ levels, cycling of C₄ photosynthesis may proceed unhindered since the initial carboxylation is not inhibited and the increased CO₂ level in the bundle sheath will allow CO₂ to compete more successfully with O₂ for ribulose-1,5-diphosphate carboxylase. At O₂ levels greater than atmospheric, inhibition of ribulose-1,5-diphosphate carboxylase would restrict the carbon flow from C₄ acids to the sugar phosphates of the Calvin cycle. Since P-enolpyruvate carboxylase is not affected by O₂, C₄ acids may accumulate at high O₂ in concentrations sufficient to inhibit P-enolpyruvate carboxylase (31), and thus inhibit photosynthesis.

Plants with C₄ photosynthesis also lack photorespiratory CO₂ evolution (3, 7). This property may also be related to the P-enolpyruvate carboxylase CO₂ pump. High CO₂ levels in the bundle sheath would inhibit the ribulose-1,5-diphosphate carboxylase-catalyzed oxidation of ribulose-1,5-diphosphate to 2-P-glycolate (25), thereby decreasing photorespiration. Since the mesophyll layers surround the bundle sheath, P-enolpyruvate carboxylase in the mesophyll would rapidly reflex any photorespiratory CO₂ and prevent leakage to the atmosphere (9).

In *Mimulus*, energy of activation values for photosynthesis of...
16 to 19 kcal per mole compared closely with values for ribulose-1,5-di-P carboxylase from this plant (32). These values are similar to those obtained with purified soybean carboxylase. The agreement between activation energies for photosynthesis and the carboxylation reaction would be expected if this reaction limits the rate of light-saturated photosynthesis in C₃ species.

Inhibitor Studies—Information concerning ribulose-1,5-di-P binding to the enzyme is contradictory. Orthophosphate and 3-P-glycerate inhibit spinach carboxylase (10). In contrast, carbamyl-P and various sugar phosphates, including fructose-1,6-di-P, reportedly did not inhibit (33). In this study, fructose-1,6-di-P was an effective competitive inhibitor of soybean carboxylase with respect to ribulose-1,5-di-P (Fig. 3), and all phosphorylated compounds tested produced inhibition (Table III). The greater inhibition caused by fructose-1,6-di-P, as compared to the monophosphates, and the lack of inhibition with nonphosphorylated sugars suggests that the two phosphate groups of ribulose-1,5-di-P are the principal groups involved in binding to the enzyme.

Since the discovery that p-CMB and IAA were inhibitors of carboxylase (14), the role of sulfhydryl groups has received much attention. Reportedly IAA inhibition could be reversed if ribulose-1,5-di-P was incubated with the enzyme and inhibitor. As neither Mg²⁺ nor HCO₃⁻ were effective in this respect, it appeared that sulfhydryls were not involved in the binding of Mg²⁺ or HCO₃⁻ but may have interacted with ribulose-1,5-di-P (17). However, fructose-1,6-di-P also protected sulfhydryls against IAA alkylation but fructose-1,6-di-P was not found to be a competitive inhibitor of carboxylase with respect to ribulose-1,5-di-P in these experiments (33). This was an objection (33) to the hypothesis that the protective mechanism involved the ribulose-1,5-di-P binding site and that sulfhydryls were located there. This objection would seem to be negated by the finding that fructose-1,6-di-P is a competitive inhibitor of soybean carboxylase (Fig. 3).

p-CMB, at low concentrations, produces uncompetitive inhibition of spinach carboxylase with respect to ribulose-1,5-di-P, while higher p-CMB concentrations give mixed inhibition (16, 34). The inhibition may be due to mercurial interaction with the enzyme-ribulose-1,5-di-P complex rather than solely with the enzyme (16), which correlates with results for soybean carboxylase showing mixed inhibition by HgCl₂ with respect to ribulose-1,5-di-P and noncompetitive inhibition with respect to CO₂. These results confirm a closer relationship of sulfhydryl groups to the ribulose-1,5-di-P than to the CO₂-binding site. Although many of the sulfhydryl groups may fulfill structural requirements (16, 33, 34), possibly a small number are involved specifically at the catalytic site (35). As HgCl₂ inhibition is mixed and noncompetitive, this suggests that sulfhydryls are not directly involved with the binding of ribulose-1,5-di-P, but may participate at the catalytic site (16) close to where ribulose-1,5-di-P binds to the enzyme.

Calvin (36) has suggested that the carboxylation of ribulose-1,5-di-P proceeds via an unstable C₄ intermediate. Support for this suggestion comes from binding studies with analogues having structures similar to that proposed for the intermediate (37, 38). The C₄ intermediate may be produced by the enediol of ribulose-1,5-di-P acting as a nucleophilic agent on CO₂ (39). A sulfhydryl group or groups could play a catalytic role, not by binding ribulose-1,5-di-P, but rather by extracting a proton from the C₅ hydroxyl of ribulose-1,5-di-P and thus acting as the initiator of the carboxylase reaction.

Acknowledgment—We gratefully acknowledge the advice and support of Dr. R. H. Hagaman.

REFERENCES

2. WEISSBACH, A., HORECKER, B. L., and HURWTITZ, J. (1955) J. Biol. Chem. 218, 705-710
34. AKAWA, T., SUGIYAMA, T., NAKAYAMA, T., and ODA, T. (1968) Arch. Biochem. Biophys. 126, 646-653
Oxygen Inhibition and Other Properties of Soybean Ribulose 1,5-Diphosphate Carboxylase
George Bowes and William L. Ogren


Access the most updated version of this article at http://www.jbc.org/content/247/7/2171

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/247/7/2171.full.html#ref-list-1