Oxygen Inhibition of Photosynthesis and Stimulation of Photorespiration in Soybean Leaf Cells

ABSTRACT

The occurrence of photorespiration in soybean (Glycine max [L.] Merr.) leaf cells was demonstrated by the presence of an O2-dependent CO2 compensation concentration, a nonlinear time course for photosynthetic 14CO2 uptake at low CO2 and high O2 concentrations, and an O2 stimulation of glycine and serine synthesis which was reversed by high CO2 concentration. The compensation concentration was a linear function of O2 concentration and increased as temperature increased. At atmospheric CO2 concentration, 21% O2 inhibited photosynthesis at 25 C by 27%. Oxygen inhibition of photosynthesis was competitive with respect to CO2 and increased with increasing temperature. The Km (CO2) of photosynthesis was also temperature-dependent, increasing from 12 μM CO2 at 15 C to 38 μM at 35 C. In contrast, the Kf(O2) was similar at all temperatures. Oxygen inhibition of photosynthesis was independent of irradiance except at 10 mM bicarbonate and 100% O2, where inhibition decreased with increasing irradiance up to the point of light saturation of photosynthesis. Concomitant with increasing O2 inhibition of photosynthesis was an increased incorporation of carbon into glycine and serine, intermediates of the photorespiratory pathway, and a decreased incorporation into starch. The effects of CO2 and O2 concentration and temperature on soybean cell photosynthesis and photorespiration provide further evidence that these processes are regulated by the kinetic properties of ribulose-1,5-diphosphate carboxylase with respect to CO2 and O2.

Oxygen inhibition of C3 photosynthesis is rapidly and completely reversible, reduced by increasing the CO2 concentration, and accompanied by the synthesis of products of the photorespiratory pathway (5, 10). These O2 effects on photosynthesis have been documented in leaves (1, 6, 14), isolated chloroplasts (7, 8) and algae (3, 25). Studies with isolated leaf cells can provide information complementing that obtained with leaves, chloroplasts and algae in that cells contain the complete photorespiratory pathway, like leaves, yet can be assayed in solution, like chloroplasts and algae, permitting reproducible modification of the aqueous environment such as the addition of photorespiratory inhibitors (20) and altering pH (21). In this paper some basic characteristics of the O2 inhibition of photosynthesis in soybean leaf cells are reported, including the effects of CO2 and O2 concentration, temperature, and irradiance. A preliminary of this work has been presented (19).

MATERIALS AND METHODS

Experiments were conducted with cells isolated from mature leaves of soybean (Glycine max [L.] Merr., cv. Wayne). Cells were isolated by the macerating enzyme technique described previously (22) and assayed at 15, 25, or 35 C. 14CO2 incorporation was assayed by adding cells to sealed vials containing 0.2 mM sorbitol, 50 mM tris-Cl (pH 7.8), 5 mM KNO3, 2 mM Ca(NO3)2, 1 mM MgCl2, and KH2CO3 (1 μCi) in a total volume of 1 ml. Vials were flushed for 5 min with the gas mixture indicated prior to the addition of buffer, bicarbonate, and cells. Illumination was 12 klux unless otherwise indicated. After 15 min, assays were terminated by addition of 6 N acetic acid. Separation and identification of 14C-labeled products were performed as described previously (22). The concentrations of CO2 and O2 in solution were calculated from standard tables (19).

RESULTS

Kinetics. O2 inhibition of photosynthesis in soybean leaf cells was completely reversible. The rate of photosynthesis in cells flushed for 3 min in the light with 100% O2 followed by a 3-min flush with 2% O2 prior to assay was identical to the rate of photosynthesis in cells flushed for 6 min with 2% O2 (data not shown). O2 had little effect on photosynthesis at 10 mM bicarbonate but greatly inhibited fixation at 0.1 mM bicarbonate (Fig. 1). Rates of 14CO2 fixation were linear with time at 0.1 mM and 10 mM bicarbonate in 2% O2, and at 10 mM bicarbonate in 100% O2. At 0.1 mM bicarbonate and 100% O2, 14CO2 fixation followed a biphasic time course, the initial rate being about twice the final rate (Fig. 1). A biphasic time course suggests the presence of photorespiration. Initially, 14CO2 is fixed by RuDP carboxylase and enters the Calvin cycle. No 14CO2 is released because the photorespiratory intermediates are not labeled. With time, at low CO2 and high O2 concentrations, labeled carbon enters compounds of the photorespiratory pathway and a portion of it is released as 14CO2. The specific radioactivity of the 14CO2 released by photorespiration gradually increases and eventually becomes constant (15, 16). At this time, the rate of 14CO2 uptake approximates the rate of apparent photosynthesis (true photosynthesis minus photorespiration). A minimum of 15 sec was required for 14CO2 to be fixed by photosynthesis and released by photorespiration in sunflower leaves, but the relative specific radioactivity of the released 14CO2 did not become constant until 15 min after exposure to 14CO2 (15, 16). Soybean leaf cells appeared to reach a constant

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net rate of $^{14}$CO$_2$ fixation about 10 min. The integrated rate of photosynthesis calculated over the interval of zero to 15 min approximates the final rate of $^{14}$CO$_2$ fixation and for simplicity this interval was used routinely for calculation of the rate of photosynthesis. Time courses at high CO$_2$ or low O$_2$ concentrations were linear because photorespiration is greatly inhibited under these conditions.

The CO$_2$ response curve of soybean leaf cell photosynthesis in 0, 21, and 100% O$_2$ showed that O$_2$ inhibition increased with increasing O$_2$ concentration and decreased at high CO$_2$ concentrations (Fig. 2). The inhibition of photosynthesis by 21% O$_2$ at 0.5 mM bicarbonate, which is equivalent to about 340 $\mu$M of CO$_2$, was 27% (Table I), equal to the amount of inhibition in leaves by atmospheric O$_2$ (1, 6, 10).

The CO$_2$ response curve extrapolated to zero in the absence of O$_2$ (Fig. 2). In the presence of O$_2$, the response curve intercepted a bicarbonate concentration greater than zero, indicating the presence of photorespiration. This intercept, $\Gamma$, increased with increasing O$_2$ concentration (Fig. 2 and Table I). Double reciprocal plots of photosynthesis versus bicarbonate concentration were linear when $\Gamma$ was subtracted from the actual bicarbonate concentration (Fig. 2). Thus soybean cell photosynthesis exhibited Michaelis-Menten kinetics with respect to CO$_2$ in the presence and absence of O$_2$. O$_2$ increased the apparent $K_m$(CO$_2$) for photosynthesis, but did not alter the $V_{\text{max}}$ (Fig. 2 and Table I), indicating that O$_2$ was a competitive inhibitor of photosynthesis with respect to CO$_2$. The $K_i$(O$_2$) value was the same at 21% and 100% O$_2$ (Table I). Analysis of the inhibition indicated that photorespiration accounts for one-third of the total O$_2$ inhibition of photosynthesis at atmospheric levels of CO$_2$ and O$_2$, and direct O$_2$ inhibition of photosynthesis, calculated from the $K_m$ and $K_i$ values in Table I, accounts for the remaining two-thirds (Table I). These values are consistent with previous measurement (15) and calculation (14) of the magnitude of the two components of O$_2$ inhibition.

**Irradiance.** The rate of photosynthesis at all bicarbonate and O$_2$ concentrations examined saturated at about 4 klux (Fig. 3). Percentage O$_2$ inhibition was constant over the entire range of irradiance except at 100% O$_2$ and 10 mM bicarbonate concentration (Fig. 3), where increasing irradiance decreased O$_2$ inhibition up to the point of light saturation.

**Temperature.** CO$_2$ response curves of photosynthesis in 2% and 50% O$_2$ were made at 15, 25, and 35°C. Double reciprocal plots were constructed (Fig. 4) and used to determine the kinetic constants of photosynthesis and O$_2$ inhibition (Table II). As was previously observed in soybean leaves (14) and cells (22), increasing temperature increased the $K_m$(CO$_2$). The $K_i$(O$_2$) was not greatly altered by temperature changes. Both $\Gamma$ and $K_i$(O$_2$) increased in the order of 15°C, 25°C, and 35°C, with the change in $K_i$(O$_2$) being proportionately greater.

**Table I.** The effect of oxygen on the kinetic parameters of cell photosynthesis at 25°C.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0%</th>
<th>21%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m$(HCO$_3^-$), mM</td>
<td>1.22</td>
<td>(1.22)</td>
<td>(1.22)</td>
</tr>
<tr>
<td>$K_m$(CO$_2$), $\mu$M</td>
<td>28</td>
<td>(28)</td>
<td>(28)</td>
</tr>
<tr>
<td>$K_i$(O$_2$), $\mu$M</td>
<td>---</td>
<td>35</td>
<td>750</td>
</tr>
<tr>
<td>$r$, $\mu$M CO$_2$</td>
<td>0.1</td>
<td>35</td>
<td>170</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>173</td>
<td>172</td>
<td>170</td>
</tr>
<tr>
<td>CO$_2$ saturated rate</td>
<td>113</td>
<td>112</td>
<td>107</td>
</tr>
<tr>
<td>APS$^2$ at 10 $\mu$M CO$_2$</td>
<td>45.9</td>
<td>33.5</td>
<td>10.0</td>
</tr>
<tr>
<td>O$_2$ inhibition (%)</td>
<td>---</td>
<td>27</td>
<td>78</td>
</tr>
<tr>
<td>TPS$^2$ at 10 $\mu$M CO$_2$</td>
<td>45.9</td>
<td>36.8</td>
<td>20.7</td>
</tr>
<tr>
<td>O$_2$ inhibition (%)</td>
<td>---</td>
<td>20</td>
<td>55</td>
</tr>
</tbody>
</table>

1Data from Figure 2
2$\mu$mol CO$_2$/mg Chl/hr
3$\mu$mol Chl/mg/hr

**Fig. 1.** Time course of soybean cell photosynthesis. Assay mixtures contained 22 $\mu$g Chl/ml. O: 2% O$_2$, 10 mM bicarbonate; □: 100% O$_2$, 10 mM bicarbonate; □: 2% O$_2$, 0.1 mM bicarbonate; ■: 100% O$_2$, 0.1 mM bicarbonate.

**Fig. 2.** A: Bicarbonate response curve of soybean cell photosynthesis; B: double reciprocal plot of soybean cell photosynthesis. Assay mixtures contained 5.5 $\mu$g Chl/ml. O: 0% O$_2$, □: 21% O$_2$, ■: 100% O$_2$.
and O₂ inhibition of photosynthesis increased with increasing temperature (Table II).

**Products of Photosynthesis.** At low bicarbonate concentrations, increasing the O₂ concentration greatly increased the percentage of label entering the basic fraction (amino acids) and acid-1 fraction (glycolate and glycerate) (Fig. 5). A correspondingly large decrease was observed in the insoluble fraction (starch). O₂ increased the percentage of label entering the acid-2 fraction (mostly sugar phosphates) to a small extent, and decreased the percentage of label in the neutral fraction (mostly sucrose). Increasing the bicarbonate concentration overcame the O₂ effect. Distribution of label in products of photosynthesis at high CO₂ concentration was similar at all O₂ concentrations, except for the neutral fraction that remained slightly depressed at high O₂.

Analysis of the basic fraction revealed that changes in O₂ and bicarbonate concentrations only altered incorporation of label into serine and glycine (Fig. 6). The percentage of labeled carbon fixed into glycine and serine was progressively inhibited with increasing bicarbonate concentration. An identical response was observed in leaves of sunflower (16), *Atriplex patula* (18), and sugar beet and tobacco (23).

**DISCUSSION**

The inhibition of soybean leaf cell photosynthesis by O₂ was equal in magnitude and character to O₂ inhibition of leaf photosynthesis. One indicator of photosynthesis in soybean...
cells was the measurement of an O₂-dependent ι, determined by extrapolating the CO₂ response curve to zero net photosynthesis. As in leaves (14), ι was directly proportional to O₂ concentration (Table I). ι was not observed in previous photosynthetic studies with tobacco cells (4, 11) or spinach protoplasts (17), probably because the assay times were too short. Time is required for isotopic saturation of ¹⁴CO₂ in intermediates of the photosynthetic and photorespiratory pathways. In short term assays, the relative specific radioactivity of photorespiratory released ¹⁴CO₂ is much less than the relative specific radioactivity of the administered ¹⁴CO₂, so the rate of apparent photosynthesis is overestimated and extrapolation of the CO₂ response curve passes through or near the origin.

The effect of photorespiration on the CO₂ response curve was largely corrected for by shifting the curve in O₂ to the origin, thus permitting the direct effect of O₂ on photosynthesis to be estimated. Cell photosynthesis followed enzyme kinetics so the inhibition constant for O₂ could be determined from double reciprocal plots. The Kι(O₂) at 25 C was about 700 μM (Tables I and II), similar to the value obtained with purified soybean RuDP carboxylase (12, 14), and suggests that O₂ inhibition of this enzyme causes the direct O₂ inhibition of cell photosynthesis. From the kinetic constants derived from Figure 2 (given in Table I), the magnitude of the two components of O₂ inhibition, photorespiration and direct inhibition, was calculated. These calculations (Table I) indicate that photorespiration accounts for one-third of the inhibition at atmospheric CO₂ concentration at 25 C, and that the remaining two-thirds is due to direct inhibition of CO₂ fixation. This agrees with previous calculations made from measurements on soybean leaves and purified RuDP carboxylase (12, 14).

Previous reports from this laboratory (12, 14, 22) have established that photosynthesis, photorespiration, ι, and O₂ inhibition of photosynthesis can be described in terms of the kinetic properties of RuDP carboxylase with respect to CO₂ and O₂. In these analyses, ι, photorespiration, and O₂ inhibition were shown to be proportional to the ratio Km(CO₂)/Kι(O₂). Increasing temperature increased ι and O₂ inhibition of photosynthesis in soybean leaves (14), and photorespiration increased at higher temperatures (15). The Km(CO₂) of soybean leaves and purified RuDP carboxylase increased at higher temperatures while the Kι(O₂) was little affected by temperature. Thus Laing et al. (14) concluded that the temperature dependence of ι, photorespiration, and O₂ inhibition of soybean photosynthesis was due to temperature-induced changes in the Km(CO₂) of RuDP carboxylase. The effect of temperature on Km(CO₂), Kι(O₂), ι, and O₂ inhibition of photosynthesis in isolated soybean leaf cells (Table II) supports this conclusion.

Table II. The effect of temperature on the kinetic parameters of cell photosynthesis and oxygen inhibition of cell photosynthesis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>15°C</th>
<th>25°C</th>
<th>35°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Km(CO₂), μM</td>
<td>12</td>
<td>26</td>
<td>38</td>
</tr>
<tr>
<td>Kι(O₂), μM</td>
<td>670</td>
<td>670</td>
<td>650</td>
</tr>
<tr>
<td>Vmax2</td>
<td>25</td>
<td>114</td>
<td>262</td>
</tr>
<tr>
<td>APS2 at 10 μM CO₂</td>
<td>2% O₂</td>
<td>2% O₂</td>
<td>2% O₂</td>
</tr>
<tr>
<td>50% O₂</td>
<td>6</td>
<td>13</td>
<td>18</td>
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<tr>
<td>O₂ inhibition, %</td>
<td>50% O₂</td>
<td>50% O₂</td>
<td>50% O₂</td>
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<tr>
<td>Km(CO₂)/Kι(O₂)</td>
<td>50</td>
<td>59</td>
<td>68</td>
</tr>
</tbody>
</table>

1 Data from Figure 4.
2 μmol CO₂/mg Chl/hr

Fig. 5. Distribution of radiocarbon into products of photosynthesis as a function of CO₂ and O₂ concentrations. Assay mixtures contained 47 μg Chl/ml. Bicarbonate saturated rate of photosynthesis was 62.5 μmol CO₂/mg Chl/hr. O₂: 100% O₂: 21% O₂: 50% O₂: 100% O₂: 100%

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for CO₂ decreases because CO₂ binds less tightly to RuDP carboxylase. The binding of O₂ to the enzyme is unchanged by temperature. Thus at higher temperatures a greater proportion of the RuDP is oxygenated and a smaller proportion of the RuDP is carboxylated, so for each absorbed quantum, which is used to regenerate RuDP, less carbon is fixed. The reduction in quantum yield at higher temperature will be proportional to the change in the ratio of carboxylation to oxygenation and this is governed by the ratio Kᵢ(O₂)/Kᵢ(CO₂) (14, 22). Because temperature does not affect Kᵢ(O₂), the change in quantum yield is determined by the change in Kᵢ(CO₂).

The effect of temperature on the quantum yield of O₂ inhibition of photosynthesis was quantitated from an Arrhenius plot and found to be 8.1 kcal/mol between 15 and 35 C (6). The temperature dependence of Φ in the same temperature range was calculated to be 7.6 kcal/mol (2). Calculation of the temperature dependence of the affinity of soybean cell photosynthesis for CO₂ between 15 and 35 C (Table II) from the Arrhenius equation gives a value of 10 kcal/mol. Calculation of the temperature dependence of the Km(CO₂) for soybean leaf photosynthesis and purified soybean RuDP carboxylase, using Km(CO₂) values published previously (14), gives values of 8 kcal/mol for both systems. Thus the effect of temperature on Φ and O₂ depression of the quantum yield of C₃ photosynthesis (2, 6) is equal to the effect of temperature on the affinity of leaf and cell photosynthesis for CO₂, and this change in affinity is the same as the effect of temperature on the Km(CO₂) of RuDP carboxylase in vitro (14).

Ku and Edwards (13) have concluded that the temperature dependence of O₂ inhibition of photosynthesis is caused by the differential effect of temperature on the solubility of O₂ and CO₂, since the O₂/CO₂ concentration ratio in solution increases as temperature increases (9). The temperature dependence of the O₂/CO₂ concentration ratio (9) can be quantitated with the Arrhenius equation and gives a value of 1.8 kcal/mol between 15 and 35 C. Thus some of the increased O₂ inhibition of photosynthesis at higher temperatures can be attributed to a differential effect of temperature on the solubility of the two gases, but this is a minor component of the total effect. The relatively small contribution of O₂/CO₂ solubility differences to the temperature response of the quantum yield of photosynthesis was noted by Ehleringer and Björkman (6).

Irradiance level affected the percentage O₂ inhibition of photosynthesis only when high concentrations of both CO₂ and O₂ were present simultaneously (Fig. 3). Under these conditions, O₂ inhibition was greatest at lowest irradiance and decreased when the amount of light was increased. A similar observation has been made in leaf photosynthesis (1). The mechanistic basis of this anomaly is unknown.

Concomitant with increased O₂ inhibition of cell photosynthesis was the partitioning of carbon into the intermediates of the photorespiratory pathway and a comparable decrease of carbon in starch. These O₂ effects were also reversed in the presence of high CO₂ concentrations. O₂ had less effect on the incorporation of label into the acid-2 fraction, which is composed mainly of the sugar phosphates. In isolated chloroplasts, high O₂ concentration severely depleted label in the sugar phosphates (8). No such depletion was observed in soybean leaf cells (Fig. 5). This difference probably occurs because glycolate is an end product of chloroplast photosynthesis, while in the cell and leaf most of the glycolate carbon can be returned to the photosynthesis cycle by peroxisomal and mitochondrial respiration (24). The O₂-induced depletion of chloroplast sugar phosphates, particularly RuDP, may explain why chloroplast photosynthesis is more sensitive to O₂ than is cell and leaf photosynthesis, and may also explain why O₂ inhibition of chloroplast photosynthesis can be partially overcome by the addition of intermediates of the photosynthetic cycle.

**LITERATURE CITED**

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16. Mohon JD, H Fock, DT Canvin 1974 Changes in the specific radioactivity of sunflower leaf metabolites during photosynthesis in $^{14}$CO$_2$ and $^{12}$CO$_2$ at three concentrations of CO$_2$. Planta 120: 245-254