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

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ABSTRACT

The occurrence of photorespiration in soybean (*Glycine max* [L.] Merr.) leaf cells was demonstrated by the presence of an O_2-dependent CO_2 compensation concentration, a nonlinear time course for photosynthetic $^{14}CO_2$ uptake at low CO_2 and high O_2 concentrations, and an O_2 stimulation of glycine and serine synthesis which was reversed by high CO_2 concentration. The compensation concentration was a linear function of O_2 concentration and increased as temperature increased. At atmospheric CO_2 concentration, 21% O_2 inhibited photosynthesis at 25 C by 27%. Oxygen inhibition of photosynthesis was competitive with respect to CO_2 and increased with increasing temperature. The Km (CO_2) of photosynthesis was also temperature-dependent, increasing from 12 μM CO_2 at 15 C to 38 μM at 35 C. In contrast, the Ki (O_2) was similar at all temperatures. Oxygen inhibition of photosynthesis was independent of irradiance except at 10 mM bicarbonate and 100% O_2, where inhibition decreased with increasing irradiance up to the point of light saturation of photosynthesis. Concomitant with increasing O_2 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 CO_2 and O_2 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 CO_2 and O_2.

Oxygen inhibition of C_3 photosynthesis is rapidly and completely reversible, reduced by increasing the CO_2 concentration, and accompanied by the synthesis of products of the photorespiratory pathway (5, 10). These O_2 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 O_2 inhibition of photosynthesis in soybean leaf cells are reported, including the effects of CO_2 and O_2 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. $^{14}CO_2$ incorporation was assayed by adding cells to sealed vials containing 0.2 M sorbitol, 50 mM tris-Cl (pH 7.8), 5 mM KNO_3, 2 mM $Ca(NO_3)_2$, 1 mM $MgCl_2$, and $KH^{14}CO_3$ (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 ^{14}C-labeled products were performed as described previously (22). The concentrations of CO_2 and O_2 in solution were calculated from standard tables (19).

RESULTS

Kinetics. O_2 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% O_2 followed by a 3-min flush with 2% O_2 prior to assay was identical to the rate of photosynthesis in cells flushed for 6 min with 2% O_2 (data not shown). O_2 had little effect on photosynthesis at 10 mM bicarbonate but greatly inhibited fixation at 0.1 mM bicarbonate (Fig. 1). Rates of $^{14}CO_2$ fixation were linear with time at 0.1 mM and 10 mM bicarbonate in 2% O_2, and at 10 mM bicarbonate in 100% O_2. At 0.1 mM bicarbonate and 100% O_2, $^{14}CO_2$ 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, $^{14}CO_2$ is fixed by RuDP[2] carboxylase and enters the Calvin cycle. No $^{14}CO_2$ is released because the photorespiratory intermediates are not labeled. With time, at low CO_2 and high O_2 concentrations, labeled carbon enters compounds of the photorespiratory pathway and a portion of it is released as $^{14}CO_2$. The specific radioactivity of the $^{14}CO_2$ released by photorespiration gradually increases and eventually becomes constant (15, 16). At this time, the rate of $^{14}CO_2$ uptake approximates the rate of apparent photosynthesis (true photosynthesis minus photorespiration). A minimum of 15 sec was required for $^{14}CO_2$ to be fixed by photosynthesis and released by photorespiration in sunflower leaves, but the relative specific radioactivity of the released $^{14}CO_2$ did not become constant until 15 min after exposure to $^{14}CO_2$ (15, 16). Soybean leaf cells appeared to reach a constant

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[2] Abbreviations: RuDP: ribulose 1,5-diphosphate; Γ: CO_2 compensation concentration; APS: apparent photosynthesis; TPS: true photosynthesis.

net rate of $^{14}CO_2$ fixation after 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 μl/l 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, Γ, increased with increasing O_2 concentration (Fig. 2 and Table I). Double reciprocal plots of photosynthesis *versus* bicarbonate concentration were linear when Γ 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 $Km(CO_2)$ for photosynthesis, but did not alter the V_{max} (Fig. 2 and Table I), indicating that O_2 was a competitive inhibitor of photosynthesis with respect to CO_2. The $Ki(O_2)$ 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 Km and Ki 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 $Km(CO_2)$. The $Ki(O_2)$ was not greatly altered by temperature changes. Both Γ

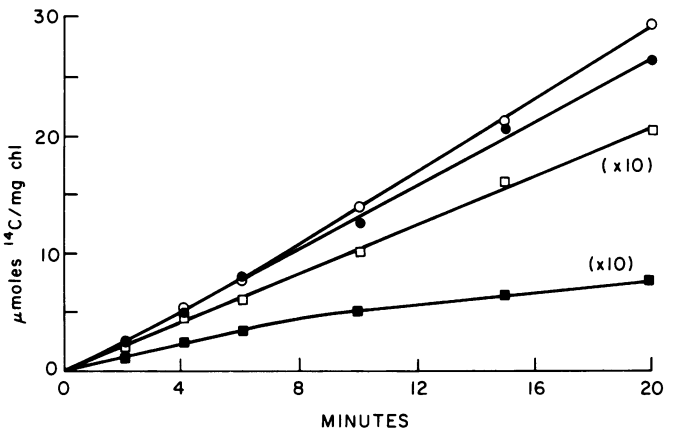

FIG. 1. Time course of soybean cell photosynthesis. Assay mixtures contained 22 μg Chl/ml. ○: 2% O_2, 10 mM bicarbonate; ●: 100% O_2, 10 mM bicarbonate; □: 2% O_2, 0.1 mM bicarbonate; ■: 100% O_2, 0.1 mM bicarbonate.

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

Parameter[1]	O_2 concentration		
	0%	21%	100%
	0 μM	258 μM	1230 μM
$Km(HCO_3^-)$, mM	1.22	(1.22)	(1.22)
$Km(CO_2)$, μM	28	(28)	(28)
$Ki(O_2)$, μM	---	750	760
Γ, μl/l CO_2	0	35	170
Vmax[2]	173	172	170
CO_2 saturated rate[2]	113	112	107
APS[2] at 10 μM CO_2	45.9	33.5	10.0
O_2 inhibition, %	----	27	78
TPS[23] at 10 μM CO_2	45.9	36.8	20.7
O_2 inhibition, %	---	20	55

[1]Data from Figure 2
[2]μmol CO_2/mg Chl·hr
[3]Calculated from the equation: TPS=VS/[S+Km(1+I/Ki)]

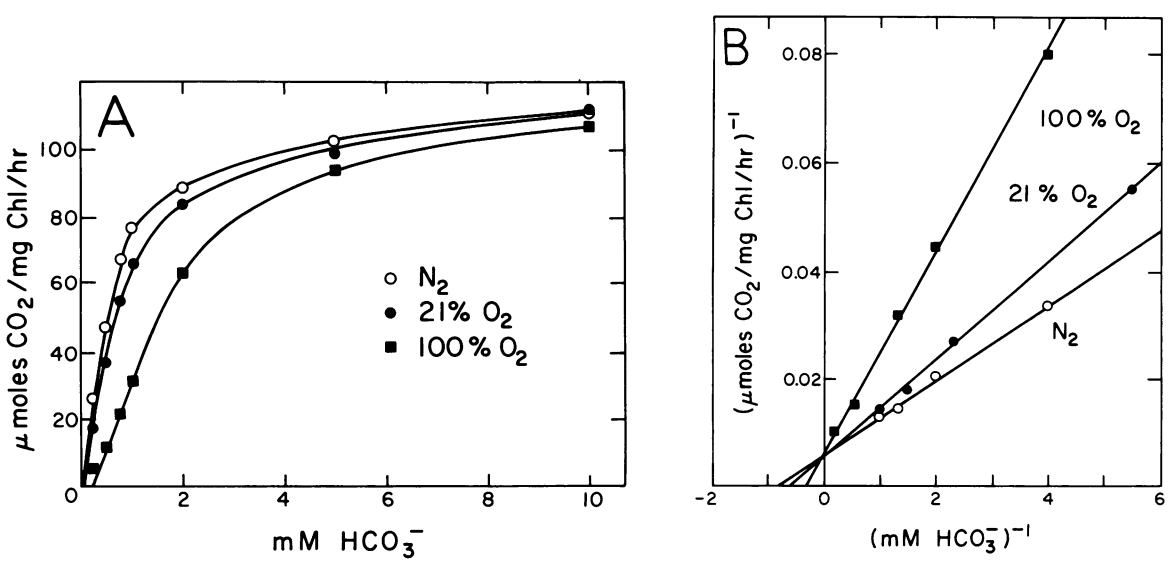

FIG. 2. A: Bicarbonate response curve of soybean cell photosynthesis; B: double reciprocal plot of soybean cell photosynthesis. Assay mixtures contained 5.5 μg Chl/ml. ○: 0% O_2; ●: 21% O_2; ■: 100% O_2.

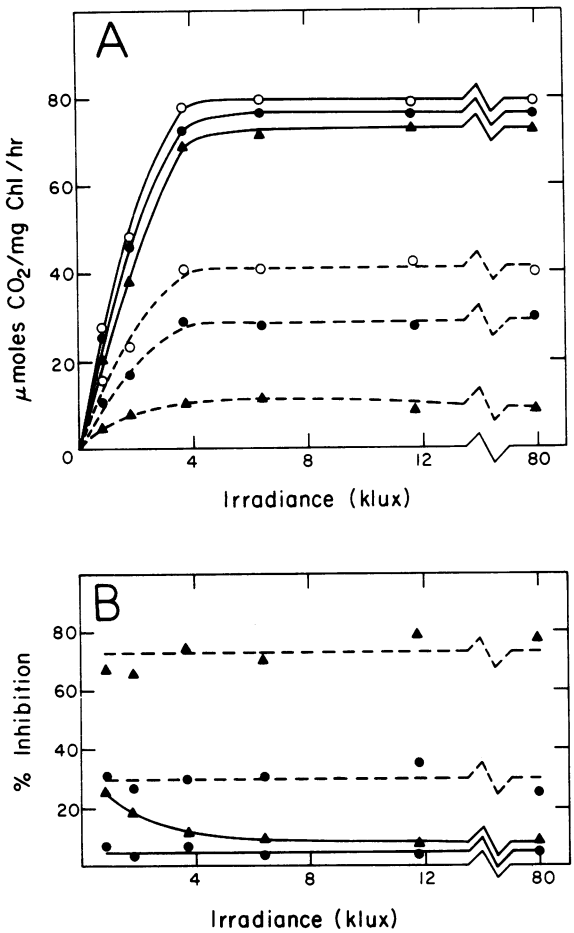

FIG. 3. A: Irradiance response curve of soybean cell photosynthesis; B: irradiance dependence of O_2 inhibition of photosynthesis. Assay mixtures contained 4.7 μg Chl/ml, 0.5 mM bicarbonate (- - -) or 10 mM bicarbonate (——), and 0% O_2 (○), 21% O_2 (●), or 100% O_2 (▲).

and O_2 inhibition of photosynthesis increased with increasing temperature (Table II).

Products of Photosynthesis. At low bicarbonate concentrations, increasing the O_2 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). O_2 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 O_2 effect. Distribution of label in products of photosynthesis at high CO_2 concentration was similar at all O_2 concentrations, except for the neutral fraction that remained slightly depressed at high O_2.

Analysis of the basic fraction revealed that changes in O_2 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 O_2 was equal in magnitude and character to O_2 inhibition of leaf photosynthesis. One indicator of photorespiration in soybean

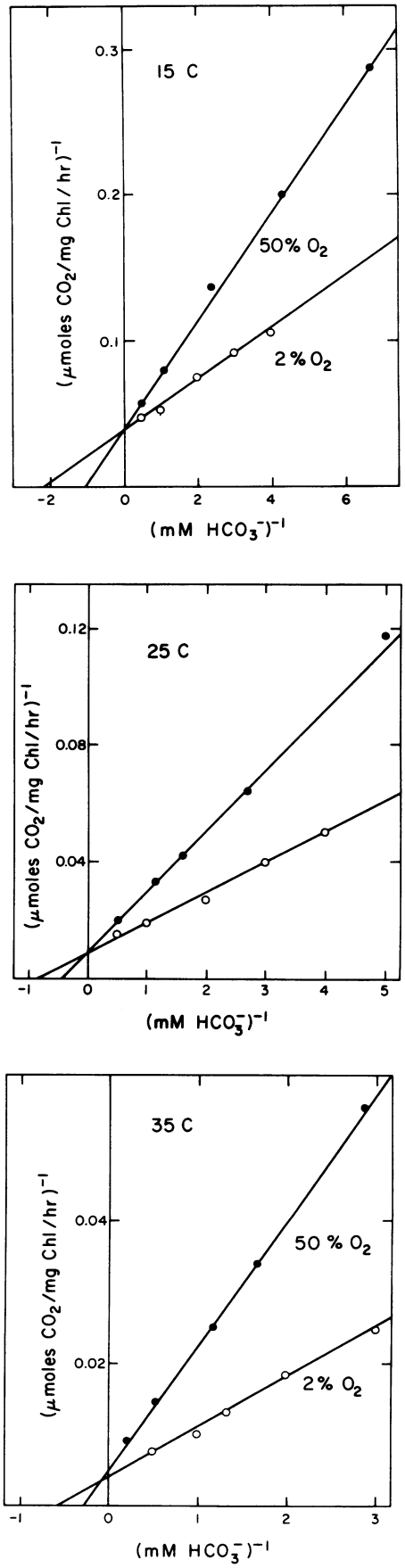

FIG. 4. Double reciprocal plots of soybean cell photosynthesis at 15 C, 25 C, and 35 C. Assay mixtures contained 11 μg Chl/ml. ○: 2% O_2; ●: 50% O_2.

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

Parameter[1]	Temperature		
	15 C	25 C	35 C
$Km(CO_2)$, μM	12	26	38
$Ki(O_2)$, μM	670	670	650
Vmax[2]	26	114	262
APS[2] at 10 μM CO_2			
2% O_2	12	32	56
50% O_2	6	13	18
O_2 inhibition, %	50	59	68
Γ, μl/l CO_2			
2% O_2	0	0	0
50% O_2	60	90	120

[1]Data from Figure 4.
[2]μmol CO_2/mg Chl·hr

cells was the measurement of an O_2-dependent Γ, determined by extrapolating the CO_2 response curve to zero net photosynthesis. As in leaves (14), Γ was directly proportional to O_2 concentration (Table I). Γ 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 $^{14}CO_2$ in intermediates of the photosynthetic and photorespiratory pathways. In short term assays, the relative specific radioactivity of photorespiratory released $^{14}CO_2$ is much less than the relative specific radioactivity of the administered $^{14}CO_2$, so the rate of apparent photosynthesis is overestimated and extrapolation of the CO_2 response curve passes through or near the origin.

The effect of photorespiration on the CO_2 response curve was largely corrected for by shifting the curve in O_2 to the origin, thus permitting the direct effect of O_2 on photosynthesis to be estimated. Cell photosynthesis followed enzyme kinetics so the inhibition constant for O_2 could be determined from double reciprocal plots. The $Ki(O_2)$ 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 O_2 inhibition of this enzyme causes the direct O_2 inhibition of cell photosynthesis. From the kinetic constants derived from Figure 2 (given in Table I), the magnitude of the two components of O_2 inhibition, photorespiration and direct inhibition, was calculated. These calculations (Table I) indicate that photorespiration accounts for one-third of the inhibition at atmospheric CO_2 concentration at 25 C, and that the remaining two-thirds is due to direct inhibition of CO_2 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, Γ, and O_2 inhibition of photosynthesis can be described in terms of the kinetic properties of RuDP carboxylase with respect to CO_2 and O_2. In these analyses, Γ, photorespiration, and O_2 inhibition were shown to be proportional to the ratio $Km(CO_2)/Ki(O_2)$. Increasing temperature increased Γ and O_2 inhibition of photosynthesis in soybean leaves (14), and photorespiration increased at higher temperatures (10). The $Km(CO_2)$ of soybean leaves and purified RuDP carboxylase increased at higher temperatures while the $Ki(O_2)$ was little affected by temperature. Thus Laing *et al.* (14) concluded that the temperature dependence of Γ, photorespiration, and O_2 inhibition of soybean photosynthesis was due to temperature-induced changes in the $Km(CO_2)$ of RuDP carboxylase. The effect of temperature on $Km(CO_2)$, $Ki(O_2)$, Γ, and O_2 inhibition of photosynthesis in isolated soybean leaf cells (Table II) supports this conclusion.

Ehleringer and Björkman (6) showed that the quantum yield of C_3 photosynthesis at atmospheric CO_2 and O_2 concentrations was temperature-dependent, decreasing as the temperature was increased. This response is readily explained by the differential effect of temperature on $Km(CO_2)$ and $Ki(O_2)$ of RuDP carboxylase. As the temperature is increased, the affinity of the leaf

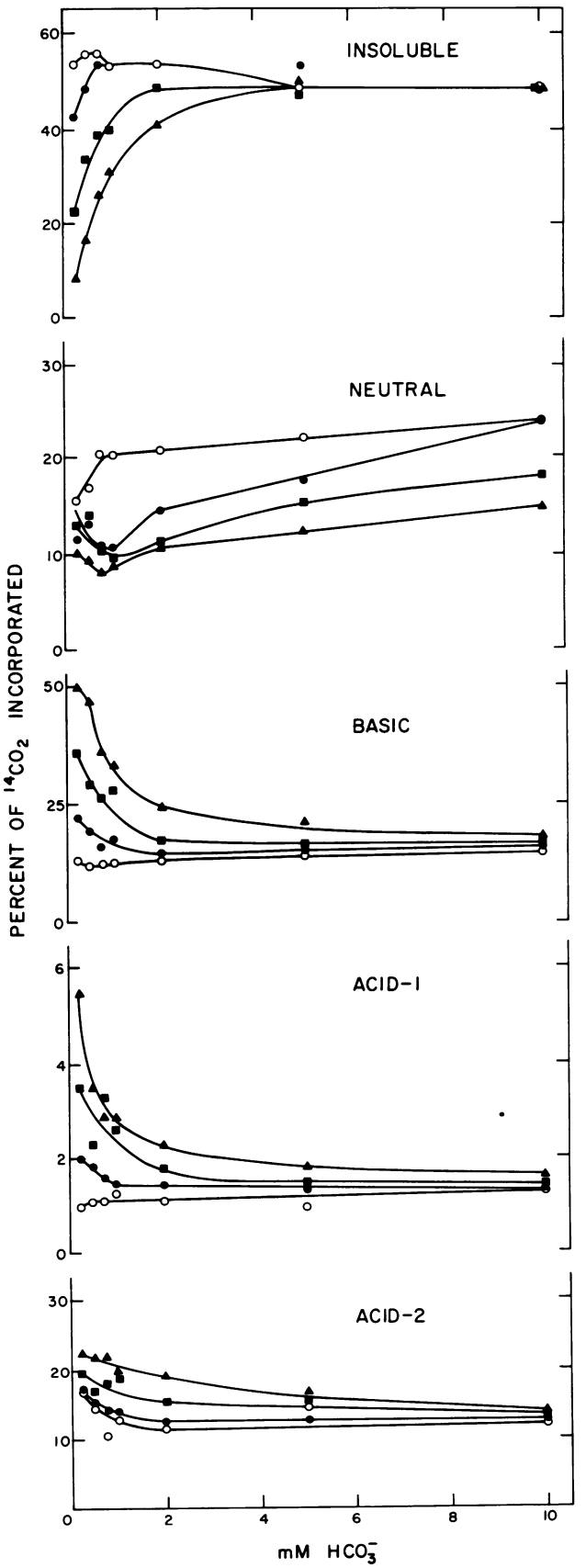

FIG. 5. Distribution of radiocarbon into products of photosynthesis as a function of CO_2 and O_2 concentrations. Assay mixtures contained 47 μg Chl/ml. Bicarbonate saturated rate of photosynthesis was 62.5 μmol CO_2/mg Chl·hr. ○: 2% O_2; ●: 21% O_2; ■: 50% O_2; ▲: 100% O_2.

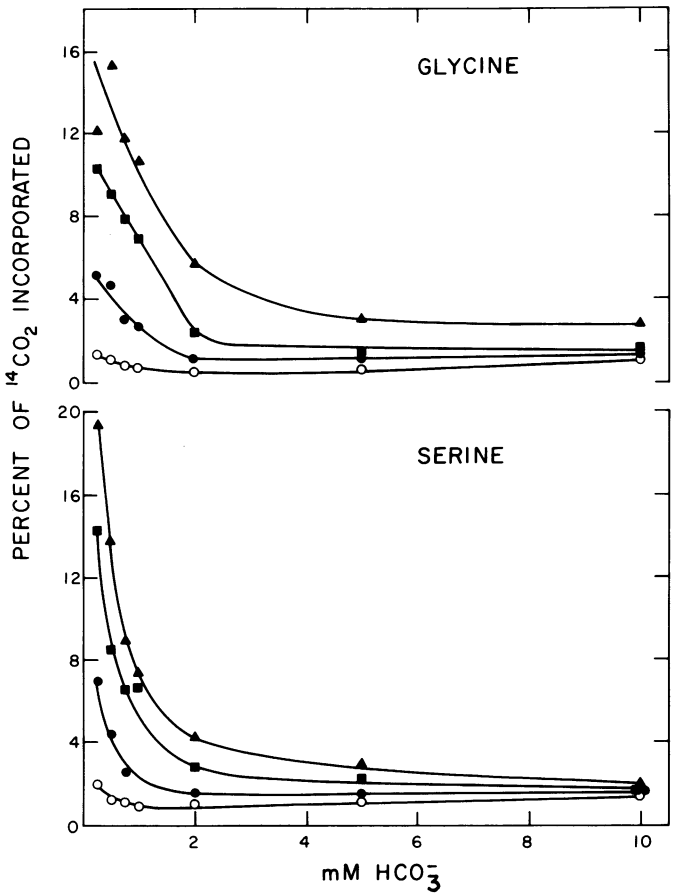

FIG. 6. Distribution of radiocarbon into glycine and serine during photosynthesis as a function of CO_2 and O_2 concentrations. This is an analysis of the basic fraction in Figure 5. O: 2% O_2; ●: 21% O_2; ■: 50% O_2; ▲: 100% O_2.

for CO_2 decreases because CO_2 binds less tightly to RuDP carboxylase. The binding of O_2 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 $Ki(O_2)/Km(CO_2)$ (14, 22). Because temperature does not affect $Ki(O_2)$, the change in quantum yield is determined by the change in $Km(CO_2)$.

The effect of temperature on the quantum yield of O_2 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 Γ 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 CO_2 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 $Km(CO_2)$ for soybean leaf photosynthesis and purified soybean RuDP carboxylase, using $Km(CO_2)$ values published previously (14), gives values of 8 kcal/mol for both systems. Thus the effect of temperature on Γ and O_2 depression of the quantum yield of C_3 photosynthesis (2, 6) is equal to the effect of temperature on the affinity of leaf and cell photosynthesis for CO_2, and this change in affinity is the same as the effect of temperature on the $Km(CO_2)$ of RuDP carboxylase *in vitro* (14).

Ku and Edwards (13) have concluded that the temperature dependence of O_2 inhibition of photosynthesis is caused by the differential effect of temperature on the solubility of O_2 and CO_2, since the O_2/CO_2 concentration ratio in solution increases as temperature increases (9). The temperature dependence of the O_2/CO_2 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 O_2 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 O_2/CO_2 solubility differences to the temperature response of the quantum yield of photosynthesis was noted by Ehleringer and Björkman (6).

Irradiance level affected the percentage O_2 inhibition of photosynthesis only when high concentrations of both CO_2 and O_2 were present simultaneously (Fig. 3). Under these conditions, O_2 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 O_2 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 O_2 effects were also reversed in the presence of high CO_2 concentrations. O_2 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 O_2 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 O_2-induced depletion of chloroplast sugar phosphates, particularly RuDP, may explain why chloroplast photosynthesis is more sensitive to O_2 than is cell and leaf photosynthesis, and may also explain why O_2 inhibition of chloroplast photosynthesis can be partially overcome by the addition of intermediates of the photosynthetic cycle.

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