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Oxygen Inhibition of Photosynthesis

II. KINETIC CHARACTERISTICS AS AFFECTED BY TEMPERATURE[1]

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ABSTRACT

The response of whole leaf photosynthesis of wheat (*Triticum aestivum* L.) in relation to soluble CO_2 available to the mesophyll cells, under low (1.5%) O_2 at 25, 30, and 35 C, followed Michaelis-Menten kinetics up to saturating CO_2 but deviated at high CO_2 levels where the experimental V_{max} is considerably less than the calculated V_{max}. The affinity of the leaves for CO_2 during photosynthesis was similar from 25 to 35 C with $Km(CO_2)$ values of approximately 3.5 to 5 μM.

In considering the effect of O_2 on photosynthesis at 25, 30, and 35 C where O_2 and CO_2 are expressed on a solubility basis: (*a*) the effect of O_2 on carboxylation efficiency was similar at the three temperatures; (*b*) increasing temperature caused only a slight increase in kinetic constants $Ki(O_2)$ and $Km(CO_2)$, while the ratio of $Ki(O_2)/Km(CO_2)$ was similar at the three temperatures; and (*c*) the reciprocal plots of apparent rate of photosynthesis *versus* ($CO_2 - \Gamma$) at various O_2 levels showed O_2 to be a competitive inhibitor of photosynthesis.

A model for separating O_2 inhibition of photosynthesis into two components, direct competitive inhibition and inhibition due to photorespiration, was presented from both simulated and experimental data of photosynthetic response curves to varying CO_2 concentrations at low O_2 *versus* 21% O_2. The photorespiratory part of O_2 inhibition is considered as a major component at Γ and increases with increasing temperature and with increase in O_2/CO_2 solubility ratio. The competitive component of O_2 inhibition is considered as a major component of O_2 inhibition under atmospheric CO_2 levels and is relatively independent of temperature at a given O_2/CO_2 ratio.

Although temperature is important in environmental regulation of photosynthesis, the degree of temperature-dependent variation in factors controlling photosynthesis in C_3 plants at the cellular level such as affinity for CO_2, nature of O_2 inhibition of photosynthesis, maximum velocity of photosynthesis based on enzyme potential, and solubility of O_2 and CO_2 is uncertain. In order to make a kinetic analysis of whole leaf photosynthesis in response to levels of CO_2 and O_2 at various leaf temperatures, it is necessary to consider the concentrations of the gases on a solubility basis in the leaf. From such an analysis, the $Km(CO_2)$, $Ki(O_2)$, V_{max} for photosynthesis, CO_2 compensation point (Γ), and effect of O_2 on carboxylation efficiency were determined in the present study for wheat at three temperatures.

In the previous study with various C_3 species (16), we reported that the magnitude of total percentage inhibition of photosynthesis by O_2 is dependent on and correlated with apparent solubility ratio of O_2/CO_2 in the leaf over a range of solubility ratios from 25 to 45 when the solubility ratio is changed either by changing CO_2 concentration, by changing O_2 level, or by changing leaf temperature. A part of the O_2 inhibition of photosynthesis is thought to be due to photorespiration (the light-stimulated oxidation of photosynthetic intermediates to CO_2) while part may be by direct inhibition of enzymes of the Calvin-Benson pathway (8, 13). It is not clear what proportion of the total percentage inhibition of photosynthesis is due to direct competitive inhibition by O_2 *versus* photorespiratory metabolism. From the kinetic analysis in this study, we also propose a method for separating the total percentage inhibition of photosynthesis by O_2 into competitive and photorespiratory components in relation to the solubility ratio of O_2/CO_2 in the leaf.

MATERIALS AND METHODS

Growth Conditions. *Triticum aestivum* L. was grown in a growth chamber at a day/night temperature of 30/25 C with other conditions as previously described (16). Newly expanded leaves of 2- to 3-week-old plants were used for all experiments.

Gas Exchange Measurements. Measurements of CO_2, O_2 and water vapor, determination of intercellular CO_2 concentration, and calculation of solubility ratio of O_2/CO_2 in the leaf were fully described in the preceding paper (16).

Carboxylation Efficiency. Carboxylation efficiency (CE) as previously defined (10) is the initial slope of response of photosynthesis to increasing CO_2:

$$CE = \frac{APS}{CO_2 - \Gamma}$$

where APS[2] is the apparent rate of photosynthesis, CO_2 is the concentration of CO_2, and Γ is the CO_2 compensation point. As previously defined, CO_2 and Γ were either atmospheric or estimated intercellular (in the leaf air spaces) measurements. We have modified the determination of CE in the present study where CO_2 and Γ are based on calculated concentration of CO_2 (μM) in the leaf which is dependent on leaf temperature and CO_2 level in the intercellular air spaces (see under "Materials and Methods" in ref. 16). Expressing CE on a solubility basis eliminates variability in carboxylation efficiency which would occur due to change in stomatal resistance in the case where atmospheric CO_2 is used and change in temperature (since increased temperature decreases CO_2 solubility) in cases where either atmospheric or intercellular CO_2 concentration is used. Carboxylation efficiency based on soluble CO_2 in the leaf more closely determines carbon assimilation efficiency per unit of CO_2 available to the photosynthetic cells and allows comparisons of CE with varying temperature. In determining CE, slopes were taken from the initial phase of apparent photosynthesis at low CO_2 levels.

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

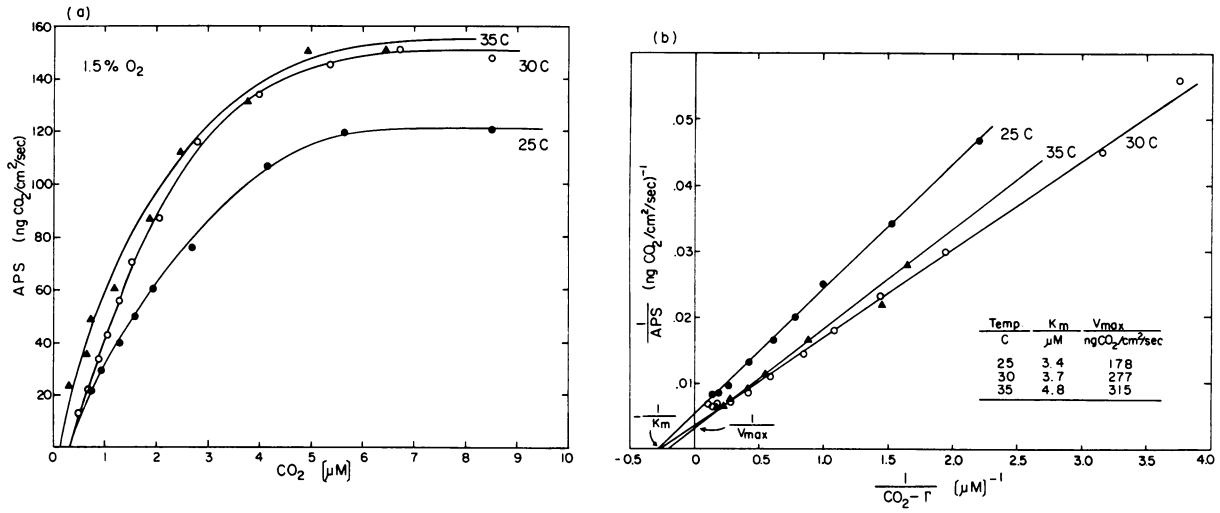

FIG. 1. a: Dependence of apparent photosynthesis on CO_2 under 1.5% O_2 at three temperatures. Measurements were made progressively from 25 to 30 to 35 C and from low to high CO_2 concentration at a given temperature. b: Double reciprocal plots of apparent rate of photosynthesis under 1.5% O_2 *versus* CO_2 concentration at three temperatures. The best fitting line for each temperature was determined by the least square method.

RESULTS

Dependence of Photosynthesis of Wheat on CO_2 under Low O_2 at Three Temperatures. Figure 1a shows the photosynthetic response of wheat under 1.5% O_2 at varying concentrations of soluble CO_2 (calculated from intercellular CO_2) at three temperatures. Low O_2 (1.5%) minimizes O_2 inhibition of photosynthesis and reduces the CO_2 compensation point (Γ). Based on soluble CO_2 in the leaf, photosynthesis saturated at around 7 μM CO_2 (Fig. 1a). From the data of Figure 1a, double reciprocal plots of APS *versus* (CO_2 − Γ) give linear plots up to CO_2 concentrations near saturation of photosynthesis (Fig. 1b). Although Γ is relatively small under 1.5% O_2, subtraction of Γ from CO_2 concentration removes the respiratory component (15). Extrapolation of the lines through the axes allows determination of *Km* for CO_2 and V_{max} (Fig. 1b). *Km* for CO_2 increased slightly with increasing temperature, being 3.4, 3.7, and 4.8 at 25, 30, and 35 C, respectively. The calculated V_{max} was 178, 277, and 315, while the V_{max} obtained experimentally (CO_2 saturated rate) was considerably lower, being 120, 150, and 155 ng $CO_2/cm^2 \cdot sec$ at 25, 30, and 35 C, respectively.

Effects of O_2 on Photosynthesis at Low CO_2 Concentrations at Three Temperatures. In a second experiment (Fig. 2), the effect of O_2 on the apparent rate of photosynthesis at 25, 30, and 35 C in wheat was measured at CO_2 concentrations just above the compensation point at various concentrations of O_2. From the data of Figure 2, several subsequent plots (Figs. 3–6) indicate the efficiency of photosynthesis at the cellular level at various temperatures on the basis of soluble CO_2. Figure 3 shows the carboxylation efficiency with the varying O_2 concentration at different temperatures. The effect of O_2 on CE measures the direct effect of O_2 on photosynthesis separate from the photorespiratory component (14, 17). As the O_2 concentration increases, the CE decreases similarly at three temperatures. This suggests that there is a similar direct inhibition of photosynthesis by O_2 at all three temperatures. Replotting the data of Figure 3 as CE *versus* temperature (Fig. 4) shows that CE increases with increasing temperature at any given O_2 level. An increased CE with increased temperature would be expected if temperature increased V_{max} of the enzymes of carbon assimilation. The parallel response of CE to temperature at different O_2 levels suggests that the leaf is not changing its affinity for CO_2 relative to O_2 at varying temperatures.

The type of inhibition of photosynthesis by O_2 with respect to decreasing CE was determined by a double reciprocal plot of APS *versus* (CO_2 − Γ) which is equivalent to a plot of $1/V$ *versus* $1/S$ in enzyme kinetics where V = velocity and S = substrate concentration. These plots, shown in Figure 5 for 25, 30, and 35 C, indicate competitive inhibition of photosynthesis by O_2 at all three temperatures.

By plotting the slopes from these reciprocal plots *versus* the concentration of O_2, the $Ki(O_2)$ and $Km(CO_2)$ can be determined at the three temperatures. This plot as given in Figure 6a is analogues to the plot of S/V (or 1/CE in this study) *versus* I where S = substrate concentration, V = velocity, and I = concentration of inhibitor. The $Ki(O_2)$ as determined from the intercept on the x-axis was 152, 190, and 215 μM O_2 at 25, 30, and 35 C, respectively, while the $Km(CO_2)$ as determined from the intercept on the y-axis was 3.2, 4.9, and 5.2 μM CO_2 at 25, 30, and 35 C, respectively. The $Km(CO_2)$ and $Ki(O_2)$ are also determined by plotting the intercepts on the x-axis (*Km* apparent for CO_2) of Figure 5 *versus* inhibitor concentration (Fig. 6b). Similar results were obtained from this type of plot. The *Km* values determined in this experiment were similar to those from a different analysis (Fig. 1). Thus with wheat, both *Ki* for O_2 and *Km* for CO_2 increase slightly with increasing temperature although the *Ki*/*Km* ratio undergoes little change with temperature variations.

Model for Partitioning O_2 Inhibition of Photosynthesis into Competitive and Photorespiratory Components. Using the kinetic constants $Ki(O_2)$, $Km(CO_2)$, and V_{max} obtained from Figures 5 and 6a and Γ from Figure 2 for wheat, we simulated various rates of photosynthesis as follows.

True rate of photosynthesis at 0% O_2:

$$TPS_{0\%O_2} = \frac{V_{max} \cdot [CO_2]}{Km + [CO_2]} \quad (1)$$

which is equivalent to:

$$V = \frac{V_{max} \cdot S}{Km + S} \quad (2)$$

for Michaelis-Menten kinetics.

True rate of photosynthesis with O_2:

$$TPS_{O_2} = \frac{V_{max} \cdot [CO_2]}{[CO_2] + Km(1 + O_2/Ki)} \quad (3)$$

which is according to the equation for competitive inhibition:

$$Vi = \frac{V_{max} \cdot S}{S + Km(1 + I/Ki)} \quad (4)$$

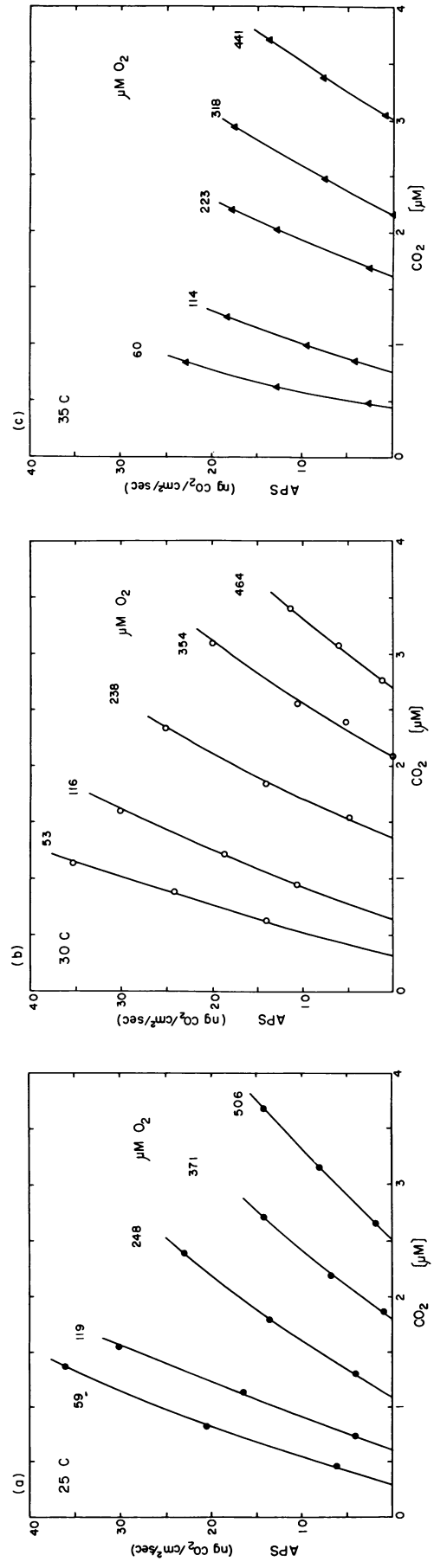

FIG. 2. Effect of O_2 concentration on the initial phase of apparent photosynthesis as a function of CO_2 concentration at three temperatures. Measurements were made progressively from 25 to 30 to 35 C, from low to high O_2 concentration at a given temperature, and from low to high CO_2 concentration at a given O_2 concentration.

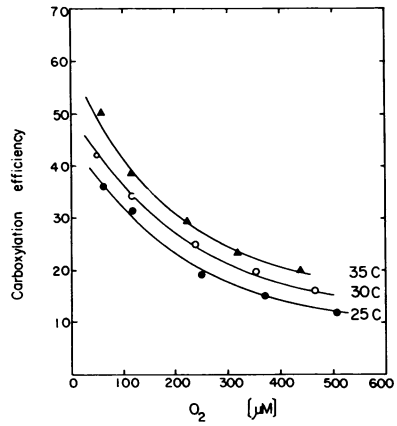

FIG. 3. Carboxylation efficiency as a function of O_2 concentration at three temperatures. Carboxylation efficiency was determined from the data of Figure 2.

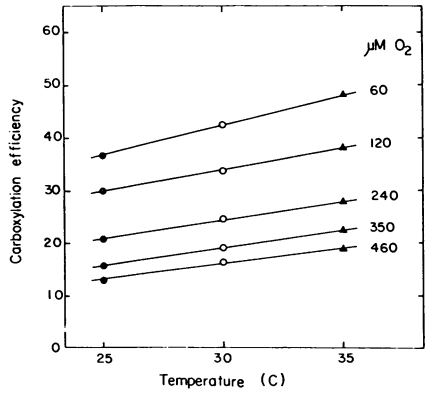

FIG. 4. Carboxylation efficiency as a function of temperature at various O_2 concentrations. Carboxylation efficiency was replotted from the data of Figure 3.

Apparent rate of photosynthesis with O_2 is given as:

$$APS_{O_2} = \frac{V_{max} \cdot (CO_2 - \Gamma)}{(CO_2 - \Gamma) + Km\,(1 + O_2/Ki)} \quad (5)$$

which essentially shifts the response curve for competitive inhibition from the origin to Γ. Using equations 1, 3, and 5 and the constants obtained from Figures 5 and 6a, we obtained the curves for true photosynthesis at 0% O_2, true photosynthesis at 21% O_2 (with competitive inhibition), and apparent photosynthesis at 21% O_2 at varying CO_2 levels for 25, 30, and 35 C (Fig. 7). The partition of total inhibition into competitive and photorespiratory components was then calculated as follows.

$$TPS_{0\%O_2} - APS_{21\%O_2} = \text{Total inhibition by 21\% } O_2$$

$$TPS_{0\%O_2} - TPS_{21\%O_2} = \text{Competitive inhibition by 21\% } O_2$$

$$TPS_{21\%O_2} - APS_{21\%O_2} = \text{Inhibition due to apparent photorespiration at 21\% } O_2$$

The data are replotted as total percentage inhibition, percentage competitive inhibition, and percentage inhibition due to apparent photorespiration at 21% O_2 as a function of the O_2/CO_2 ratio (Fig. 8). In each case, percentage inhibition is calculated with reference to $TPS_{0\%O_2}$.

The total percentage inhibition of photosynthesis by O_2 at the three temperatures is very similar at solubility ratios of O_2/CO_2 from 15 to 50, but at higher solubility ratios the percentage inhibition increases much more at the higher temperatures.

The percentage competitive inhibition of photosynthesis by O_2 increases with increasing solubility ratio in a similar manner for

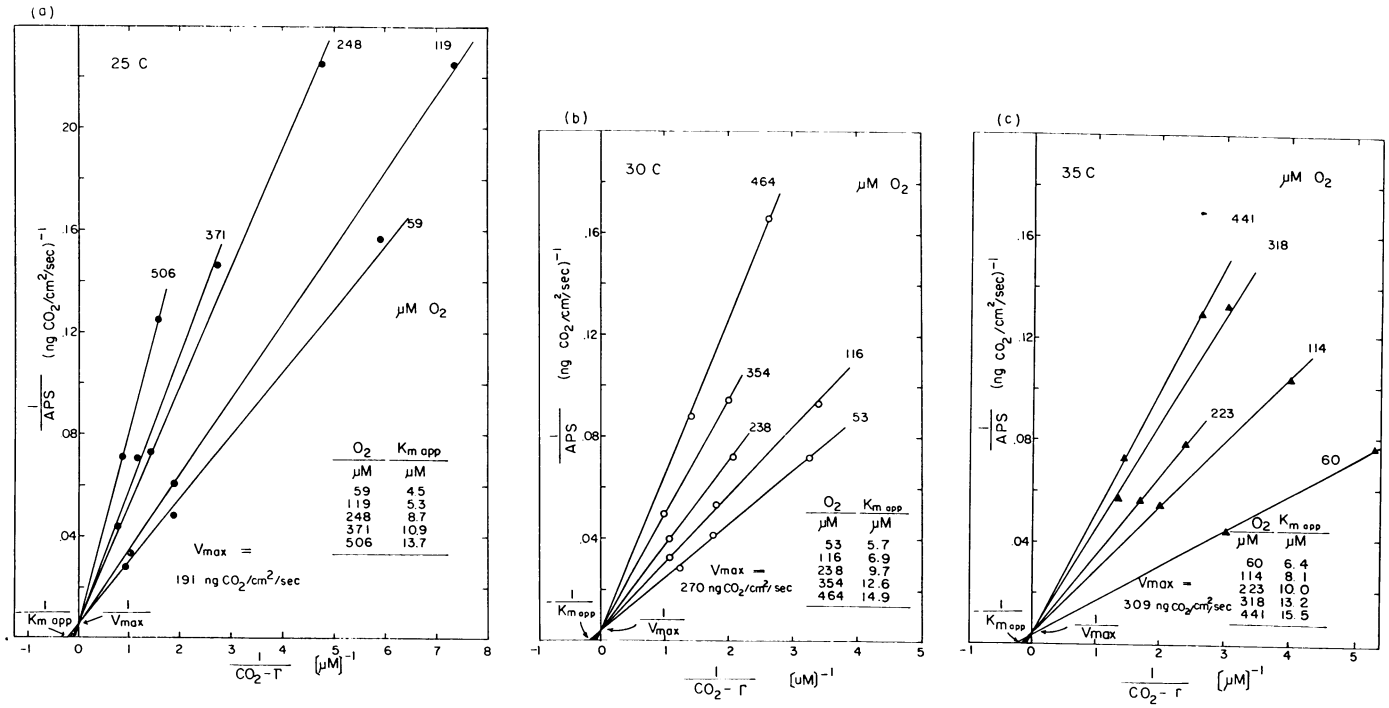

FIG. 5. Double reciprocal plots of apparent rate of photosynthesis *versus* CO_2 concentration at various O_2 concentrations at three temperatures. Apparent rates of photosynthesis were taken from the data of Figure 2.

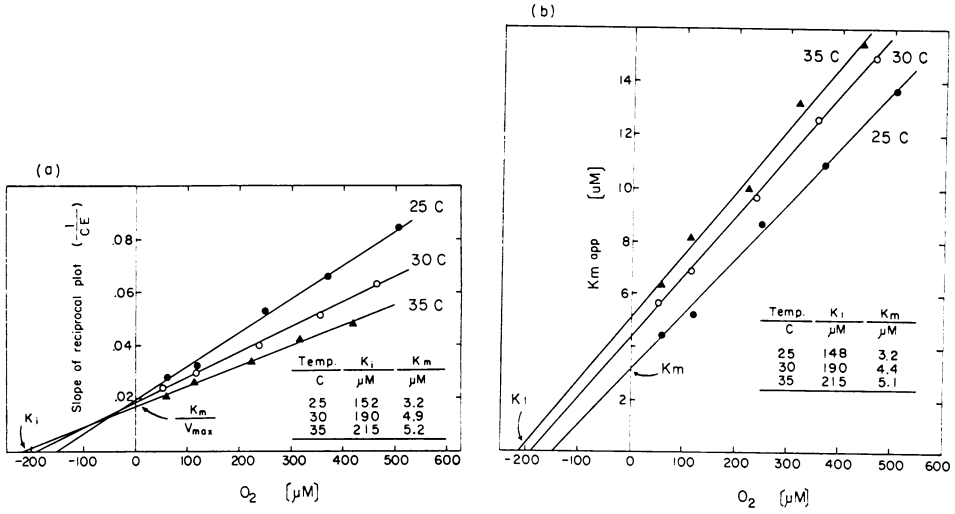

FIG. 6. a: Relationship between the slope of double reciprocal plots from Figure 5 (1/CE) and O_2 concentration at three temperatures. For the determination of Km (CO_2) from the intercept on y-axis ($Km(CO_2)/V_{max}$) in this plot the V_{max} for each temperature was taken from the data of Figure 5. b: Relationship between the apparent $Km(CO_2)$ from Figure 5 and O_2 concentration at three temperatures. In both plots, the best fitting line for each temperature was determined by the least squares method.

all three temperatures. This similar increase with temperature is expected if $Ki(O_2)/Km(CO_2)$ is not changing with varying temperatures. With competitive inhibitors, the percentage inhibition is dependent on both the ratio I/S and the substrate concentration S (23) according to the equation:

$$i = \frac{I}{I + Ki(1 + S/Km)}(100) \qquad (6)$$

where i is the percentage competitive inhibition, I is the concentration of inhibitor, and S is the substrate concentration. Thus the data obtained from this equation are the same as those shown in Figure 8b. Because of the dependence of percentage competitive inhibition on S as well as I/S, at lower substrate concentrations it requires a relatively higher ratio of I/S to attain a certain percentage inhibition. This explains the leveling off of the percentage competitive inhibition at higher solubility ratio of O_2/CO_2 (Fig. 8b). Although the rate of photosynthesis may vary with temperature, the percentage competitive inhibition is not dependent on velocity (equation 6).

The percentage inhibition of photosynthesis due to apparent photorespiration increases in a linear manner with increasing I/S and also increases with increasing temperature at any given I/S (Fig. 8c). Percentage competitive inhibition is relatively independent of temperature while the percentage inhibition due to apparent photorespiration is temperature-dependent.

Photosynthesis of Wheat at Varying CO_2 Levels, 1% and 21% O_2, and Three Temperatures. To test further the above proposal for separating competitive and photorespiratory components of

O_2 inhibition, we measured the O_2 inhibition of photosynthesis (21% *versus* 1% O_2) in a third set of experiments over a wide range of CO_2 concentrations from Γ to saturating levels at 25, 30, and 35 C (Fig. 9). The reason for measuring photosynthesis at 1% O_2 instead of 0% O_2 is that stomata of wheat were unstable in an O_2-free atmosphere (1) and O_2 is necessary for both opening and closing of wheat stomata (2). From the difference between photosynthesis at 1% and 21% O_2 we determined the total percentage inhibition as shown in Figure 10a. Competitive inhibition was separated from inhibition due to apparent photorespiration by shifting the curve for $APS_{21\%O_2}$ from Γ to the intercept on x-axis of $APS_{1\%O_2}$. The percentage inhibition due to these two components is plotted in Figure 10, b and c. The trends of total percentage inhibition, percentage competitive inhibition, and percentage inhibition due to apparent photorespiration were the same for data from simulated plots based on kinetic constants (Fig. 8) in comparison to the data from experimental plots (Fig. 10). However, quantitatively, the total percentage inhibition was lower from experimental data (Fig. 10) than from the simulated data (Fig. 8). In the experimental determinations, $APS_{1\%O_2}$ was taken to indicate $TPS_{0\%O_2}$. This would tend to underestimate the total percentage inhibition since $APS_{1\%O_2}$ would be lower than the actual $TPS_{0\%O_2}$.

As expected, the CO_2-saturated rate of photosynthesis under 1% O_2 (experimental V_{max}) in this experiment, being 140, 165, 170 ng $CO_2/cm^2 \cdot sec$ at 25, 30, and 35 C, respectively (Fig. 9), was also considerably lower than the calculated V_{max} in Figures 1b and 7. This is not expected to affect the percentage competitive inhibition since velocity is not a component of the equation for competitive inhibition (equation 6).

At high I/S (low CO_2 concentrations), apparent photorespiration is the main component of O_2 inhibition, whereas at low I/S (high CO_2 concentrations), competitive inhibition is the main component of O_2 inhibition (Figs. 8 and 10). This is further illustrated in Figure 11 (replotting data of Fig. 10) where the proportion of the inhibition due to competitive inhibition and inhibition due to apparent photorespiration is given at various solubility ratios of O_2/CO_2. At CO_2 around atmospheric conditions as indicated by the arrows in the figures, the main component of O_2 inhibition is suggested to be due to competitive inhibition and the proportion of inhibition due to apparent photorespiration increased with increasing leaf temperature.

DISCUSSION

Affinity of Wheat Leaf for CO_2 during Photosynthesis. Under low O_2 the CO_2 required for saturation of photosynthesis of

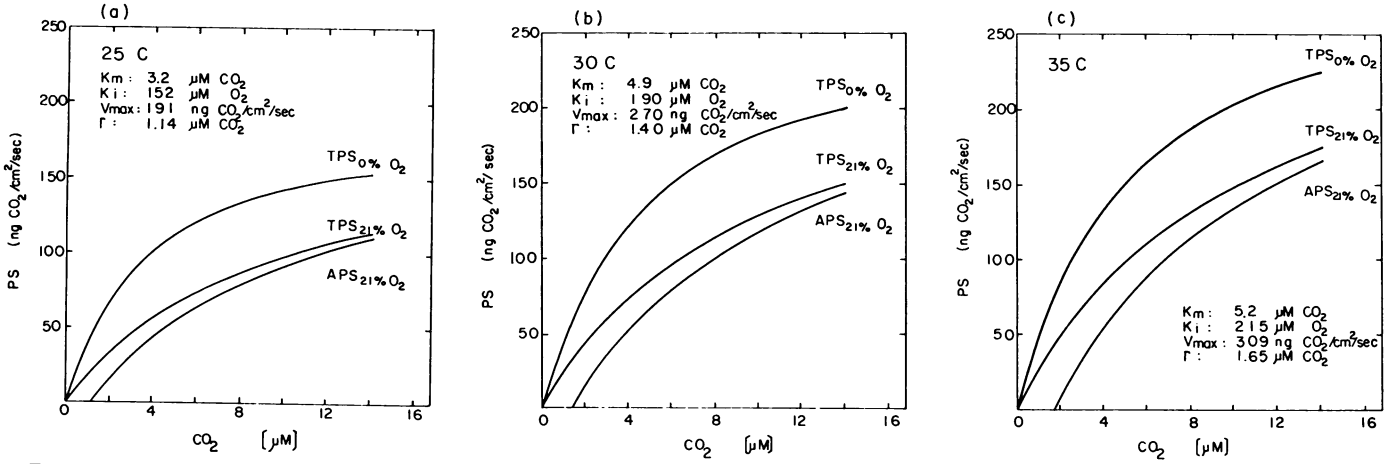

FIG. 7. Simulated CO_2 response curves for photosynthetic rates at three temperatures. Calculations of various photosynthetic rates were according to equations 1, 3, and 5 as described in the text. Kinetic constants $Km(CO_2)$, $Ki(O_2)$, and V_{max} were taken from the data of Figures 5 and 6a, and Γ from Figure 2.

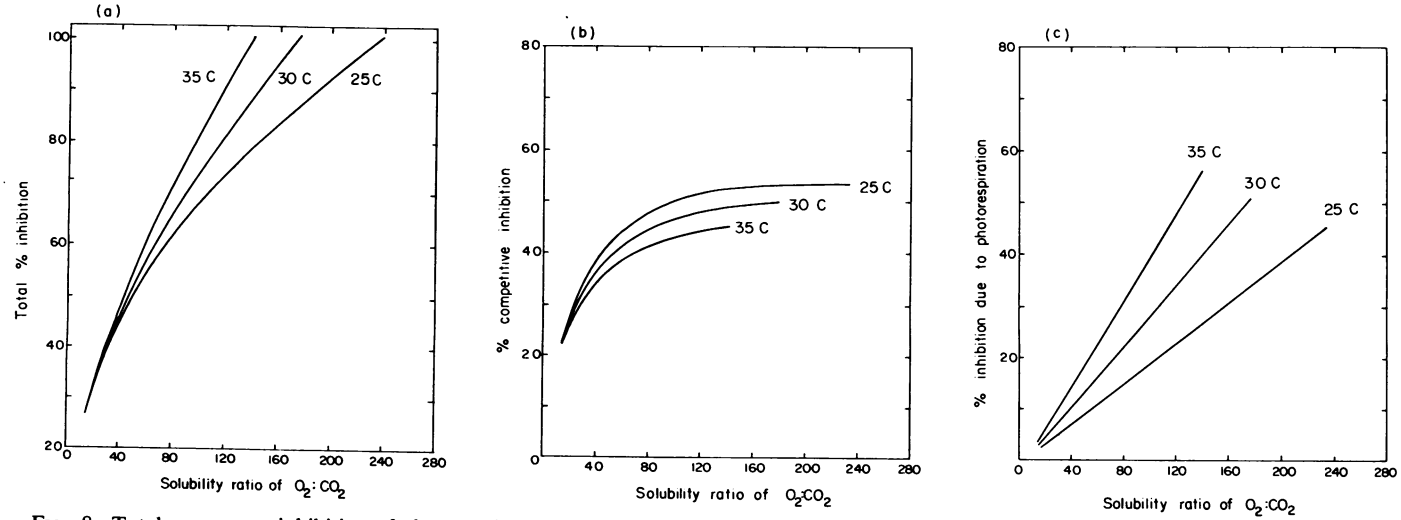

FIG. 8. Total percentage inhibition of photosynthesis by O_2 (a), percentage competitive inhibition of photosynthesis by O_2 (b), and percentage inhibition due to apparent photorespiration (c) as a function of solubility ratio of O_2/CO_2 at three temperatures. Percentage inhibition of photosynthesis by O_2 was calculated from the simulated data of Figure 7 as described in the text. Calculation of O_2/CO_2 solubility ratio from Figure 7 was as described in ref. 16.

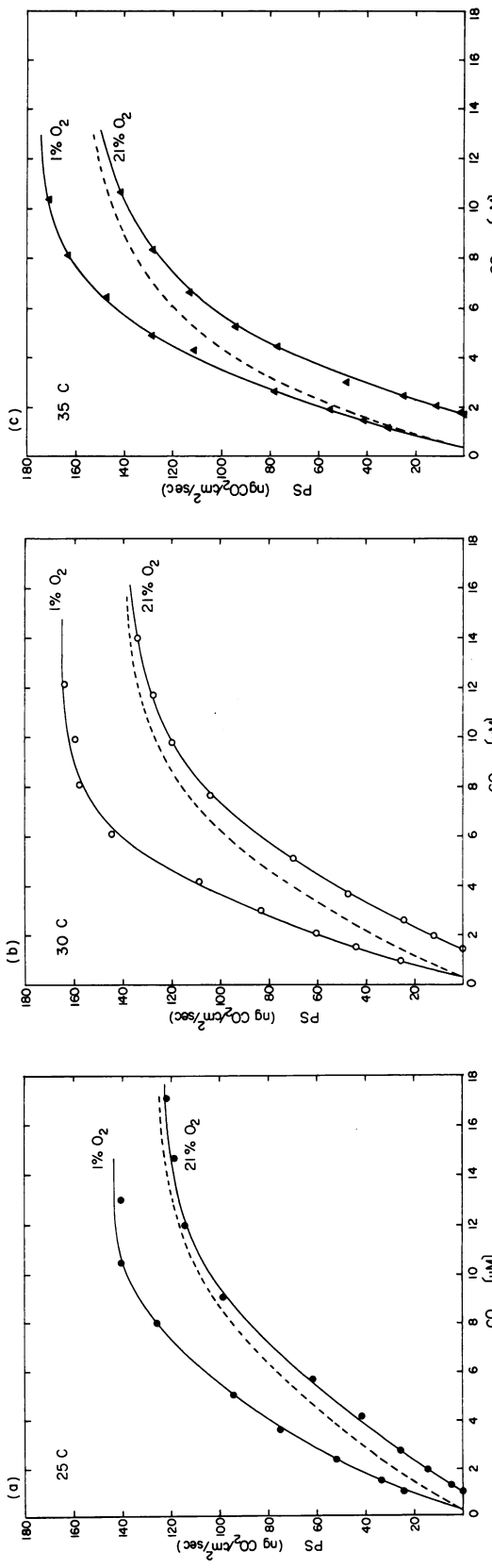

FIG. 9. Dependence of apparent photosynthesis on CO_2 under 1% and 21% O_2 at three temperatures. Measurements were made progressively from 25 to 30 to 35 C, from low to high CO_2 levels at a given temperature, and from 21% to 1% O_2 at a given CO_2 level. Broken line, taken as curve for true photosynthesis at 21% O_2, was obtained by shifting the curve for apparent photosynthesis at 21% O_2 from Γ to the intercept on x-axis of apparent photosynthesis at 1% O_2.

wheat ranged from 7 to 10 μM CO_2 at three temperatures (Figs. 1a and 9), which is within the range reported by Jones and Slatyer (15) for cotton (about 5-14.5 μM CO_2 at 25-28 C). The photosynthetic response of wheat leaves is similar to that of spinach chloroplasts (18) in that the response to CO_2 follows Michaelis-Menten kinetics at lower CO_2 concentrations but deviates at higher CO_2 concentrations near saturation of photosynthesis. Lilley and Walker (18) suggested that this deviation from Michaelis-Menten kinetics is due to a much higher capacity for RuDP carboxylase than for *in vivo* photosynthesis with a major limitation on photochemical capacity at saturating CO_2. We also found that leaf extracts of wheat have substrate-saturated activity of RuDP carboxylase exceeding the CO_2-saturated rate of photosynthesis (Ku and Edwards, unpublished). Goldstein *et al.* (11) and Edwards *et al.* (9) also recently reported levels of RuDP carboxylase in some C_3 species well above the expected maximum rates of leaf photosynthesis.

Previous studies (15, 22) have considered intracellular flux of CO_2 on the basis of transfer resistance from the cell wall to the chloroplasts and carboxylase resistance. The high affinity of wheat leaves for CO_2 under low O_2 (K*m* 3-5 μM, Figs. 1 and 6) suggests that the transfer resistance may be a minor component, since these K*m* values are lower than those thus far determined *in vitro* for RuDP carboxylase (4, 6, 17). The absolute values of K*m* for RuDP carboxylase *in vitro* are dependent on degree of activation of the enzyme (19) although the lowest values thus far determined are 7 to 20 μM CO_2 (4, 6, 17). In the present study, we consider the K*m*(CO_2) determination approximating maximum values for RuDP carboxylase since the CO_2 solubility is based on calculated intercellular levels of CO_2 in the air spaces of the leaf. Without a CO_2 concentrating mechanism in the leaf, the actual soluble CO_2 in the chloroplast may be equivalent to or somewhat lower than the solubility values as determined depending on the transfer resistance. Lilley and Walker (18), who considered a limitation on the carboxylase activity *in vivo* at CO_2 saturation of photosynthesis, also suggested that the transfer resistance is a minor component (also see ref. 22). Jones and Slatyer (15) initially suggested that transfer resistance of CO_2 from the cell wall to the chloroplast may be a larger component than the carboxylation resistance during photosynthesis. However, their analysis assumed experimental V_{max} represents substrate saturation of the carboxylase, predicted very low K*m* values for the carboxylase of about 0.2 to 1.8 μM CO_2 on a soluble basis, and suggested a transport limitation rather than a carboxylase limitation at V_{max}.

Goldsworthy (12) previously determined the apparent Michaelis constants for CO_2 during photosynthesis in several species by experimentally measuring the V_{max} and the atmospheric CO_2 concentration required to give $^1/_2$ V_{max} under nitrogen. However, such Michaelis constants would not be indicative of carboxylation efficiency or affinity of the photosynthetic cells for CO_2 since both boundary layer and stomatal resistances to CO_2 transfer and photochemical limitations at high CO_2 were not considered. Thus, Michaelis constants based on atmospheric levels of CO_2 cannot be reasonably compared with those of the present study. Akita and Moss (2) also noted some limitations in using experimental V_{max} for whole leaf photosynthesis to determine apparent Michaelis constant for CO_2.

Oxygen Effect on Carboxylation Efficiency, K*m*(CO_2), and K*i*(O_2) in Wheat. It is necessary, when analyzing the effect of O_2 on leaf photosynthesis with respect to CE, K*m*(CO_2), and V_{max} at varying temperatures, to make comparisons on a solubility basis for O_2 and CO_2. Previous methods of determining CE have used either atmospheric or calculated intercellular levels of CO_2 (3, 10, 17). If CE is to represent the rate of true photosynthesis per unit of available CO_2 as an indication of biochemical efficiency, then determinations should be made on the basis of soluble CO_2. CE determinations from external CO_2 concentra-

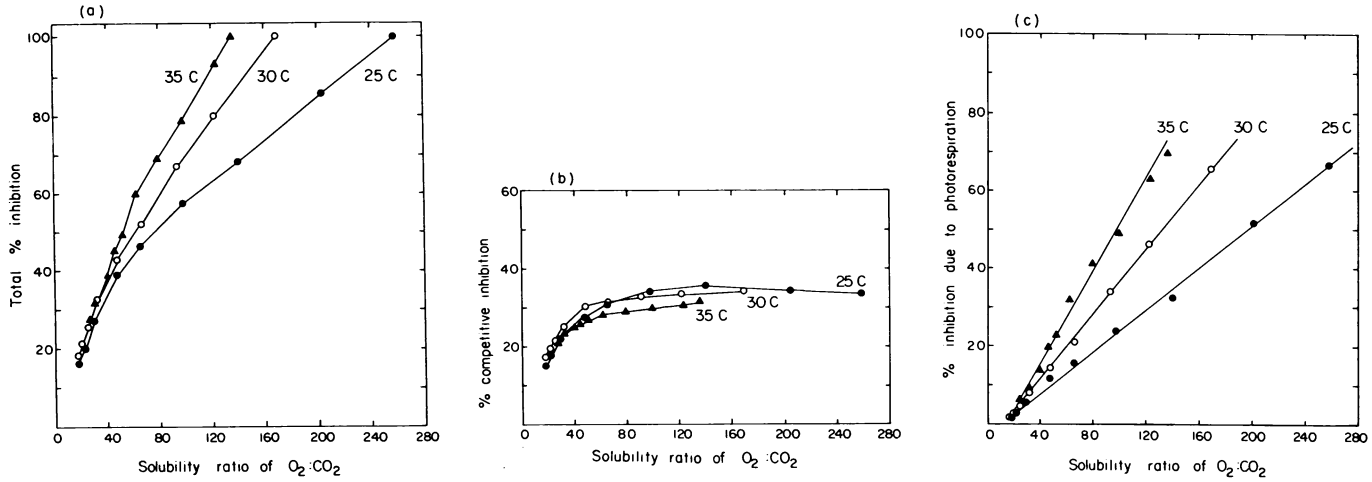

FIG. 10. Total percentage inhibition of photosynthesis by O_2 (a), percentage competitive inhibition of photosynthesis by O_2 (b), and percentage inhibition due to apparent photorespiration (c) as a function of solubility ratio of O_2/CO_2 in the leaf at three temperatures. Percentage inhibition of photosynthesis by O_2 was calculated from the data of Figure 9 and O_2/CO_2 solubility ratio as described in ref. 16.

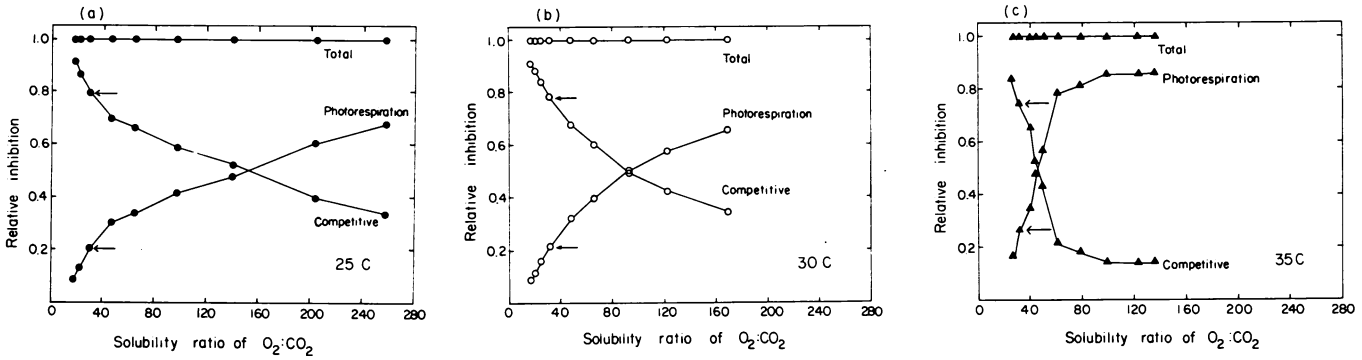

FIG. 11. Relative proportion of the O_2 inhibition of photosynthesis due to competitive inhibition and due to apparent photorespiration as a function of solubility ratio of O_2/CO_2 in the leaf at three temperatures. Calculations were made from the data of Figure 10. The total percentage inhibition of photosynthesis by O_2 at various solubility ratios of O_2/CO_2 was always taken as 1.0. Arrows indicate the relative proportion of the two components at atmospheric conditions while data at highest solubility ratio indicate proportion of two components at CO_2 compensation point.

tions will give lower values than use of intercellular levels. In particular, use of atmospheric levels of CO_2 would underestimate CE when stomatal resistance increases as may occur at higher CO_2 levels and higher temperatures. Use of intercellular CO_2 concentrations for determining CE will also underestimate CE as temperature increases due to a decreased solubility of CO_2. Since as temperature increases the solubility ratio of O_2/CO_2 increases (Fig. 1 of ref. 16), use of intercellular levels of O_2 and CO_2 to determine the sensitivity of CE to O_2 would overestimate the effect of O_2 on CE at higher temperatures. Thus, when CE is expressed on the basis of atmospheric levels of CO_2 and O_2, CE could become increasingly more sensitive to atmospheric O_2 with increasing temperature either due to increased stomatal resistance which gives lower intercellular CO_2, and/or due to increased solubility ratio of O_2/CO_2. This could in part be the basis for the previously reported higher sensitivity of CE to O_2 with increasing temperature in soybean (17).

The kinetic analysis of the effect of O_2 on photosynthesis at varying temperatures when considered on the basis of soluble CO_2 and O_2 (Figs. 2–6) allowed several conclusions: (*a*) part of the O_2 inhibition of photosynthesis is by competitive inhibition (Fig. 5); (*b*) there is a similar decrease in CE at 25, 30, 35 C as O_2 is increased (Fig. 3); and (*c*) both the inhibition constant of photosynthesis for O_2 (Ki) and the kinetic constant for CO_2 (Km) increase slightly with increasing temperature from 25 to 35 C, while the ratio of $Ki(O_2)/Km(CO_2)$ with varying temperature is similar (Fig. 6). The Ki/Km ratio did not change in favor of increased O_2 inhibition at higher temperature.

From equation 4 for competitive inhibition, rearranging gives:

$$\frac{S}{Vi} = \frac{1}{CE} = \frac{S + Km(1 + I/Ki)}{V_{max}} \quad (7)$$

From equation 2 for Michaelis-Menten kinetics without inhibitor, rearranging gives:

$$\frac{S}{V} = \frac{1}{CE_0} = \frac{S + Km}{V_{max}} \quad (8)$$

where CE_0 is the carboxylation efficiency without inhibitor. Combining equations 7 and 8 gives:

$$\frac{1}{CE} = \frac{1}{CE_0} + \frac{Km \cdot I}{Ki \cdot V_{max}} \quad (9)$$

Therefore, the plot of 1/CE *versus* I is expected to give a linear relation as shown in Figure 6a, where $1/CE_0$ is the intercept on y-axis. Rearranging and combining equations 2 and 4 gives:

$$CE = \frac{CE_0(S + Km)}{S + Km(1 + I/Ki)} \quad (10)$$

Since O_2 is a competitive inhibitor of photosynthesis and since reciprocal plots of APS *versus* ($CO_2 - \Gamma$) give linear relations (Fig. 5), as expected from equation 4 for a competitive inhibitor, equation 10 appears to be a reasonable expression of the effect of O_2 on CE. Laing *et al.* (17) used an empirical equation to describe CE which they adapted from a derivation of Forrester *et al.* (10) in analyzing the apparent rate of photosynthesis *versus*

O_2 concentration. This equation

$$CE = CE_0 e^{-0/k}$$

or

$$\ln CE = \ln CE_0 - 0/k$$

suggests a linear relationship between ln CE *versus* O where O = oxygen and k = an arbitrary constant. However, the log CE *versus* I does not give a linear plot either using the velocity equation for competitive inhibition

$$CE = \frac{V_{max}}{S + Km(1 + I/Ki)}$$

with theoretical values of Km, Ki, and V_{max} or using the experimental data in this paper (for example, from Fig. 2). In both cases, with increasing O_2 there is a decrease in log CE in a curvelinear manner. Thus, any determination of k as an indication of relative effect of O_2 on CE at various temperatures by this method is rather arbitrary, and also fails to provide a quantitative evaluation of Ki. Since 1/CE *versus* O_2 provides a linear plot (equation 9) as expected for a competitive inhibitor (Fig. 6a), the empirical equation derived by Forrester *et al.* (10) is not necessary for defining CE in relation to O_2 effect. Peisker and Apels (22) also reported a linear increase in intracellular resistance, r_i (equivalent to 1/CE in the present study), with increasing O_2, which supports a model of photosynthesis proposed by Peisker (21) based on competitive interactions of CO_2 and O_2 with RuDP carboxylase-oxygenase.

In vitro assays of RuDP carboxylase-oxygenase activities of soybean suggested that $Ki(O_2)$ decreased, $Km(CO_2)$ increased, and RuDP oxygenase-carboxylase activities increased with increase in temperature (17). These results are not compatible with calculated changes in $Ki(O_2)$ and $Km(CO_2)$ with wheat in the present study (Figs. 1 and 6) where both $Ki(O_2)$ and $Km(CO_2)$ increased with increase in temperature. A change in Ki and Km in the same direction with variable temperature is generally expected for reactions involving competitive inhibition (26). For example, Singer *et al.* (24) found that Ki for malonate increased as a competitive inhibitor of succinate dehydrogenase from 20 to 38 C as did the Km for succinate. Recently, Chollet and Anderson (7) also found that there is no differential regulation of the RuDP carboxylase-oxygenase by physiological levels of some plastid metabolites.

Competitive and Photorespiratory Components of O_2 Inhibition of Photosynthesis. The results of the present study support previous suggestions that O_2 is a direct inhibitor of photosynthesis and O_2 inhibits photosynthesis through photorespiration (14, 17). Around atmospheric conditions, most of the O_2 inhibition appears to be due to competitive inhibition (Fig. 11). Ludwig and Canvin (20) suggested that under atmospheric conditions about $^2/_3$ of the O_2 inhibition was due to direct inhibition and $^1/_3$ due to photorespiration in sunflower. With wheat the results suggest that if a constant O_2/CO_2 ratio is maintained at varying temperatures the percentage competitive inhibition is similar (Figs. 8 and 10). Also at a given solubility ratio around atmospheric conditions (20 to 45) the total percentage inhibition of photosynthesis by O_2 is the same at varying temperatures (Figs. 8 and 10, and ref. 16), since competitive inhibition is the main component under these conditions. Lack of temperature effect on percentage competitive inhibition at a given solubility ratio of O_2/CO_2 is expected with similar Ki/Km and similar effect of O_2 on CE at varying temperatures. The percentage competitive inhibition of photosynthesis by O_2 is similar in both the theoretical plots generated from kinetic constants (Fig. 8b) and the experimental plots (Fig. 10b). It seems that the rate of true photosynthesis under O_2 is equivalent to the response curve of APS shifted from Γ to the origin.

The percentage inhibition due to apparent photorespiration shows a linear increase with increasing O_2/CO_2 solubility ratio and also increases with temperature as well. These responses may be characteristics of photorespiration but they have not been interpretated on a biochemical basis. Laing *et al.* (17) have suggested that Γ may be a function of V_{max} oxygenase/V_{max} carboxylase and $Km(CO_2)/Km(O_2)$. If temperature does not have differential effects on these parameters, then an additional temperature-dependent component may be involved in photorespiration.

The temperature-dependent increase in percentage inhibition of photosynthesis by O_2 due to apparent photorespiration as well as rate of photorespiration becomes particularly significant at low CO_2. The total percentage inhibition reaches 100% (effectively CO_2 compensation point) at increasing solubility ratios of O_2/CO_2 as the temperature decreases (Fig. 10a). Recalculating the data of Bjorkman *et al.* (5) and Laing *et al.* (17) for Γ at varying temperatures also shows that the O_2/CO_2 solubility ratio at Γ increases as temperature decreases. Ludwig and Canvin (20) suggested that the rate of photorespiration is constant with increasing CO_2 up to atmospheric levels in sunflower, although results of the present study would suggest a progressive decrease in the rate of apparent photorespiration with increasing CO_2. Ludwig and Canvin (20) determined the level of photorespiration as the difference between true photosynthesis, measured as direct fixation of $^{14}CO_2$, and apparent photosynthesis by IR gas analysis. However, the true rate of photosynthesis may be underestimated at low external CO_2 concentrations due to increased dilution of $^{14}CO_2$ by high photorespiration and recycling of $^{12}CO_2$. This could lead to an underestimation of photorespiration at low CO_2 concentrations. Tamas and Bidwell (25) suggest that the rate of photorespiration decreases with increasing CO_2 in bean leaves.

In the present study, the carboxylation efficiency (Fig. 3), V_{max} (Fig. 5), and percentage inhibition of photosynthesis due to apparent photorespiration (Figs. 10 and 11) all increased with increasing temperature. However, the kinetics of these increases needs to be evaluated over a broader temperature range.

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LITERATURE CITED

1. Akita S, A Miyasaka 1969 Studies on the differences of photosynthesis among species. II. Effect of oxygen-free air on photosynthesis. Proc Crop Sci Soc Jap 38: 525-534
2. Akita S, DN Moss 1973 The effect of oxygen-free atmosphere on net photosynthesis and transpiration of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) leaves. Plant Physiol 52: 601-603
3. Augustine JJ, MA Stevens, RW Breidenbach, DF Paige 1976 Genotypic variation in carboxylation of tomatoes. Plant Physiol 57: 325-333
4. Badger MR, TJ Andrews 1974 Effects of CO_2, O_2 and temperature on a high-affinity form of ribulose diphosphate carboxylase-oxygenase from spinach. Biochem Biophys Res Commun 60: 204-210
5. Bjorkman O, E Gauhl, MA Nobs 1970 Comparative studies of *Atriplex* species with and without β-carboxylation photosynthesis and their first-generation hybrid. Carnegie Inst. Yearbook 68: 620-633
6. Bowes G, WL Ogren, RH Hageman 1975 pH dependence of the $Km(CO_2)$ of ribulose-1,5-diphosphate carboxylase. Plant Physiol 56: 630-633
7. Chollet R., LL Anderson 1976 Regulation of ribulose-1,5-biphosphate carboxylase-oxygenase activities by temperature pretreatment and chloroplast metabolites. Arch Biochem Biophys 176: 344-351
8. Chollet R., WL Ogren 1975 Regulation of photorespiration in C_3 and C_4 species. Bot Rev 41: 137-179
9. Edwards GE, SC Huber, M. Gutierrez 1976 Photosynthetic properties of plant protoplasts. *In* JF Peberdy, AH Rose, HJ Rogers, EC Cocking, eds, Microbial and Plant Protoplasts. Academic Press, New York pp 299-322
10. Forrester ML, G Krotkov, CD Nelson 1966 Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves. I. Soybean. Plant Physiol 41: 422-427
11. Goldstein DD, TB Ray, DP Kestler, BC Mayne, RH Brown, CC Black 1976 Biochemical characterization of *Panicum* species which are intermediate between C_3 and C_4 photosynthesis plants. Plant Sci Lett 6: 85-90

12. Goldsworthy A 1968 Comparison of the kinetics of photosynthetic carbon dioxide fixation in maize, sugarcane and tobacco, and its relation to photorespiration. Nature 217: 62
13. Jackson WA, RJ Volk 1970 Photorespiration. Annu Rev Plant Physiol 21: 385–432
14. Jolliffe PA, EB Tregunna 1973 Environmental regulation of the oxygen effect on apparent photosynthesis in wheat. Can J Bot 51: 841–853
15. Jones HG, RO Slatyer 1972 Estimation of the transport and carboxylation components of the intracellular limitation to leaf photosynthesis. Plant Physiol 50: 283–288
16. Ku SB, GE Edwards 1977 Oxygen inhibition of photosynthesis. I. Temperature dependence and relation to O_2/CO_2 solubility ratio. Plant Physiol 59: 986–990
17. Laing WA, WL Ogren, RH Hageman 1974 Regulation of soybean net photosynthetic CO_2 fixation by the interaction of CO_2, O_2, and ribulose-1,5-diphosphate carboxylase. Plant Physiol 54: 678–685
18. Lilley RM, DA Walker 1975 Carbon dioxide assimilation by leaves, isolated chloroplasts, and ribulose biphosphate carboxylase from spinach. Plant Physiol 55: 1087–1092
19. Lorimer GL, MR Badger, TJ Andrews 1976 The activation of ribulose-1,5-diphosphate carboxylase by carbon dioxide and magnesium ions. Equilibria, kinetics, a suggested mechanism, and physiological implications. Biochemistry 15: 529–536
20. Ludwig LJ, DT Canvin 1971 The rate of photorespiration during photosynthesis and the relationship of the substrate of light respiration to the products of photosynthesis in sunflower leaves. Plant Physiol 48: 712–719
21. Peisker M 1974 A model describing the influence of oxygen on photosynthetic carboxylation. Photosynthetica 8: 47–50
22. Peisker M, P Apel 1975 Influence of oxygen on photosynthesis and photorespiration in leaves of *Triticum aestivum* L. I. Relationship between oxygen concentration, CO_2 compensation point, and intracellular resistance to CO_2 uptake. Photosynthetica 9: 16–23
23. Segel IH 1975 Enzyme Kinetics. Wiley-Interscience, New York
24. Singer TP, EB Kearney, V Massey 1957 Newer knowledge of succinic dehydrogenase. Adv Enzymol 18: 65–111
25. Tamas IA, RGS Bidwell 1970 $^{14}CO_2$ fixation in leaf discs of *Phaseolus vulgaris*. Can J Bot 48: 1259–1263
26. Webb JL 1963 *In* Enzyme and Metabolic Inhibitors Vol 1. Chap 15. Academic Press, New York