Effects of Irradiance on the in Vivo CO$_2$:O$_2$ Specificity Factor in Tobacco Using Simultaneous Gas Exchange and Fluorescence Techniques

Richard B. Peterson

Department of Biochemistry and Genetics, the Connecticut Agricultural Experiment Station,
New Haven, Connecticut 06504

ABSTRACT

The effects of gas phase O$_2$ concentration (1%, 20.5%, and 42.0%, v/v) on the quantum yield of net CO$_2$ fixation and fluorescence yield of chlorophyll a are examined in leaf tissue from Nicotiana tabacum at normal levels of CO$_2$ and 25 to 30°C. Detectable decreases in nonphotochemical quenching of absorbed excitation occurred at the higher O$_2$ levels relative to 1% O$_2$ when irradiance was nearly or fully saturating for photosynthesis. Photochemical quenching was increased by high O$_2$ levels only at saturating irradiance. Simultaneous measurements of CO$_2$ and H$_2$O exchange and fluorescence yield permit estimation of partitioning of linear photosynthetic electron transport between net CO$_2$ fixation and O$_2$-dependent, dissipative processes such as photorespiration as a function of leaf internal CO$_2$ concentration. Changes in the in vivo CO$_2$:O$_2$ ‘specificity factor’ ($K_{sp}$) with increasing irradiance are examined. The magnitude $K_{sp}$ was found to decline from a value of 85 at moderate irradiance to 68 at very low light, and to 72 at saturating photon flux rates. The results are discussed in terms of the applicability of the ribulose bisphosphate carboxylase/oxygenase enzyme model to photosynthesis in vivo.

Many studies over the years have confirmed that atmospheric (i.e. 21% v/v) levels of O$_2$ are inhibitory to photosynthesis in plants possessing the C$_3$ pathway of CO$_2$ fixation when CO$_2$ is present at rate-limiting concentrations. The primary biochemical basis for O$_2$ inhibition is the process of photorespiration during which glycolate-P is metabolized with consequent evolution of CO$_2$ inside the leaf (14, 22, 28). Glycolate-P arises by oxygenation of RuBP$^{1}$ as catalyzed by the enzyme Rubisco (17). The individual enzyme activities are related to photorespiratory CO$_2$ release (PR) and gross CO$_2$ fixation (GPS) by $t$, the ratio of moles CO$_2$ produces per mole of glycolate-P metabolized (14). Thus,

$$PR/GPS = t([O_2]/[CO_2])/K_{sp}.$$  (1)

The constant $K_{sp}$ (specificity factor) clearly determines the relative enzyme-catalyzed velocities of carboxylation ($v_a$) and oxygenation ($v_o$) as [O$_2$] and [CO$_2$] are varied. The $K_{sp}$ equals $V_{c}K_{c}/V_{o}K_{o}$ where $V_{c}$ and $V_{o}$ are maximal velocities and $K_{c}$ and $K_{o}$ are Michaelis constants for the respective activities of carboxylation and oxygenation. The magnitude of $K_{sp}$ is relatively constant for enzymes isolated from C$_3$ species and is temperature dependent (1, 14).

Simultaneous determination of CO$_2$ exchange and PSII Chl a fluorescence yield at room temperature offers a new opportunity for assessment of the proportion of total linear electron transport expended for collective O$_2$-dependent processes such as photorespiration and the Mehler reaction (21, 22). Difficulties inherent in estimation of photorespiration have been discussed previously (22). A recently developed modulation technique permits separation and quantitation of major fluorescence quenching processes during steady state CO$_2$ fixation (25). Specifically, ‘photochemical’ quenching (expressed as the coefficient $q_p$) is related to the redox state of QA, the first stable quinone electron acceptor in PSI (2, 24). ‘Non-photochemical’ quenching is dominated by reversible processes which regulate the extent of thermal dissipation of absorbed excitation in PSI (9, 13). Alternative means of expression of this quantity are as the coefficient, $q_N$, or variable fluorescence yield, $F_{v}'/F_{m}'$. Recent evidence (5) indicates that the quantum yield of linear electron transport is directly related to the proportion of PSII centers which are open (i.e. $q_p$) and to the efficiency of energy capture by these centers (i.e. $F_{v}'/F_{m}'$).

This study examines interactive effects of [O$_2$] and irradiance on quantum yield of net CO$_2$ uptake, $q_p$, $q_N$, and $F_{v}'/F_{m}'$ in leaf tissue from Nicotiana tabacum at normal levels of CO$_2$. At elevated O$_2$ levels the product $q_p \times F_{v}'/F_{m}'$ is employed as an empirical means of predicting the quantum yield of total linear electron transport (5). Thus, allocation of photosynthetic reducing equivalents to O$_2$-dependent, dissipative processes may be assessed as the difference between the predicted quantum yield of PSII electron transport based on fluorescence yield and that observed based on net CO$_2$ uptake.
exchange. This approach constitutes a more accurate means of studying effects of O$_2$ on carbon metabolism since it takes into account changes in the partitioning of excitation in PSII which may result from associated changes in acceptor availability and P$i$-recycling as the [O$_2$] is varied. This is in contrast to conventional measurements of O$_2$ inhibition which rely on gas exchange alone. Assessment of allocation of reducing equivalents to O$_2$-dependent processes combined with measurements of leaf internal [O$_2$]/[CO$_2$] permits calculation of the in vivo specificity factor, $K_p$. An earlier study (21) indicated that discrepancies between observed electron allocation and that predicted by the Rubisco model occurred at saturating irradiance. This could indicate enhanced Mehler reaction activity (19) or a change in the proportion of glycolate-P carbon converted to CO$_2$ at very high light intensity. The dependence of $K_p$ on irradiance is examined in greater detail in this study.

MATERIALS AND METHODS
Nicotiana tabacum var Havana seed was grown in a greenhouse as described previously (21). For each experiment a leaf was removed at 0730 (EST), washed with hand soap, and rinsed with distilled H$_2$O. Leaf discs were prepared and CO$_2$ and H$_2$O exchange measurements were conducted using an open, flow-through system using a gas flow rate of 2.0 L min$^{-1}$ (23). The light responses of steady state net CO$_2$ assimilation, transpiration, and fluorescence yield of Chl $a$ were determined following stepwise increases in irradiance of continuous white light over the range of $\sim$80 to 1900 $\mu$mol photons m$^{-2}$s$^{-1}$. The O$_2$ concentrations in the gas stream were 1.6, 20.5, and 42.0% (v/v) corresponding to 16, 210, and 430 mbar, respectively. The mean steady state partial pressure for CO$_2$ in the chamber gas phase was 355 (SD = 8) $\mu$bar. The water vapor pressure deficit was 10 to 11 mbar. Three replicate experiments were performed at each [O$_2$] for each of two leaf temperatures of 25 and 30°C (18 experiments total).

Gas phase (i.e., combined boundary layer and stomatal) conductances to H$_2$O vapor ($q_{H_2O}$) and CO$_2$ ($q_{CO_2}$) were calculated according to Long and Hallgren (16). The value of $q_{CO_2}$ differs from that of $q_{H_2O}$ over the same diffusion pathway due to differences in diffusivity between the two gases. As described previously (16), the H$_2$O:CO$_2$ conductance ratio across the stomatal barrier is 1.61 and that across the boundary layer (where turbulent transfer contributes) is 1.37. Inter-cellular partial pressure of CO$_2$ (C$i$) was calculated using $q_{H_2O}$ and includes a slight correction for effects of entrainment of CO$_2$ molecules by high rates of transpirational H$_2$O efflux (Eq. 6.20 of ref. 16). Dissolved leaf internal O$_2$ concentrations were assumed to be in equilibrium with the partial pressure of O$_2$ in the bulk gas phase. Perturbation of the internal [O$_2$] by photosynthetic O$_2$ evolution was neglected. Molar aqueous phase ratios of leaf internal [O$_2$]/[CO$_2$] were computed using tabular data on the respective solubilities of these gases in H$_2$O at the leaf temperatures ($\pm$0.05°C) employed (7).

Changes in Chl fluorescence yield were monitored using a weak modulated measuring beam of red light (H. Walz, Effeltrich, FRG). The procedures employed are described in detail in (23). Maximal fluorescence yields ($F_{max}$ and $F_{m}'$ for a fully dark-adapted leaf and during continuous actinic illu-

mination, respectively) were recorded during a saturating (7500 $\mu$mol photons m$^{-2}$s$^{-1}$ for 0.7 s) flash of white light. Initial fluorescence yields were recorded for a fully dark-adapted leaf ($F_{ad}$) and during steady state photosynthesis ($F_{s}'$, by imposition of a 2 to 4 s dark interval). The value of $F_{max}$ (and associated $F_{ad}$) were measured just prior to the beginning of the experiment. The mean variable fluorescence yield associated with full dark-adaptation ($F_{max} - F_{ad}$)/$F_{max}$ for the experiments reported herein was 0.833 (SD = 0.010). Photochemical ($q_p$) and nonphotochemical ($q_N$) fluorescence quenching coefficients were computed as ($F_{m}' - F_s'$/($F_{m}' - F_{s}'$) and ($F_{max} - F_{m}'$/($F_{max} - F_{s}'$), respectively (25). The $F_s$ and $F_{m}'$ fluorescence yields were recorded at a measuring beam modulation frequency of 100 kHz. All other measurements were performed at a modulation frequency of 1.6 kHz.

RESULTS

Figure 1 shows mean rates of net assimilation of CO$_2$ for the combined data obtained in the experiments performed at 25° and 30°C. Three-way ANOVA indicated that the main effects of irradiance and [O$_2$] were highly significant ($P < 0.001$). Neither the main effect of temperature nor the temperature × [O$_2$] interaction were significant ($P > 0.1$). The increase in CO$_2$ assimilation with irradiance was accompanied by an increase in the total gas phase conductance to H$_2$O (not shown). Mean values of $q_{H_2O}$ increased from 132 mmol H$_2$O m$^{-2}$s$^{-1}$ (SD = 52) at the lowest irradiance level to 545 mmol H$_2$O m$^{-2}$s$^{-1}$ (SD = 110) at the highest. Three-way ANOVA showed that, as with $A$, the main effects of irradiance and [O$_2$] were highly significant ($P < 0.001$). When averaged across all irradiance levels and both temperatures, $q_{H_2O}$ declined from 384 to 304 and 284 mmol H$_2$O m$^{-2}$s$^{-1}$ at 1.6, 20.5, and 42.0% O$_2$, respectively.

Effects of irradiance on fluorescence quenching parameters are shown in Figure 2. Only the effect of irradiance was significant ($P < 0.05$) in accounting for overall variation in photochemical quenching ($q_p$; Fig. 2, top). Nevertheless, an increase in $q_F$ of $\sim$25% was associated with elevated O$_2$ levels at the highest irradiance employed. Nonphotochemical

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Plots of mean net CO$_2$ assimilation rate versus incident irradiance at three levels of gas phase [O$_2$] for the experiments reported here with *N. tabacum*. The mean gas phase [CO$_2$] was 355 (SD = 8) $\mu$bar. Each point is a mean of six determinations. Error bars indicate $\pm$SE.
for each \([\text{O}_2]\) employed are presented in Figure 3. Similar results were obtained for the experiments performed at 25°C except that the slopes of the linear regression fits to the data (see legend to Fig. 3) were somewhat higher in accordance with the small effect of temperature on nonphotochemical quenching. Clearly, elevated \([\text{O}_2]\) levels result in lower values of \(\Phi_s\) for any given \(q_P \times F_v'/F_m'\) due to diversion of a proportion of linear photosynthetic from net fixation of \(\text{CO}_2\) toward \(\text{O}_2\)-dependent processes such as photorespiration. Since \(\text{O}_2\)-dependent processes are likely to be suppressed at 1.6% \(\text{O}_2\) the associated regression line constitutes a prediction of the quantum yield of total linear electron transport versus \(q_P \times F_v'/F_m'\).

As defined previously (21, 22), the proportion of total noncyclic electron transport which is partitioned to \(\text{O}_2\)-dependent processes is \(P_{\text{diss}}\). Furthermore, the predicted quantum yield of linear electron transport at any elevated \(\text{O}_2\) concentration (\(\Phi_v'\), as \(\text{CO}_2\) equivalents) is calculated by substitution of the associated value of \(q_P \times F_v'/F_m'\) into the linear regression equation obtained at 1.6% \(\text{O}_2\) (Fig. 3). Thus, \(P_{\text{diss}} = (\Phi_v' - \Phi_s)/\Phi_v'\) where \(\Phi_s\) is the observed \(A/I\) at the specified high \(\text{O}_2\). Such estimates of \(P_{\text{diss}}\) are free of any independent effects of changing \([\text{O}_2]\) on photochemical or nonphotochemical quenching of absorbed excitation and therefore constitute unbiased estimates of allocation of photosynthetic reducing power.

The dependencies of \(P_{\text{diss}}\) and intercellular aqueous phase molar \([\text{O}_2]/[\text{CO}_2]\) at 20.5% and 42.0% \(\text{O}_2\) upon irradiance are shown in Figure 4 (top and bottom, respectively). Mean values for \(C_{\text{i}}\) of 235 (SD = 51), 271 (SD = 26), and 301 (SD =

---

Figure 2. Changes in the photochemical fluorescence quenching coefficient \(q_P\) (top panel), nonphotochemical quenching coefficient \(q_N\) (middle panel), and steady-state variable fluorescence yield \(F_v'/F_m'\) (bottom panel) with increasing irradiance. The data shown are for the three experiments performed at each \(\text{O}_2\) concentration and 30°C (error bars indicate ±se). The corresponding plots for replicate experiments performed at 25°C were very similar.

The quenching of fluorescence yield showed significant \((P < 0.001)\) effects of irradiance and \([\text{O}_2]\) as shown by plots of \(q_P\) and \(F_v'/F_m'\) versus irradiance from the 30°C experiments. The higher levels of \(\text{O}_2\) resulted in substantially lower values of \(q_P\) as light-saturation was approached (Fig. 2, middle). Likewise, elevated \([\text{O}_2]\) resulted in a detectable increase in \(F_v'/F_m'\) over the same range of irradiance levels (Fig. 2, bottom). A small, yet statistically significant main effect of temperature was indicated by three-way ANOVA. However, partitioning of the sums of squares indicated that temperature accounted for only about 2% of the observed variation in nonphotochemical quenching. The temperature \times [\text{O}_2] interaction was not significant.

Plots of \(\Phi_s \approx (A/I)\) versus the product \(q_P \times F_v'/F_m'\) at 30°C are presented in Figure 3. The relationship between \(\Phi_s\) and the product \(F_v'/F_m' \times q_P\) for the three replicate experiments performed at each \(\text{O}_2\) concentration shown and 30°C. Data are from Figures 1 and 2. (Note that for each experiment a single value of \(\Phi_s\) was computed by linear regression as the slope of \(A\) versus \(I\) response for the two lowest irradiance levels examined. This was done to minimize errors due to the presence of dark respiration at the low irradiances. Corresponding values of \(F_v'/F_m' \times q_P\) were based on mean values for the respective quantities at the two irradiance levels.) The solid lines are linear regression fits to data. For the data shown the equations are: for 1.6% \(\text{O}_2\), \(y = 0.07867x + 0.00264\) \((R = 0.983)\); for 20.5% \(\text{O}_2\), \(y = 0.0446x - 0.00093\) \((R = 0.937)\); for 42.0% \(\text{O}_2\), \(y = 0.0305x - 0.00284\) \((R = 0.950)\). The linear regression equation for the experiments performed at 25°C and 1.6% \(\text{O}_2\) was \(y = 0.08983x + 0.00254\) \((R = 0.979)\).
Figure 4. Changes in the partitioning of linear electron transport to \(O_2\)-dependent, dissipative processes and in the ratio of leaf internal \([O_2]/[CO_2]\) with increasing irradiance for the experiments of Figures 1 and 2. **Top panel.** \(P_{\text{diss}}\) was calculated as \((\Phi_v - \Phi_d)/\Phi_v\), where \(\Phi_v\) is the predicted quantum yield of linear electron transport (expressed as \(CO_2\) equivalents, i.e. \(4e^{-}\cdot[CO_2]\) based on substitution of the observed \(F_v/F_m\times q_E\) into the corresponding linear regression equation calculated for data obtained at 1.6% \(O_2\) and the same leaf temperature (see legend to Fig. 3). The quantity \(\Phi_d(=A/I)\) is the observed quantum yield of net \(CO_2\) fixation at either 20.5% or 42.0% \(O_2\) based on gas exchange. The mean values shown (error bars indicate \(\pm \text{se}\)) are differentiated according to leaf temperature; i.e. 25°C (circles) and 30°C (squares). The solid lines represent predictions of \(P_{\text{diss}}\) based on the photosynthesis model presented by Farquhar et al. (4) (see Table I). **Bottom panel.** the corresponding leaf intercellular (open symbols) and chloroplast (closed symbols) aqueous phase molar \([O_2]/[CO_2]\) levels for the data in the top panel. The effect of leaf temperature on the respective quantities was exceedingly small so the data points represent means (error bars indicate \(\pm \text{se}\)) of data obtained at both temperatures. See text for further explanation.

20) \(\mu\)bar were computed for \(O_2\) the levels of 1.6, 20.5, and 42.0% in Figure 1, respectively. Aqueous phase molar ratios of \([O_2]/[CO_2]\) based on estimates of \(C_c\) do not accurately reflect the respective concentration ratio of dissolved gases in the chloroplast where \(CO_2\) fixation occurs. The certain existence of a finite mesophyll diffusive resistance will result in a smaller partial pressure of \(CO_2\) in the chloroplast relative to the intercellular air spaces. The mesophyll resistance cannot be measured by conventional gas exchange procedures so that the accurate magnitude of this quantity is not known.

Evans et al. (3) used an isotopic procedure to estimate the mesophyll resistance in wheat. They concluded that the mesophyll resistance to diffusion of \(CO_2\) fell within the range of 1.2 to 2.5 \(m^2\) s \(-1\) bar\(^{-1}\). Thus, a value of 2.0 \(m^2\) s \(-1\) bar\(^{-1}\) was employed as an estimate of the mesophyll resistance in these experiments with tobacco. The partial pressure of \(CO_2\) in the chloroplast (\(C_c\) in \(\mu\)bars) is given by \(C_c = C_i - 2.0\). The irradiance dependencies of the aqueous phase molar ratios of \([O_2]/[CO_2]\) in the chloroplast at 20.5 and 42.0% are also shown in Figure 4 (bottom panel).

The observed dependence of \(P_{\text{diss}}\) on irradiance was compared to that predicted based on the photosynthesis model proposed by Farquhar et al. (4). According to the model, when the [RuBP] is saturating the velocities of carboxylation \((v_c)\) and oxygenation \((v_o)\) are expressed as \(V_cC_i/(C_c+K_c(1+O_i/K_c))\) and \(V_oO_i/(O_i+K_o(1+C_i/K_o))\), respectively. The linear electron transport rate \(J\) supporting net \(CO_2\) fixation, glycyrretic metabolism, and \(NH_4^+\) fixation is \((4 + 4\phi)v_o\) where \(\phi = V_o/K_c(V_o/K_c+K_o)\). Net \(CO_2\) assimilation \(A\) is \(v_c - 0.5v_o\) assuming that photorespiratory \(CO_2\) is released exclusively during the conversion of glycine to serine in the mitochondrion (11). As defined previously, \(P_{\text{diss}} = (J - 4A)/J\). The predicted \(P_{\text{diss}}\) (Table I and Fig. 4, top) may thus be expressed in terms of the Rubisco kinetic constants (10) and the mean chloroplast \(O_2\) and \(CO_2\) concentrations,

\[
P_{\text{diss}} = \frac{\phi}{1 + \phi} \cdot \left[1 + \frac{0.5 K_o(C_c + K_o(1 + O_i/K_o))}{K_c(O_i + K_o(1 + C_i/K_c))}\right]
\]

(2)

As an alternative means of analysis the in vivo \(K_{sp}\) may be calculated directly using a formula derived previously (21). By again assuming that photorespiratory \(CO_2\) arises solely during glycine oxidation, then \(P_{\text{diss}}, K_{sp}\), and the dissolved molar \([O_2]/[CO_2]\) in the chloroplast are related by

\[
K_{sp} = ([O_2]/[CO_2]) \cdot (1.5 - P_{\text{diss}})/P_{\text{diss}}
\]

(3)

Changes in mean \(K_{sp}\) with irradiance for the data of Figure 4 are shown in Figure 5.

**Table I. Calculation of \(P_{\text{diss}}\) Based on the Photosynthesis Model of Farquhar et al. (4)**

<table>
<thead>
<tr>
<th>Photons Flux Rate</th>
<th>20.5% (O_2)</th>
<th>42.0% (O_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu)mol m(^{-2}) s(^{-1})</td>
<td>(C_c)</td>
<td>(P_{\text{diss}})</td>
</tr>
<tr>
<td>81</td>
<td>312 (11)</td>
<td>0.359</td>
</tr>
<tr>
<td>229</td>
<td>276 (19)</td>
<td>0.394</td>
</tr>
<tr>
<td>331</td>
<td>250 (25)</td>
<td>0.423</td>
</tr>
<tr>
<td>472</td>
<td>232 (22)</td>
<td>0.448</td>
</tr>
<tr>
<td>577</td>
<td>224 (19)</td>
<td>0.457</td>
</tr>
<tr>
<td>691</td>
<td>217 (17)</td>
<td>0.468</td>
</tr>
<tr>
<td>907</td>
<td>217 (15)</td>
<td>0.468</td>
</tr>
<tr>
<td>1114</td>
<td>217 (19)</td>
<td>0.468</td>
</tr>
<tr>
<td>1880</td>
<td>230 (13)</td>
<td>0.449</td>
</tr>
</tbody>
</table>
DISCUSSION

A potentially important practical application of room temperature fluorescence yield measurements is to provide a rapid, noninvasive, and economical means of assessing photosynthetic efficiency in response to varying environmental conditions. The occurrence of a simple linear, albeit empirical, relationship between \( \Phi \) and the product \( q_p \times F_v' / F_o' \) (Fig. 3) provides support for the feasibility of the method. It should be pointed out, however, that that radiant energy utilization in PSII is likely to be influenced by numerous factors including \( Q_A \) redox state, \( \Delta pH \)-dependent thermal dissipation, photo-inhibition, PSII antennae size, PSII electron cycling, and occurrence of inactive units. Therefore, one should not assume without verification that the same simple relationship between photochemical and fluorescence yields is valid for all physiological conditions including the occurrence of stress. Ultimate validation of the use of fluorescence measurements to predict linear electron transport rates must await a clearer understanding of mechanisms which regulate energy partitioning and fluorescence emission in PSII.

Photorespiration is undoubtedly the dominant \( O_2 \)-dependent process competing with net \( CO_2 \) fixation for photosynthetically generated NADPH and ATP in \( C_3 \) leaves such as tobacco at normal \( CO_2 \) levels. Changes in partitioning of electron transport with varying \([O_2]/[CO_2]\) and irradiance (Fig. 4) will be discussed within the context of the Rubisco enzyme model based on \textit{in vitro} kinetics (Eq. 1). Specifically, comparison of \textit{in vivo} values of \( K_{sp} \) obtained over varying environmental conditions with reported \textit{in vitro} values provides a framework for identifying and assessing the significance of any suspected deviations from the model.

Figure 5 shows mean \( K_{sp} \) was stable at \( \approx 85 \) for irradiances ranging from 350 to 700 \( \mu \)mol photons m\(^{-2}\)s\(^{-1}\). A value for \( K_{sp} \) of 85 is representative of those reported for purified Rubisco preparations from \( C_3 \) plants (1, 10). The calculated mean \textit{in vivo} \( K_{sp} \) in Figure 5 declined, however, to 68 at low irradiance and to 72 at the highest irradiance tested. The photosynthesis model proposed by Farquhar et al. (4) provides additional support for these conclusions. Based on the model, predicted values of \( P_{\text{in}} \) \((t = 0.5) \) agreed well with observed values at intermediate photon flux rates but were significantly lower than those observed at the lowest (with the exception of 42% \( O_2 \) and 30°C, Fig. 4) and highest irradiances tested. The kinetic constants employed (see ref. 10) are consistent with an \textit{in vitro} \( K_{sp} \) of 82 (Eq. 1) based on dissolved molar concentrations of \( O_2 \) and \( CO_2 \).

Various explanations for the observed variation in \textit{in vivo} \( K_{sp} \) will be considered. If it may be assumed that the kinetic constants \( (V_o, V_c, K_o, K_c) \) for Rubisco from a particular source are invariant then a decline in the apparent \textit{in vivo} \( K_{sp} \) could indicate a diversion of reducing equivalents from \( CO_2 \) fixation to intermediary and secondary metabolism or to reduction of alternate acceptors such as \( NO_3^- \), \( O_3^- \), and \( SO_4^{2-} \). Direct photoreduction of \( O_2 \) by PSI (19) at very high irradiance would increase \( P_{\text{in}} \) for a given \( V_o/V_c \) and chloroplast \([O_2]/[CO_2]\) so that the apparent \textit{in vivo} \( K_{sp} \) would decline (Eq. 2). Recent work by Weis and collaborators (15) raises some doubt as to the feasibility of this hypothesis. They reported that rate-limiting \([CO_2] \) electron donation from reduced plastoquinone to \( P700^+ \) is regulated by the transthyrakoid \( pH \) gradient. Thus, both the donor side of PSI and the NADP/ NADPH pool become more oxidized with increasing irradiance. Since it is generally assumed that PSI-mediated photoreduction of \( O_2 \) requires an excess of reducing equivalents on the acceptor side, the evidence does not unequivocally support enhanced Mehler reaction activity as leading to the decline in \( K_{sp} \) at very high irradiance. As an alternative to PSI-mediated pseudocyclic electron flow, reduced quinones of the intersystem electron transport chain have been shown to donate electrons to \( O_2 \) (18). Also, evidence for synthesis of glycolate from externally provided pyruvate in a pathway involving the enzyme isocitrate lyase has been reported for tobacco leaf tissue (29). The quantitative significance of these latter processes relative to photorespiration resulting from Rubisco activity is not known, however.

The foregoing discussion and the results of Figure 5 are based on the premise that photorespiratory \( CO_2 \) arises only during the glycine \( \rightarrow \) serine conversion so that \( t = 0.5 \) (11). This may not always be true. Oxidative decarboxylation of \( \alpha \)-ketoads by \( H_2O_2 \) produced during photorespiration has been frequently suggested to contribute to \( CO_2 \) evolution (6, 22, 28). Formate and \( CO_2 \) are produced by reaction of hydrogen peroxide with glyoxylate. Formate dehydrogenase is normally present at low levels in leaf tissue (20) so that the probable fate of any formate synthesized is entry into the \( C_4 \) pool following ATP-dependent activation by formyltetrahydrofolate synthetase (27). Methylene tetrahydrofleolate is condensed with glycine to form serine as catalyzed by serine hydroxy-methyltransferase. Thus, glyoxylate peroxidation need not result in \( t > 0.5 \) \textit{in vivo}. Peroxidation of hydroxypyruvate to produce glycolate and \( CO_2 \) during photorespiration could theoretically result in quantitative conversion of the carbon...
ACKNOWLEDGMENTS

I wish to thank Nancy Burns for skillful technical assistance and Israel Zelitch for helpful comments on the manuscript.

LITERATURE CITED


19. Mehler AH (1951) Studies on reactions of illuminated chloro-
plasts. I. Mechanism of the reduction of oxygen and other Hill reagents. Arch Biochem Biophys 33: 65–77