Effects of O₂ and CO₂ Concentrations on Quantum Yields of Photosystems I and II in Tobacco Leaf Tissue

Richard B. Peterson
Department of Biochemistry and Genetics, The Connecticut Agricultural Experiment Station, 123 Huntington St., New Haven, Connecticut 06504

ABSTRACT

The interactive effects of irradiance and O₂ and CO₂ levels on the quantum yields of photosystems I and II have been studied under steady-state conditions at 25°C in leaf tissue of tobacco (Nicotiana tabacum). Assessment of radiant energy utilization in photosystem II was based on changes in chlorophyll fluorescence yield excited by a weak measuring beam of modulated red light. Independent estimates of photosystem I quantum yield were based on the light-dark in vivo absorbance change at 830 nanometers, the absorption band of P700⁺. Normal (i.e. 20.5%, v/v) levels of O₂ generally enhanced photosystem II quantum yield relative to that measured under 1.6% O₂ as the irradiance approached saturation. Photorespiration is suspected to mediate such positive effects of O₂ through increases in the availability of CO₂ and recycling of orthophosphate. Conversely, at low intercellular CO₂ concentrations, 41.2% O₂ was associated with lower photosystem II quantum yield compared with that observed at 20.5% O₂. Inhibitory effects of 41.2% O₂ may occur in response to negative feedback on photosystem II arising from a build-up in the thylakoid proton gradient during electron transport to O₂. Covariation between quantum yields of photosystems I and II was not affected by concentrations of either O₂ or CO₂. The dependence of quantum yield of electron transport to CO₂ measured by gas exchange upon photosystem II quantum yield as determined by fluorescence was unaffected by CO₂ concentration.

Nonphotochemical quenching of fluorescence is associated with enhanced thermal deactivation of excited Chl states. Although the mechanism is unclear, nonphotochemical quenching is dependent on the formation of a proton gradient across the thylakoid membrane during photosynthetic electron transport (3). Increased nonphotochemical quenching is manifested as a decrease in Fₐ' accompanied by a much smaller relative decrease in F₂'. This results in a decrease in F₂'/(Fₐ' - F₂').

Kitajima and Butler (14) provided early evidence that the ratio of F₂' to Fₐ' fluorescence yield provides a measure of photochemical efficiency of PSII. Genty et al. (8) have proposed that the quantity F₂'/Fₐ' is a measure of the efficiency of radiant energy capture by the PSII reaction centers so that the overall Φₚₗₛᵢ is simply the product of the proportion of open units (qp) times the F₂'/Fₐ'. This product is numerically equal to the quantity (Fₐ' - F₂)/Fₐ'. The magnitude of Φₚₗₛᵢ has been shown to be closely related to the quantum yield of photosynthetic electron transport as measured by gas exchange (8, 9, 19). Note that the magnitude of the F₂'/Fₐ' does not depend upon the Fₐ'. Furthermore, the F₂'/Fₐ' is inversely (yet nonlinearly) related to the alternative measure "photochemical" and "nonphotochemical" (26). A PSII unit in which the primary quinone electron acceptor is reduced (i.e. Q₀) is closed to further productive photochemistry and possesses high fluorescence yield (Fₐ'). An open PSII unit (i.e. Q₀ nonreduced) is photochemically competent and exhibits low fluorescence yield (F₂'). The prime symbol denotes that the fluorescence yields are recorded under steady-state conditions of photosynthesis, i.e. in the light-adapted state (26).

For a leaf sample, the proportion of PSII units in the open state (qp) may be estimated by transiently converting all units to closed state with a brief pulse of saturating white light. This is accompanied by a rise in fluorescence yield from F₁ to Fₐ'. Thus, qp = (Fₐ' - F₀)/Fₐ' - F₂'.

Numerous reports from several laboratories during recent years have established that a complex feedback regulation system exists between the stromal carbon-fixing reactions and the primary photochemical reactions situated on the thylakoid membrane (4, 5, 8, 9, 19, 27, 28). These regulatory processes serve to match the rates of production of NADPH and ATP by the light reactions to the prevailing ability of the dark reactions to utilize these products as determined by CO₂ availability, degree of enzyme activation, and carbohydrate assimilation capacity. Changes in the steady-state quantum yields of PSII and PSI in vivo under physiologically relevant conditions are detectable by Chl fluorescence and absorbance changes in the far red region of the spectrum, respectively (9).

Variation in fluorescence yield is due to a combination of distinct quenching processes operating in PSII termed "photochemical" and "nonphotochemical" (26). A PSII unit in which the primary quinone electron acceptor is reduced (i.e. Q₀) is closed to further productive photochemistry and possesses high fluorescence yield (Fₐ'). An open PSII unit (i.e. Q₀ nonreduced) is photochemically competent and exhibits low fluorescence yield (F₂'). The prime symbol denotes that the fluorescence yields are recorded under steady-state conditions of photosynthesis, i.e. in the light-adapted state (26).

For a leaf sample, the proportion of PSII units in the open state (qp) may be estimated by transiently converting all units to closed state with a brief pulse of saturating white light. This is accompanied by a rise in fluorescence yield from F₁ to Fₐ'. Thus, qp = (Fₐ' - F₀)/Fₐ' - F₂'.

Nonphotochemical quenching of fluorescence is associated with enhanced thermal deactivation of excited Chl states. Although the mechanism is unclear, nonphotochemical quenching is dependent on the formation of a proton gradient across the thylakoid membrane during photosynthetic electron transport (3). Increased nonphotochemical quenching is manifested as a decrease in Fₐ' accompanied by a much smaller relative decrease in F₂'. This results in a decrease in F₂'/(Fₐ' - F₂').

Kitajima and Butler (14) provided early evidence that the ratio of F₂' to Fₐ' fluorescence yield provides a measure of photochemical efficiency of PSII. Genty et al. (8) have proposed that the quantity F₂'/Fₐ' is a measure of the efficiency of radiant energy capture by the PSII reaction centers so that the overall Φₚₗₛᵢ is simply the product of the proportion of open units (qp) times the F₂'/Fₐ'. This product is numerically equal to the quantity (Fₐ' - F₂)/Fₐ'. The magnitude of Φₚₗₛᵢ has been shown to be closely related to the quantum yield of photosynthetic electron transport as measured by gas exchange (8, 9, 19). Note that the magnitude of the F₂'/Fₐ' does not depend upon the Fₐ'. Furthermore, the F₂'/Fₐ' is inversely (yet nonlinearly) related to the alternative measure

2 Abbreviations: Fₐ, Fₐ', maximal fluorescence yield in the dark-adapted and light-adapted states, respectively; Cᵢ, intercellular CO₂ concentration (µbar); Cₑ, external CO₂ concentration (µbar); F₀', minimal fluorescence yield in the light-adapted state; Fₐ, steady state fluorescence yield; F₂', variable fluorescence yield in the light-adapted state (Fₐ' - F₀'); qₚ, photochemical quenching coefficient; RuBP, ribulose bisphosphate; Φₚₗₛᵢ, photochemical quantum yield of PSII; Φₚₗₛᵢ, quantum yield of electron transport to CO₂ (mol CO₂ fixed: mol incident visible photons).

---

1 This work was supported by grant No. 9000663 from the Competitive Research Grants Office of the U.S. Department of Agriculture and by Hatch funds from The Connecticut Agricultural Experiment Station.
of nonphotochemical quenching, $q_n = (F_m - F_m')/(F_m - F'_m)$, employed in some studies (18, 27, 28).

Complementary information regarding the quantum yield of PSI may be based on in vivo absorbance changes around 830 nm, which is the absorption band for the oxidized form of the PSI reaction center Chl, P700 (10). The thylakoid proton gradient has been postulated to regulate photosynthetic electron transport by affecting energy capture in PSII and by controlling electron donation from plastoquinol to PSI (5, 9). Excitation captured by a photooxidized (i.e. P700') PSI reaction center is dissipated as heat. Thus, the in vivo proportion of PSI reaction centers in the nonoxidized state constitutes a measure of the $\Phi_{\text{PSII}}$ (9, 28).

This report characterizes quantum yields of PSI and PSII based on the optical methods described above and quantum efficiency of linear electron transport as irradiance and [CO$_2$] are varied. Special emphasis is placed on the interactive effects of [O$_2$] on $\Phi_{\text{PSI}}$ and $\Phi_{\text{PSII}}$. The results are discussed with regard to the roles of photorespiration and the Mehler reaction in mediating the observed effects of O$_2$ on photochemical quantum yield.

### MATERIALS AND METHODS

#### Plant Material and Gas Exchange

All experiments were conducted with leaf discs from greenhouse-grown tobacco (Nicotiana tabacum var Havana Seed). Sample preparation and measurement of CO$_2$ and H$_2$O exchange by infrared gas analysis have been described previously (18, 19). Only modifications to the basic system used earlier will be described here.

A gas phase recirculation system was added to assure a rapid flow rate over both surfaces of the leaf disc (illuminated area, 10 cm$^2$). A pump forced the gas around a loop composed of the assimilation chamber and an empty Plexiglas mixing chamber at a flow rate of about 6 L min$^{-1}$. The contents of the mixing chamber (5 L) were blended continuously by a fan. The low turnover time (approximately 60 ms) for the gas phase of the assimilation chamber ensured that the boundary layer resistance to diffusion was minimal and that no appreciable gradients of CO$_2$ or H$_2$O existed across the leaf during measurements. The boundary layer conductance to H$_2$O (2.1 mol m$^{-2}$ s$^{-1}$ bar$^{-1}$) was estimated from the gas velocity using the formula of Monteith (17). The mixing chamber was incorporated into the open flow-through system upstream from the measuring cells of the infrared analyzers and flushed at a rate of 1 L min$^{-1}$. The H$_2$O vapor pressure deficit was maintained at 4 to 5 mbar and the leaf temperature was 25°C.

#### Fluorescence and in Vivo Absorbance Measurements

Assessments of Chl fluorescence yield in leaf tissue were performed using a weak, modulated measuring beam of red light (Chlorophyll Fluorescence System, H. Walz, Effeltrich, Germany). A detailed description of the system and procedures has been presented (20). The $F_i$ was recorded in continuous white light (tungsten-halogen) supplied by a Schott KL1500 Cold Light Source. $F'_{m'}$ and $F'_m$ in vivo were measured during a brief (2-4 s) interruption in illumination and during a superimposed saturating pulse of white light (7500 $\mu$mol photons m$^{-2}$ s$^{-1}$ for 0.7 s), respectively. All fluorescence yields were measured with respect to the signal obtained when the measuring beam was off.

Measurements of light-induced absorbance changes around 830 nm were made by a modification (ED 800 T emitter-detector unit) of the Walz system. Absorbance increases at 830 nm in vivo have been shown to be the result of photooxidation of P700 to P700$^+$ (10). The modulated (100 kHz) far red measuring beam was emitted from the fiberoptic probe, which was positioned near the upper surface of the leaf disc. An increase in in vivo absorbance results in a reduction in backscattered measuring light reaching the collecting channel of the fiberoptic probe (i.e. remission mode of detection, see ref. 23). The amplified analog signal was digitized (20) every 2 ms and the mean of 500 consecutive conversions was stored in a microcomputer every 1 s. The change (Δ$A_{380}$) in signal was recorded during a brief cessation in white, actinic illumination, which was imposed subsequent to associated gas exchange and fluorescence measurements. Fluorescence and in vivo absorbance measuring beams were directed to the leaf surface with the same fiberoptic assembly because the appropriate emitter and collector leads could be conveniently switched between the red and far red emitter-detector units. Hence, the geometric relationship between the leaf surface and the fiberoptic probe was undisturbed over the course of the measurements. The white actinic light was passed through heat-absorbing (Schott KGI) and heat-reflecting filters to effectively eliminate artifacts arising from overloading of the detectors by far red light.

The magnitude of the signal change associated with full photooxidation of P700 was estimated on the fully dark-adapted leaf in each experiment by application of continuous far red illumination (Schott RG9 filter plus KGI and heat-reflecting filters) to the leaf. The light-dark signal change was measured for several intensities of background far red light. These data were fitted to a rectangular hyperbola so as to assess the signal change associated with infinite intensity of far red light (i.e. $\Delta A_{380,\text{max}}$). The proportion of P700 in the nonoxidized state (i.e. $\Phi_{\text{PSII}}$) is thus given by $(1 - \Delta A_{380}/\Delta A_{380,\text{max}})$.

#### Experimental Protocol

For a single leaf sample maintained at a fixed external CO$_2$ concentration (mean values of 203, 389, and 1109 $\mu$mbar) and 1.4 to 1.8% (v/v) O$_2$ (14.4-18.5 mbar), measurements were recorded after steady-state photosynthesis was attained (i.e. after at least 15 min) at each of successively increasing levels of irradiance. The [O$_2$] was then increased to 20.4 to 20.5% (212 mbar) or 41.0 to 41.4% (424-428 mbar) at the highest irradiance and the same measurements were performed as the irradiance was lowered in steps.

#### RESULTS

Figures 1 through 3 show the irradiance response of $q_n$ and $F'_m/F'_{m'}$ for tobacco leaf tissue at three levels each of CO$_2$ and O$_2$. The concentrations of O$_2$ and CO$_2$ were chosen to produce an associated wide range of levels of inhibition of net photosynthesis by O$_2$. As observed previously (19), the rate of net
Uptake of CO₂ increased hyperbolically with irradiance. At the highest mean irradiance employed (2074 μmol photons m⁻² s⁻¹), mean rates of net photosynthesis (±SD) at 1.6% O₂ and 203, 389, and 1109 μbar CO₂ were 24.7 ± 2.5, 45.4 ± 6.8, and 42.2 ± 3.2 μmol CO₂ m⁻² s⁻¹, respectively. Likewise, inhibition of net photosynthesis by high [O₂] relative to the rate measured at 1.6% O₂ ranged from a minimum of 1.9 ± 12.5% at 1109 μbar CO₂ to a maximum of 77.2 ± 3.7% at 203 μbar CO₂ (both at 41.2% O₂). Gas phase conductance to H₂O (averaged across the four highest irradiance levels and all O₂ concentrations) declined from a mean of 0.30 ± 0.06 mol m⁻² s⁻¹ bar⁻¹ at 203 μbar CO₂ to 0.18 ± 0.06 mol m⁻² bar⁻¹ at 1109 μbar CO₂. Analysis of variance indicated that the effect of [CO₂] was significant (P < 0.001) but the effect of [O₂] was not (P > 0.05).

The decline in qₚ and Fᵥ'/Fₘ' with irradiance was influenced significantly by the [O₂] at each CO₂ level tested. Elevated [O₂] was associated with higher qₚ as irradiance increased for Cᵥ values of 203 and 1109 μbar CO₂ but had no effect at 389 μbar CO₂.

The interactive effects of [O₂] on the Fᵥ'/Fₘ' were more complex compared to the associated effects on qₚ. Variations in Fᵥ'/Fₘ' with irradiance and [O₂] were the result primarily of changes in Fₘ'. The largest O₂-dependent increases in the Fᵥ'/Fₘ' at 203 μbar were observed at 20.5% O₂ and approx-

Figure 1. Steady-state responses of net CO₂ uptake (top panel), qₚ (middle panel), and Fᵥ'/Fₘ' (bottom panel) to irradiance at a mean external CO₂ concentration of 203 μbar (SD = 17) for tobacco leaf tissue. Error bars = ± SE.

Figure 2. Steady-state responses of net CO₂ uptake (top panel), qₚ (middle panel), and Fᵥ'/Fₘ' (bottom panel) to irradiance at a mean external CO₂ concentration of 389 μbar (SD = 27) for tobacco leaf tissue. Error bars = ± SE.
EFFECTS OF O₂ ON QUANTUM YIELDS OF PSI AND PSII

Figure 3. Steady-state responses of net CO₂ uptake (top panel), qₚ (middle panel), and Fv'/Fm' (bottom panel) to irradiance at a mean external CO₂ concentration of 1109 µbar (sd = 49) for tobacco leaf tissue. Error bars = ± se.

Figure 4. Dependence of φₚₛᵢ on φₚₛᵢ for experiments performed at mean external CO₂ concentrations of 203 and 1109 µbar. The results were similar at 389 µbar CO₂ (not shown). Within the limits imposed by the errors of the methods, no effects of O₂ or CO₂ concentrations on covariation between the quantum yields of PSI and PSII were detected. Note that the mean value of φₚₛᵢ measured for the leaf samples used in these experiments when in the fully dark-adapted state (i.e. 12 h dark) was 0.819 (sd = 0.014).

Approximately 1000 µmol photons m⁻² s⁻¹ (Fig. 1). The positive effect of 20.5% O₂ diminished as the irradiance either increased or declined. In contrast, an irradiance of approximately 2000 µmol photons m⁻² s⁻¹ was required for a maximal effect of 20.5% O₂ on the Fv'/Fm' when the Cₒ was 389 µbar (Fig. 2). The enhancing effect of elevated [O₂] on Fv'/Fm' was virtually absent at 1109 µbar CO₂ (Fig. 3). Furthermore, 41.2% O₂ was much less effective than 20.5% O₂ in increasing the Fv'/Fm' at the lower concentrations of CO₂ (Figs. 1 and 2). The data of Figures 1 through 3 clearly indicate a decline in φₚₛᵢ (qₚ × Fv'/Fm') with increasing irradiance. The O₂-dependent increases in qₚ and Fv'/Fm' imply increases in φₚₛᵢ.

Appreciable O₂- or CO₂-dependent changes in qₚ and Fv'/Fm' did not occur at limiting irradiance (150–260 µmol photons m⁻² s⁻¹). This is based on results obtained with tobacco leaf tissue in separate experiments conducted under the same levels of O₂ and CO₂ indicated in Figures 1 through 3. The mean qₚ was 0.947 (sd = 0.009) and the mean Fv'/Fm' was 0.758 (sd = 0.006), and effects of gas phase composition on either of these quantities (although significant, i.e. P < 0.05) never exceeded 2.2%.

The quantum yield of PSI based on the redox state of P700 (i.e. φₚₛᵢ) declined with irradiance, as did φₚₛᵢ (not shown). Figure 4 shows the strong (albeit somewhat sigmoidal) dependence of φₚₛᵢ on φₚₛᵢ. Within the limits of detectability of the methods employed, no effects of [O₂] or [CO₂] on covariation of φₚₛᵢ with φₚₛᵢ were observed in these experiments.

Values of φₚₛᵢ were calculated from the data obtained at the four highest irradiances of Figures 1 through 3. The results are presented in Figure 5 as a function of the associated mean Cₒ. At subsaturating irradiance (panels A and B), the internal [CO₂] seldom limited φₚₛᵢ. Effects of [O₂] under these conditions were relatively small. In contrast, as irradiance approached saturation and the [O₂] was 20.5% or less (Fig. 5, panels C and D) CO₂ availability limited φₚₛᵢ up to at least 200 µbar. These results illustrate an interesting paradox con-
Figure 5. Dependence of ΦPSII on the gas phase O2 level and the intercellular CO2 concentration at four mean irradiances ranging from 420 to 2074 μmol photons m⁻² s⁻¹. Data were obtained from the experiments of Figures 1 through 3. Error bars = ± se.

cerning the effects of O2 on photosynthetic efficiency. For a constant and relatively low C0 (<400 μbar), an increase in gas phase [O2] results in a decline in net CO2 flux from the atmosphere into the leaf. This is caused predominantly by oxygenation of RubBP and consequent internal release of CO2 during photorespiration. In these experiments, stomatal conductance to diffusion of CO2 did not change significantly (see above) with [O2], so that the Ci increased with O2 level when C0 was constant. The results of Figures 1 and 2 (top panels) and Figure 5 (panels B–D) indicate that, whereas net photosynthesis is inhibited by 20.5% O2 when C0 < 400 μbar, ΦPSII increased due largely to a rise in Ci.

Very high levels of CO2 can cause a reduction in ΦPSII (Fig. 5, panel D). The stimulatory effects of both 20.5 and 41.2% O2 on ΦPSII persisted, however, at nearly saturating irradiances and internal CO2 concentrations >650 μbar. This indicates that O2 can stimulate ΦPSII by means other than enhancement of CO2 availability. The inhibitory effects of 41.2% O2 at the lower CO2 levels are clearly reversed by increasing the [CO2].

The interactive effects of irradiance, [O2], and [CO2] on ΦPSII shown in Figure 5 also reflect variation in the Φs, as shown in Figure 6. At low levels of O2 (1.6%), photorespiration is suppressed so that measurements of CO2 uptake provide a reasonable estimate of coupled noncyclic photosynthetic electron transport. The results indicate that, over a wide range of availabilities of light and CO2, a nearly linear dependence of Φs with ΦPSII is obtained (see also refs. 8, 19).

The protocol employed (see "Materials and Methods") in these experiments was devised to reduce the influence of biological variability among leaves with regard to O2 effects. However, the magnitudes of qE and Fv'/Fm' recorded at high [O2] and irradiances <2000 μmol photons m⁻² s⁻¹ may include effects of slowly relaxing photoprotective energy dissipation due to prior exposure to more intense illumination. Evaluation of such hysteresis effects is based on the following considerations. First, both qE and Fv'/Fm' always showed a strong tendency to increase as the irradiance was lowered at high [O2]. Second, in several of the experiments performed, a final set of measurements was repeated at low [O2] and approximately 400 μmol photons m⁻² s⁻¹ to serve as a check for hysteresis effects. On average, the magnitude of ΦPSII measured at the end of the experiment was 87% (SD = 11%) of the prior value measured under the same conditions. Nearly all of this decline was due to reduction in the Fv'/Fm'.

Figure 6. Dependence of Φs (expressed as CO2 equivalents) on ΦPSII [= (Fv' − Fm')/Fm'] at 1.6% O2 (v/v) and three external CO2 levels at 25°C. Error bars = ± se.
of $\Phi_{PSI}$ and $\Phi_{PSII}/\Phi_{PSI}$ measured at the end divided by those measured at the beginning of the experiments indicated little change in these quantities (106 ± 15% and 96 ± 5%, respectively). No clear effect of [CO$_2$] or prior [O$_2$] was evident in these measurements. Thus, uncontrolled effects of prior illumination do contribute modestly to the effects of [O$_2$] at approximately 400 $\mu$mol photons m$^{-2}$ s$^{-1}$ described here, and these effects are expected to be of even lesser significance at the higher irradiances.

**DISCUSSION**

Molecular oxygen interacts with the photosynthetic apparatus at several points, particularly in C$_3$ systems such as tobacco. The substantial effect of elevated [O$_2$] on net photosynthesis due to the occurrence of photorespiration has been well documented (13, 19, 30). The oxygenation of RuBP as catalyzed by RuBP carboxylase/oxygenase is considered to represent the first step in photorespiration (13). Metabolism of the resulting phosphoglycolate to 3-phosphoglycerate is accompanied by O$_2$ uptake. Oxygen can serve as an oxidant on the reducing side of PSI at probably more than one specific site, although antioxidizable reduced ferredoxin is the most efficient electron donor (7). Some evidence (2, 15) has been presented for electron transfer to O$_2$ by quinols on the acceptor side of PSII. In addition, O$_2$-dependent photoactivation processes occur, but these are not well understood (12). A fundamental objective of this study was to examine effects of O$_2$ on photosynthetic efficiency using probes that are not subject to the limitations inherent in gas exchange methods, which arise due to fixation of photorespiratory CO$_2$.

The effects of O$_2$ on $\Phi_{PSII}$ described here clearly are both stimulatory and inhibitory. Enhancement of photochemical efficiency as indicated by both $q_p$ and $F_v'/F_m'$ was most evident at low [CO$_2$] and atmospheric levels of O$_2$ (i.e. 20.5%). Considerable evidence has accumulated suggesting that the recycling of orthophosphate from starch and sucrose synthesis can limit the rate of formation of ATP by phosphorylation in the chloroplast (11, 24, 25). Hydrolysis of phosphoglycolate could be expected to substantially increase the availability of inorganic phosphate for phosphorylation, resulting in discharge of the thylakoid $\Delta$pH (25). Enhancement of $\Phi_{PSII}$ in these experiments by 20.5% O$_2$ at high [CO$_2$] and high irradiance is readily explained in terms of the phosphate recycling model. Rates of CO$_2$-dependent photosynthetic electron transport were maximal under these conditions, thereby placing a high demand for ATP.

The inhibitory effects of hyperbaric O$_2$ levels (41.2%) on photochemical quantum yield are of special interest because such occurrences are not readily observable by gas exchange methods alone. These effects were most apparent at high irradiance and relatively low [CO$_2$] (Fig. 5) and could be due to Mehler reaction activity (16). During this process, O$_2$ is reduced to superoxide (O$_2$-) by PSI. Superoxide anion is converted to H$_2$O$_2$ by superoxide dismutase, followed by detoxification of peroxide in the chloroplast by the ascorbate-glutathione reductase system (6). This process supports whole chain electron transport from PSI and is accompanied by the pumping of protons across the thylakoid membrane (7). However, the Mehler reaction results in no consumption of ATP so that, if not dissipated by some other process, the resulting build-up of the $\Delta$pH could result in negative feedback on the magnitude of $\Phi_{PSII}$. Also, a Mehler reaction-dependent increase in $\Delta$pH could stimulate conversion of the xanthophyll pigment violaxanthin to zeaxanthin (29). Zeaxanthin has been postulated to play a central role in the creation of excitation "quenching centers" in the Chl antennae complex (4).

The quantitative significance of the Mehler reaction under steady-state conditions of photosynthesis is uncertain (22). However, regulatory aspects of this process may be inferred on the bases of *in vitro* studies and recent results concerning control of redox states of PSI components. Mehler reaction activity is favored by high O$_2$ levels, which compete more favorably for reduced ferredoxin on the acceptor side of PSI (7). Weis and Lechtenberg (28) reported that the acceptor side of PSI is relatively oxidized when $\Delta$pH-dependent quenching of excitation is high, as is observed in the presence of strong light and low [CO$_2$]. This should suppress reduction of O$_2$ by PSI.

The results presented here offer little indication that quantitatively significant Mehler reaction activity occurs at 20.5% O$_2$ because $\Phi_{PSII}$ at this [O$_2$] is similar to that observed at 1.6% O$_2$, when irradiance is low (Fig. 5, panels A and B). At high irradiance (Fig. 5, panels C and D), $\Phi_{PSII}$ appears to be determined primarily by a similar response to the C$_i$ (for values <200 $\mu$bar) at both 1.6 and 20.5% O$_2$. The parameters of this response appear to interact with the irradiance level, however. In the presence of 41.2% O$_2$, enough Mehler reaction activity could occur to account for the suppressive effects of this [O$_2$] at low C$_i$. The stimulation of the Mehler reaction may partly explain lower values of $\Phi_{PSII}$ observed at the limiting irradiances and 41.2% O$_2$ (Fig. 5, panels A and B). The apparent stimulatory effect of 41.2% O$_2$ (relative to 1.6% O$_2$) on $\Phi_{PSII}$ at very high [CO$_2$] and high irradiance is most likely due to the O$_2$-dependent reversal of inhibition of photorespiration by high [CO$_2$] (13). This would restore efficient recycling of orthophosphate as described above. The negative effect of diminished Mehler-type activity on $\Phi_{PSII}$ at 20.5% O$_2$ relative to 41.2% O$_2$ is offset by a lower capacity for phosphate recycling at 20.5% O$_2$ as the C$_i$ increases.

Finally, the effect of the Mehler reaction on $\Phi_{PSII}$ may be amplified if it is capable of regulating redox poise of electron carriers involved in PSI-catalyzed cyclic photophosphorylation (1). If an imbalance in the ratio of cyclic to noncyclic electron flow were induced at very high [O$_2$], then a negative effect on $\Phi_{PSII}$ could result. It is worth noting that the Mehler reaction is viewed in this discussion as having negative impact on photochemical quantum yield. However, the Mehler reaction promotes overall photosynthetic efficiency during photosynthetic induction (21) or in any instance in which ATP consumption exceeds that supplied by coupled cyclic and noncyclic electron flow.

A model of coordinate control of $\Phi_{PSI}$ and $\Phi_{PSII}$ by the thylakoid $\Delta$pH has been presented (5). According to the model, a $\Delta$pH-dependent restriction to electron flow exists between PSII and PSI at the Cyt b/f complex. Thus, the $\Delta$pH simultaneously regulates quantum yield of each photosystem by controlling the redox state of the PSII acceptor pool ($q_A$) and by limiting electron flow to P700$^+$. The results presented here are consistent with this model and demonstrate that $\Phi_{PSI}$...
and $\Phi_{PSI}$ vary in a nearly linear manner over a wide range of O$_2$ and CO$_2$ levels. In agreement with recent evidence (20), the results of Figure 4 indicate that oxidation of quinols of the intersystem electron transport chain is quantitatively insignificant. If elevated [O$_2$] did oxidize quinols in these experiments, then an O$_2$-dependent decline in $\Phi_{PSI}$ could be expected for a given value of $\Phi_{PSII}$. No such O$_2$-dependent deviation in the relationship between $\Phi_{PSI}$ and $\Phi_{PSII}$ was observed.

The results presented here show that nonphotochemical energy quenching processes work in concert with photochemistry, as governed by availability of the substrates CO$_2$ and O$_2$, so as to minimize damaging effects of overexcitation. Indeed, the presence of adequate CO$_2$ is vital to efficient photoprotection. Evidence has been presented (12) describing irreversible photoactivation of chloroplasts maintained in the absence of CO$_2$. It follows that efforts to enhance photosynthesis by abolishing photorespiration (30) through elimination of the oxygenase function of RuBP carboxylase/oxygenase (and, hence, CO$_2$ recycling) could result in catastrophic susceptibility to water stress-induced stomatal closure. This need not preclude, however, the possibility that partial reductions in oxygenase activity could result in superior and stable photosynthesis. Better information is needed concerning (a) the dependence of the rate of photoactivation versus the degree of reduction of the electron transport system in the light, and (b) the possibly protective effects provided by dark respiratory CO$_2$ release and cuticular diffusion of CO$_2$, before conclusions are justified concerning the consequences of suppression of photorespiration on plant survival.

ACKNOWLEDGMENT

The author wishes to thank I. Zelitch for helpful comments on the manuscript.

LITERATURE CITED