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Relationship between Steady-State Fluorescence Yield and Photosynthetic Efficiency in Spinach Leaf Tissue[1]

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ABSTRACT

The relationship between steady-state photosynthetic efficiency, as moles CO_2 per mole of incident visible photons under 2% O_2, and chlorophyll fluorescence quenching has been investigated in intact leaf tissue of Spinacia oleracia. Fluorescence yield was measured using a pulse amplitude modulation technique that permitted rapid and sensitive resolution and quantitation of photochemical and nonphotochemical quenching coefficients. A highly linear relationship was observed between photosynthetic efficiency and the ratio of photochemical:nonphotochemical quenching coefficients for values of the latter less than 1.6. This relationship applied whether irradiance or CO_2 concentration was varied. The observed relationships between photochemical yield and fluorescence yield were compatible with the photosystem II model proposed by Butler and Kitajima (1975 Biochim Biophys Acta 376: 116–125). The results are discussed with respect to the proposed role of nonphotochemical quenching in regulating radiant energy utilization and also the applicability of fluorescence measurements as a means of estimation of the rate of photosynthetic electron transport.

The relationship between *in vivo* Chl fluorescence and the quantum efficiency of photosynthetic electron transport is a subject which is both fundamental to an understanding of the mechanism of function of PSII and one which may be of great practical importance. Despite the obvious advantages of sensitivity and nonintrusiveness, successful application of fluorescence as a probe of photosynthetic efficiency *in vivo* has been hampered by ambiguities concerning the relationship between changes in fluorescence intensity and events at the molecular level. Fluctuations in fluorescence are generally interpreted in terms of processes that quench it relative to the maximal fluorescence yield possible (12). One of the quenching mechanisms is referred to as "Q-quenching" or "photochemical quenching" which senses the redox state of the first quinone electron acceptor of PSII, Q_A.[2] When the acceptor of a PSII unit is reduced (Q_A^-), the unit is termed "closed," implying that photochemistry cannot occur. A quantum of visible light trapped by a closed unit will be reemitted as fluorescence or dissipated as heat. Thus, Q_A is a quencher and Q_A^- is a nonquencher of fluorescence (3, 6, 12).

Considerable recent evidence indicates that a potent "nonphotochemical" fluorescence quenching mechanism exists in chloroplasts (3, 10–12, 16). This nonphotochemical quenching is associated with thylakoid membrane energization as indicated by the transthylakoid ΔpH. The introduction of methodology utilizing a modulated measuring beam to excite fluorescence *in vivo* has greatly facilitated resolution of these quenching mechanisms during photosynthesis under physiologically relevant conditions (3, 16, 17). Quenching analysis has already been useful (18) in interpretation of induction effects in photosynthesis associated with a dark-light transition or a sudden change in ambient CO_2 concentration (19, 20).

It is clear that the excitation density in PSII will influence the degree of reduction of Q_A. Photooxidation of Q_A^- proceeds via plastoquinone and PSI so that availability of $NADP^+$, and hence Calvin cycle activity, will ultimately affect the redox state of Q_A and thus photochemical quenching. Dietz *et al.* (6) demonstrated a nonlinear, inverse relationship between *Q*-quenching and quantum efficiency of CO_2 fixation over a range of CO_2 concentrations in an intact leaf of spinach. Weis *et al.* (22, 23) have presented evidence that nonphotochemical quenching exerts a simultaneous effect on quantum efficiency of CO_2 fixation in sunflower and bean leaves by increasing the rate constant for thermal deactivation of excitation at the PSII reaction center. Horton and Hague (9) have similarly reported on a decline in the quantum yield for PSII which was associated with the presence of energy-dependent quenching during induction of photosynthesis in protoplasts from barley.

This study examines the relationships among quantum efficiency, *Q*-quenching, and nonphotochemical quenching in leaf discs from spinach. A linear relationship between quantum efficiency and the ratio of photochemical:nonphotochemical quenching coefficients is demonstrated over a wide range of conditions of light intensity and CO_2 concentration. The results are discussed with reference to a model relating fluorescence to PSII photochemistry (4) and the potential use of fluorescence measurements in estimating the rate of total photosynthetic electron transport in intact systems.

MATERIALS AND METHODS

Plant Material. *Spinacia oleracia* was grown hydroponically (21) in a glasshouse with supplemental lighting under a short day regime (11 h light, 13 h dark). Fully expanded leaves were excised, and leaf discs (14 mm diameter) were cut for use in the experiments in this report.

CO_2 Exchange. A custom-built IRGA (Analytical Develop-

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[2] Abbreviations: Q_A, primary electron acceptor of PSII; IRGA, infrared gas analyzer; Hz, Hertz (s^{-1}); *F*, steady state fluorescence signal; F_o, initial fluorescence; F_v, variable fluorescence; F_m, maximum total fluorescence; $(F_v)_s$, light saturated variable fluorescence; $(F_v)_m$, maximum variable fluorescence; q_Q, photochemical quenching coefficient; q_E, nonphotochemical quenching coefficient.

ment Co., Ltd., Hoddesdon, U.K.) was situated downstream from the assimilation chamber and the system was operated in the conventional open flow-through mode. The flow rate through the chamber was maintained at about 50 mL min^{-1}.

The assimilation chamber was constructed of Perspex with aluminum water jackets to provide cooling. The temperature was maintained at 20° to 21°C. The leaf disc was floated top side up on a thin film of distilled H_2O. Since the flushing gas was maintained at or near H_2O saturation, little or no water vapor concentration differential should have developed in the system so that no corrections for the presence of water vapor have been applied to the IRGA response.

Fluorescence Measurements. Chl fluorescence was studied using a PAM 101 Fluorometer (Heinz Walz, Effeltrich, FRG). The system provided a low intensity, pulsed measuring beam (peak wavelength, 650 nm) from a light-emitting diode at frequencies of 1.6 kHz or 100 kHz. The measuring beam intensities incident upon the leaf sample were 0.03 and 1.8 μmol photons m^{-2} s^{-1} at 1.6 kHz and 100 kHz, respectively. A four-armed fiber optic carried the measuring beam to the upper Perspex window of the chamber and also collected the fluorescence, supplied continuous white actinic light, and provided a white light saturation pulse. Further details of the system are provided elsewhere (6, 17).

The methods of Schreiber *et al.* (17) were employed to estimate q_Q and q_E. F_o was determined for the leaf sample at steady state after at least 10 min of darkness with the measuring beam frequency set at 1.6 kHz. F_v was that proportion of the observed F in excess of F_o that occurred when the actinic light source was on and the measuring beam frequency was set at 100 kHz (*i.e.* $F_v = F - F_o$). F_m was determined by supplying a saturating pulse of white light (7000 μmol photons m^{-2} s^{-1} for 0.7 s) to a replicate leaf sample that had been predarkened for several hours. A fully dark adapted sample was used to ensure that fluorescence quenching was totally relaxed. Thus, $(F_v)_m$ was equal to $F_m - F_o$. The F_m:F_o ratios for predarkened leaves were typically about 5. As shown elsewhere (2, 6, 10), the quantities of F_v, F_o, and $(F_v)_m$ are related by $F_v = (F_v)_m (1 - q_Q)(1 - q_E)$.

Under steady-state conditions of photosynthesis in the presence of the actinic light, the saturating pulse was superimposed upon the actinic light at 100 s intervals to transiently reduce Q_A, thus removing photochemical quenching. The result was an associated rise in F to a new level, F'. From this information another quantity termed $(F_v)_s$ was calculated such that $(F_v)_s = F' - F_o$. Fluorescence quenching coefficients were given by $q_Q = [(F_v)_s - F_v]/(F_v)_s$ and $q_E = [(F_v)_m - (F_v)_s]/(F_v)_m$. At a specified irradiance and $[CO_2]$, the leaf sample was allowed to achieve steady state conditions of photosynthesis and fluorescence output. Mean values for quenching coefficients were determined based on fluorescence changes associated with three successive saturating pulses as described above.

F_o is frequently suppressed after a period of intense illumination (F_o'). An associated quenching coefficient $q_o = 1 - F_o'/F_o$ has been defined by Bilger and Schreiber (2). Since accurate estimates of q_E and q_Q must take fluctuations in the level of this basal fluorescence intensity into account, F_o' was determined in the experiments described. The leaf sample was provided with continuous far-red background illumination (Schott RG715 filter) at an intensity of 178 μmol photons m^{-2} s^{-1}. The actinic illumination was briefly (2–3 s) interrupted during an experiment and F_o' was recorded at 1.6 kHz in the presence of the far-red illumination (23). Alternatively, F_o' could be estimated from the minimum in the transient of modulated fluorescence which occurred within 5 s of stopping the actinic illumination.

Miscellaneous. Irradiances of the white light (400–700 nm) sources were measured with a Li-Cor Quantum Meter (Lincoln, NB). The sensor was positioned flush with the center of the upper Perspex window. The irradiance of far-red illumination transmitted via the lower Perspex window was estimated with a quantum sensor specially adapted for use in this spectral region (Skye Instruments Ltd., Llandrindod Wells, UK).

RESULTS

For all of the results presented here the $[O_2]$ in the gas phase was 2%. We assume that under these conditions photorespiration is suppressed and that CO_2 provided externally is the only quantitatively significant terminal acceptor for photosynthetic electron flow (*i.e.* $4e^-$:CO_2).

Figures 1 and 2 describe changes in CO_2 uptake, q_Q, and q_E as irradiance and CO_2 are varied, respectively. At an external $[CO_2]$ of 392 μL/L the photosynthesis rate saturated at about 750 μmol photons m^{-2} s^{-1} (Fig. 1). Nevertheless, q_Q continued to decline linearly to a value of 0.5 at an irradiance of 1735 μmol photons m^{-2} s^{-1}. Thus, even when the irradiance was more than twice the saturating level, Q_A was only 50% reduced. As the $[CO_2]$ was increased to saturating levels at two irradiances (Fig. 2) q_Q also increased, indicating that Q_A became more oxidized as the availability of acceptor increased. Nevertheless, the value of q_Q did not significantly exceed 0.5 at 1735 μmol photons m^{-2} s^{-1}. At

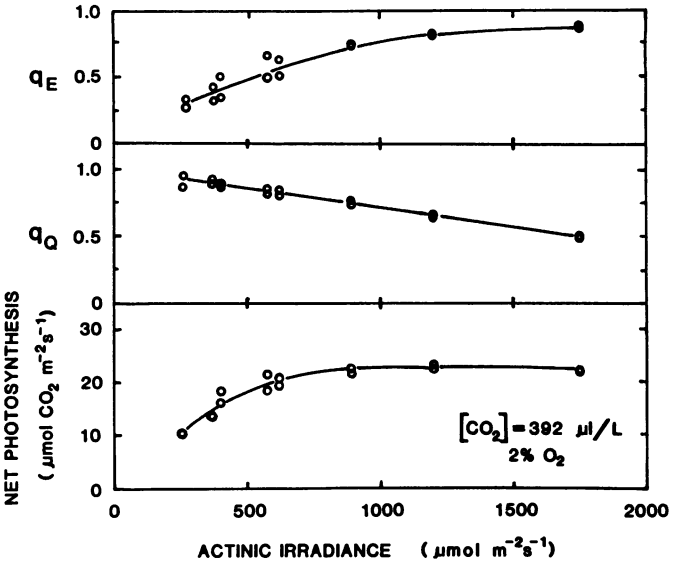

FIG. 1. Relationship among net photosynthetic rate, q_Q, and q_E as the actinic irradiance is varied for leaf tissue from spinach. Experimental conditions are noted in the figure and in "Materials and Methods."

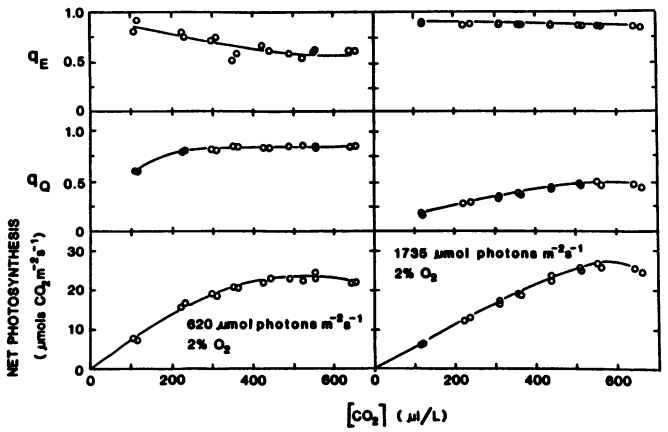

FIG. 2. Relationship among net photosynthetic rate, q_Q, and q_E as the CO_2 concentration is varied at two levels of actinic irradiance.

620 μmol photons m^{-2} s^{-1}, q_Q remained relatively constant at 0.8 at all but the lowest [CO_2] examined.

The relationship between the quantum effectiveness of CO_2 fixation (Φ_s in mols CO_2 mol photons^{-1}) and q_Q for the data of Figures 1 and 2 is presented in Figure 3. Quantum efficiency (Φ_s) is defined as the ratio of the rate of CO_2 uptake:actinic irradiance. Clearly, over the range of conditions examined, Φ_s varied nonlinearly with q_Q. A single curvilinear relationship accounted for 96% of the variation in Φ_s and q_Q regardless of whether irradiance or [CO_2] was varied. An even stronger empirical correlation was observed when Φ_s was plotted *versus* q_Q/q_E for values of the latter < 1.6 (Fig. 4). Values of q_Q/q_E in excess of 1.6 were predominantly recorded at the lower irradiances. This relationship among photosynthetic efficiency, q_Q, and q_E was examined further in the context of the model for PSII fluorescence and photochemistry set forth by Butler and Kitajima (4) (see inset to Fig. 5, top).

Weis and Berry (23) have proposed that effects of energy-dependent quenching of excitation (q_E) alone can best be examined by expressing the PSII quantum efficiency on the basis of the fraction of PSII centers in the open configuration. Thus, the quantum efficiency of open centers (Φ_p) is given by $\Phi_p = \Phi_s/q_Q$.

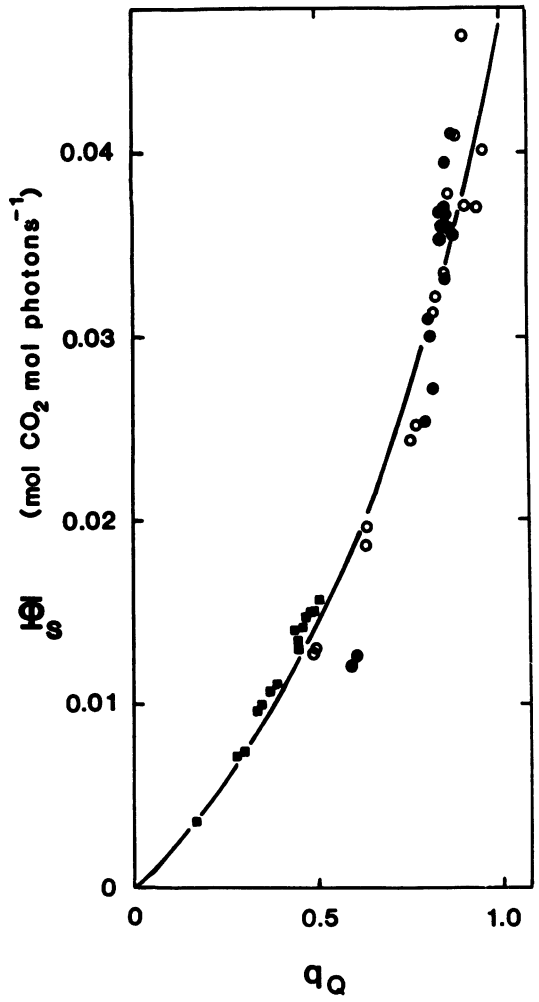

FIG. 3. Relationship between photosynthetic efficiency (*i.e.* Φ_s = CO_2 uptake rate: incident actinic irradiance) and q_Q for the data in Figures 1 and 2. The various symbols refer to: (○), [CO_2] = 392 μL L^{-1}, irradiance varied; (●), irradiance = 620 μmol photons m^{-2} s^{-1}, [CO_2] varied; (■), irradiance = 1735 μmol photons m^{-2} s^{-1}, [CO_2] varied. The solid line is a regression fit to the equation $Y = K[X/(2\text{-}X)]^a$ where a = 1.0621, K = 0.04665, and the correlation coefficient = 0.980, P < 0.001.

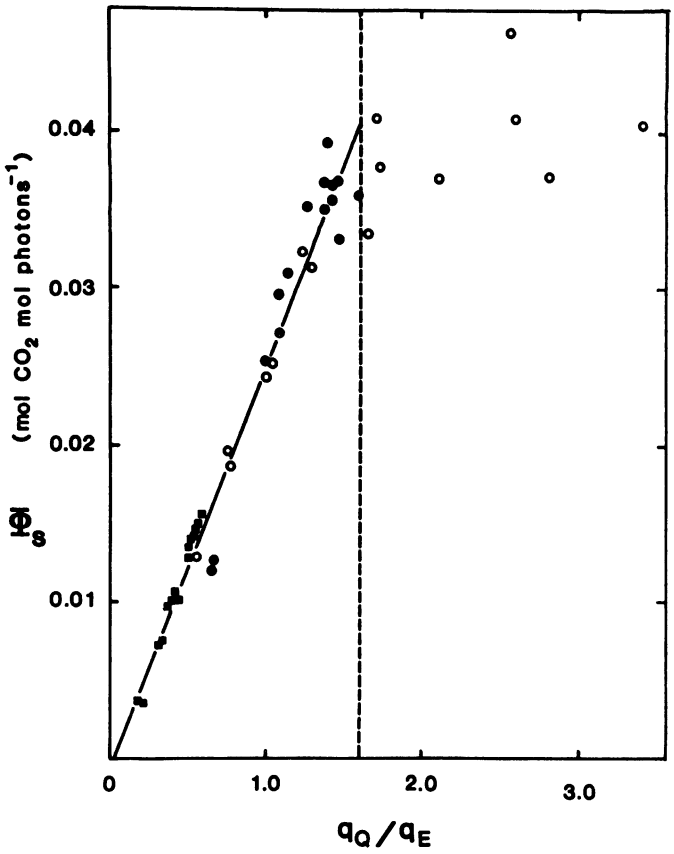

FIG. 4. Relationship between photosynthetic efficiency (Φ_s) and q_Q/q_E for the data in Figures 1 and 2. The symbols are defined in the legend to Figure 3. The solid line is a regression fit to the equation $Y = KX + b$ for the ordered pairs associated with q_Q/q_E values of less than 1.6 (dashed line). The correlation coefficient was 0.985 (P < 0.001), $K = 2.59 \times 10^{-2}$, and $b = -6.63 \times 10^{-4}$.

According to the aforementioned model (4), the yield of fluorescence (Φ_F) and the yield of photochemistry (Φ_p) are given by the following:

$$\Phi_F = \frac{k_F}{k_F + k_D + k_T}\left[\frac{1}{1 - \Psi_T\Psi_{tc}}\right] \quad (1)$$

$$\Phi_p = \Psi_T\left[\frac{\Psi_p}{1 - \Psi_{to}}\right] \quad (2)$$

All fluorescence is assumed to originate from the antennae Chl. The rate constants for fluorescence (k_F), thermal deactivation (k_D), and transfer to a PSII reaction center (k_T) refer to processes occurring in the antennae complex (4). The rate constants k_d and k_p refer to processes of thermal deactivation and photochemistry, respectively, at the reaction center. The variables Ψ_T, Ψ_{tc}, Ψ_{to}, and Ψ_p are the probabilities for (a) transfer of a quantum of excitation from the antennae Chl to a PSII reaction center, (b) backtransfer from a closed (Ψ_{tc}, *i.e.* $k_p = 0$) or an open reaction center (Ψ_{to}) to the antennae, and (c) photochemistry, respectively. In terms of the rate constants, the probability terms are given by $\Psi_T = k_T/(k_T + k_D + k_F)$, $\Psi_t = k_t/(k_t + k_d + k_p)$, and $\Psi_p = k_p/(k_p + k_d + k_t)$.

The normalized values of Φ_p calculated from the data of Figures 1 and 2 are plotted *versus* q_E in Figure 5 (top). Using reasonable values for the rate constants (see Ref. 23 and legend to Fig. 5), the predicted relationship between normalized yields of photochemistry (Φ_p') and fluorescence ($\Phi_F' = 1 - q_E$ when $k_p = 0$) are shown for two values of k_t. This was accomplished by

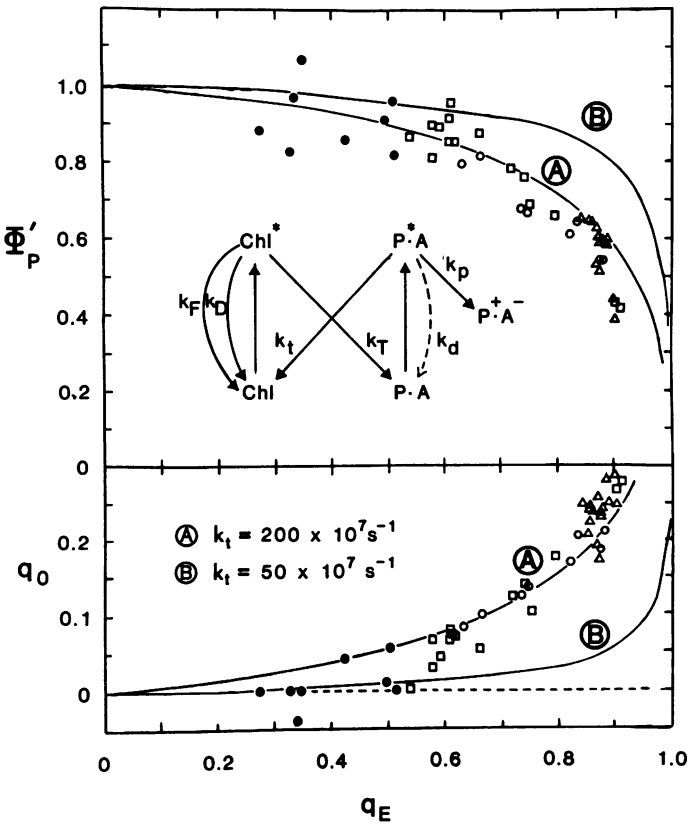

FIG. 5. Analysis of the results in Figures 1 and 2 with respect to the photochemical model of PSII proposed by Butler and Kitajima (4) (inset, top panel). The elements P and A of the model refer to the reaction center Chl of PSII and the primary electron acceptor, respectively. The rate constants for the model are described in the text. The symbols refer to results from Figure 1 (○, ●) and Figure 2 [(□), irradiance = 620 μmol photons m^{-2} s^{-1}; (△), irradiance = 1735 μmol photons m^{-2} s^{-1}]. Solid symbols (●) are differentiated from open symbols (○) based on the method employed (see below) to estimate the fluorescence level associated with open PSII centers (F_o') which is, in turn, used to calculate q_Q. In the top panel, $\Phi_p = \Phi_s/q_Q$ and the results were collectively normalized such that a value of Φ_p = 0.048 mol CO_2 mol photons^{-1} (estimated from the mean of the four highest values of Φ_p) corresponds to Φ_p' = 1.00. The solid lines are predicted dependencies of Φ_p' on q_E (=1-Φ_F') when k_d was varied from 0 to 250 × 10^7 s^{-1} in Equations 1 and 2 for the two values of k_t shown. Other values for the rate constants were: k_F = 2.0 × 10^7 s^{-1}, k_D = 10 × 10^7 s^{-1}, k_T = 144 × 10^7 s^{-1}, and k_p = 100 × 10^7 s^{-1}. The lower panel compares predicted and observed variation in q_o with q_E (*i.e.* all PSII reaction centers open) for the same values of the rate constants as indicated for the top panel. The q_o values (open symbols) were estimated from the steady state fluorescence observed during brief intervals of far-red illumination only. The solid symbols are values of q_o based on examination of light-dark fluorescence transients. See text for further information.

varying k_d (the rate constant for thermal dissipatation of excitation at the reaction center) from 0 (*i.e.* Φ_F' = 1) to 250 × 10^7 s^{-1} (Φ_F' = 0.0183 when k_t = 200 × 10^7 s^{-1}). With allowance for random experimental error, curve A in Figure 5 represents a satisfactory fit to the data. Note that the rate constants employed predict a F_m:F_o ratio of 5 (see Eq. 7 of Ref. 4). An F_m:F_o ratio of 5 was consistently observed with spinach in these experiments.

The four data points situated at the extreme right in Figure 5 and associated with q_E = 0.9 were obtained at 2% O_2 and a chamber [CO_2] of ~100 μL/L. The occurrence of some photorespiration under these conditions cannot be ruled out due to the comparatively high gas phase O_2:CO_2 (15). Thus, the associated Φ_p' values may be somewhat underestimated. These data points also appear as the two outlying values (●) shown in each of Figures 3 and 4.

A further test of the model is provided by analysis of the effect of changes in q_E upon suppression of the initial fluorescence level in the absence of actinic light (q_o), as shown in Figure 5 (bottom). The value of F_o is given by Equation 1 when $k_d > 0$ and assuming that the PSII centers are open (k_p = 100 × 10^7 s^{-1}). If k_d = 0 (*i.e.* q_E = 0) then F_o' = F_o. Thus, the coefficient relating F_o and F_o' (*i.e.* q_o) was calculated over the range of k_d values mentioned above. Again, when k_t = 200 × 10^7 s^{-1}, a satisfactory agreement was obtained between predicted and observed effects of q_E and q_o.

Values of F_o' were determined during a brief interruption of actinic illumination in the presence of far-red illumination which should preferentially excite PSI (23). Thus, Q_A was assumed to become rapidly photooxidized with the consequent conversion of the PSII centers to the open state. The occasional observation of anomalous negative q_o values was generally associated with q_E < 0.5 at actinic irradiances ≤ 620 μE m^{-2} s^{-1} (not shown). These are best explained by a finite degree of excitation of PSII due to slight transmission of shorter wavelength (*i.e.* <700 nm) light by the RG715 filter and weak far-red absorption by PSII resulting in a low (~3%) steady state level of reduction of Q_A. This interpretation was supported by examining transient changes in fluorescence accompanying interruption of actinic light in the absence of the far-red background illumination as in Bilger and Schreiber (2). The q_o values estimated from the minimum in these transients were (with one exception) positive and were substantially greater than those estimated during brief far-red illumination for q_E values less than 0.5. When q_E > 0.5, values of q_o were similar regardless of whether the estimates were based on steady state fluorescence during far-red illumination or examination of light-dark transients. Though the effects of the alternative means of estimating F_o' on q_o could in some cases be large, the effects on calculated values of q_Q and q_E were small (<3%) in these experiments.

The magnitude of the estimates of Φ_p shown in Figure 5 (top) warrants comment. The maximal value observed, 0.051 mol CO_2 mol photons^{-1}, is somewhat lower than expected. This is most likely due to loss of actinic radiation through the transport walls of the assimilation chamber combined with reflection and transmission losses by the leaf sample. Note that the maximum rate of 27 μmol CO_2 m^{-2} s^{-1} at high [CO_2] and irradiance is quite acceptable for spinach (Fig. 2).

DISCUSSION

There is growing evidence that energy-dependent quenching of fluorescense is a manifestation of a regulatory mechanism designed to match the rate of photosynthetic electron transport to the ability of the Calvin cycle to utilize the products NADPH and ATP (6, 7, 10, 22, 23). The purpose of the regulatory mechanism is to ensure that adequate rates of electron transport are maintained without leading to harmful side reactions associated with overreduction of electron transport components in the thylakoid membrane. This is accomplished by controlling the magnitude of the rate constant for harmless thermal dissipation of excitation at the PSII reaction center (k_d). The mechanism by which changes in q_E are related to changes in k_d is uncertain, but evidence has been presented implicating displacement of Mg^{2+} ions by protons at the inner thylakoid membrane (13). This would result in a conformational change in the membrane with a consequent change in k_d. Lack of a strict association of q_E with ΔpH has been reported, however (14).

An important observation concerns the relationship among quantum efficiency, q_Q, and q_E. Over a wide range of photosyn-

thetic rates, the function relating quantum efficiency and q_Q/q_E is the same whether the irradiance or [CO_2] is varied (Fig. 4). Thus, variations in the level of excitation or availability of terminal electron acceptors ultimately affect PSII photochemistry (a) through their influence on the redox state of Q_A and (b) by changes in k_d or by means equivalent to this (see below). This lack of interaction between availability of excitation or electron acceptor could prove to be of practical benefit by establishing a basis for estimation of total photosynthetic electron transport rate under conditions when CO_2 provided externally is not the sole electron acceptor. At elevated [O_2] both photorespiratory CO_2 and O_2 act as terminal acceptors, in addition to external CO_2. Recent reports (5, 18) have discussed the possible occurrence of electron transport of O_2 mediated by the violaxanthin cycle. The quantitative contribution of such a pathway to total electron flow together with its regulation remains uncertain, however. Thus, fluorescence, in conjunction with gas exchange measurements, could provide a new means of study of regulation of electron transport associated with collective dissipative processes such as photorespiration and pseudocyclic electron flow.

Application of fluorescence as an effective indicator of quantum efficiency may be limited to conditions when $q_Q \leq 0.85$ (Fig. 3). At q_Q values above 0.85, energy-dependent regulation of PSII activity is considererably diminished. This is apparent in Figure 4 where quantum efficiency estimates associated with high photochemical quenching ($q_Q \geq 0.85$) and $q_Q/q_E \geq 1.6$ deviated substantially from the highly linear relationship that applies when $q_Q/q_E < 1.6$. The points associated with q_Q/q_E values above 1.6 in Figure 4 were all taken from experiments performed at a [CO_2] of 392 μL/L and irradiances $\leq$ 620 μmol photons m^{-2} s^{-1}.

The kinetic model for PSII fluorescence and photochemistry presented by Butler and Kitajima (4) (Fig. 5, top, inset) predicts a nonlinear relationship between quantum efficiency and q_E such that only at q_E values substantially higher than 0.5 does photochemical efficiency decline significantly. The predictions of this model as well as the observed results are consistent with the proposed protective effect of energy-dependent quenching on the photosynthetic apparatus. At high q_Q values, certainly Q_A and most likely all other components of the intersystem electron transport chain, such as plastoquinone, are relatively oxidized, and the potential for harmful processes associated with overreduction are slight. The low but significant q_E values observed under such conditions arise because thermal deactivation (*i.e.* k_d) at the reaction center still competes effectively with deactivation processes occurring the antennae Chl (*i.e.* k_F and k_D). However, thermal deactivation competes poorly with the process of photochemistry (*i.e.* $k_d/k_p < 0.1$). There is no reason to suggest that Φ_p' and q_o (Fig. 5) should not vary independently based on assumptions inherent in the methodology and formulae used in their calculation (6, 17). Since the same set of rate constants satisfactorily explains the dependencies of both Φ_p' and q_o with q_E, the probability of a coincidental fit of predicted and observed results seems low, thus affirming the applicability of the model.

The model simulations appearing in Figure 5 were generated using the rate constants listed by Weis and Berry (23) with the exception of a significantly higher value (200×10^7 s^{-1} *versus* 50×10^7 s^{-1}) for the rate constant for excitation backtransfer (k_t) for curve A. The effect of altering the value of k_t from 200×10^7 s^{-1} (curve A) to 50×10^7 s^{-1} (curve B) is substantial in terms of the fit to observed changes in both Φ_p' and q_o. Although the data in Figure 5 do not permit an accurate estimate of k_t, the magnitude of k_t is, at least, comparable to that of the rate constant for the forward process (k_T). This is contrary to the expected function of the reaction center as an energy trap yet is supported by independent estimates of these rate constants based on fluorescence decay profiles obtained with chloroplasts and algae (1).

The possible role of transfer of excitation directly from PSII to nonfluorescent PSI should be considered as a possible contributor to observed q_E values. A growing body of evidence suggests that cation (*i.e.* Mg^{2+}) induced changes in thylakoid membrane organization and phosphorylation of the PSII Chl *a/b* light harvesting complex (*i.e.* state 1/state 2 transitions) can regulate such an energy transfer (7, 12). If, as some studies suggest (8, 13), such a PSII $\rightarrow$ PSI transfer were to occur at the level of the antennae Chl an equal diminution of both F_v and F_o would be expected. In these experiments and others (2, 9, 22, 23), F_v is more strongly affected than F_o indicating an important role for the PSII reaction center (*i.e.* k_d) in regulating fluorescence yield. As pointed out in (4), however, excitation transfer from the PSII reaction center to PSI would mimic an increase in k_d. Nevertheless, Horton and Hague (9) have reported that the proportion of total nonphotochemical quenching due to PSII $\rightarrow$ PSI excitation transfer is comparatively small at all but very low irradiance levels (<100 μmol photons m^{-2} s^{-1}) in protoplasts from barley. Furthermore, a reduction in quantum efficiency due to photoinhibition is unlikely in these experiments since net photosynthetic rates were consistently high and no time-dependent loss of activity was apparent even at the highest irradiance employed (1735 μmol photons m^{-2} s^{-1}, see Figs. 1 and 2). We thus assume that ΔpH-dependent quenching is the main component of nonphotochemical quenching in this report.

Weis and Berry (23) have investigated the relationships between quantum yield of CO_2 fixation and q_E in leaves of sunflower and bean. They obtained a linear decline in Φ_p' versus q_E over a range of q_E values similar to that investigated here. The results were not directly compatible with the bipartite model of Butler and Kitajima (4). The discrepancy was reconciled by postulating that PSII centers exist in two interconvertible forms (normal [$PSII_o$] and quenched [$PSII_e$]), which respectively possess significantly different values of k_d. It is unclear presently how these units correspond to the kinetically distinct $PSII_\alpha$ and $PSII_\beta$ units that may be involved in regulation of transfer of energy from PSII to PSI (1).

In contrast to results reported by Weis and Berry (23), the results observed in the experiments presented here with spinach (Fig. 5; see also Ref. 9) suggest a nonlinear relationship between Φ_p' and q_E similar to that predicted in (4). Our results could thus be accounted for without assuming PSII heterogeneity. Nevertheless, unequivocal support for either PSII model (*i.e.* heterogeneous or homogeneous) is not provided by existing evidence. For instance, significant transfer of excitation among heterogeneous PSII units could result in nonlinear plots of Φ_p' *versus* q_E (E Weis, personal communication). Differences between our observations and those of Weis and Berry (23) may reflect the different species investigated or growth conditions employed. The results presented here and previously (9, 10, 23) are consistent, however, with the proposal that thylakoid membrane energization is an important and rapidly reversible mechanism regulating utilization of radiant energy by PSII.

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