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End product feedback effects on photosynthetic electron transport

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Abstract

The inhibition of photosynthetic electron transport when starch and sucrose synthesis limit the overall rate of photosynthesis was studied in *Phaseolus vulgaris* L. and *Xanthium strumarium* L. The starch and sucrose limitation was established by reducing photorespiration by manipulation of the partial pressure of O_2 and CO_2. Chlorophyll *a* fluorescence quenching, the redox state of Photosystem I (estimated by the redox status of NADP-dependent malate dehydrogenase), and the intermediates of the xanthophyll cycle were investigated. Non-photochemical fluorescence quenching increased, NADP-dependent malate dehydrogenase remained at 100% activity, and the amount of violaxanthin decreased when starch and sucrose synthesis limited photosynthesis. In addition, O_2-induced feedback caused a decrease in photochemical quenching. These results are consistent with a downward regulation of photosynthetic electron transport during end product feedback on photosynthesis. When leaves were held in high CO_2 for 4 hours, the efficiency of Photosystem II was reduced when subsequently measured under low light. The results indicate that the quantum efficiency of open Photosystem II centers was reduced by the 4 hour treatment. We interpret the results to indicate that feedback from starch and sucrose synthesis on photosynthetic electron transport stimulates mechanisms for dissipating excess light energy but that these mechanisms do not completely protect leaves from long-term inhibition of photosynthetic electron transport capacity.

Abbreviations: MDH – malate dehydrogenase; $p(CO_2)$ – partial pressure of CO_2; $p(O_2)$ – partial pressure of O_2; PFD – photon flux density; PS I-Photosystem I; PS II – Photosystem II; q_N – non-photochemical quenching; q_P – photochemical quenching; RuBP – ribulose-1,5-bisphosphate; rubisco – RuBP carboxylase/oxygenase; F_o – initial fluorescence yield of dark-adapted leaves; F_m – maximal fluorescence yield of dark-adapted leaves

Introduction

Photosynthesis is a complex process which requires light-driven electron transport, carbon reduction cycle reactions, and end product (primarily starch and sucrose) synthesis in strict stoichiometries (Woodrow and Berry 1988). The exact stoichiometries vary with conditions; for example, when photorespiration decreases, relatively less photosynthetic electron transport but more end product synthesis is required. Presumably, there exist mechanisms for adjusting the rates of the component processes of photosynthesis so that electron transport, carbon fixation, and starch and sucrose synthesis occur at the required rate. In addition to changes in gene expression which undoubtably occur, some of these mechanisms for adjustment must operate

over short time scales. It has been hypothesized that decarbamylation of rubisco is the primary mechanism used to adjust rubisco activity when end product synthesis limits the overall rate of photosynthesis (Sharkey 1990). The mechanism which adjusts electron transport when end product synthesis limits the rate of photosynthesis is not known.

Various methods have been developed to study the interaction between carbon metabolism and photosynthetic electron transport (Schreiber et al. 1986, Weis and Berry 1987, Scheibe and Stitt 1988, Horton 1989, Krause and Weis 1991). When more light energy and electron transport capacity is available than can be used by the carbon metabolism of photosynthesis, the excess light energy is dissipated. This dissipation is associated with high levels of non-photochemical quenching of chlorophyll fluorescence, and may involve the xanthophyll cycle in which violaxanthin is converted to antheraxanthin and then zeaxanthin (Demmig-Adams 1990).

The restriction of photosynthetic electron transport imposed by the lack of CO_2 has been well studied (e.g. Dietz et al. 1985, Weis and Berry 1987). However, less is known about the limitation of photosynthetic electron transport when end product synthesis limits photosynthesis (cf. Sharkey et al. 1988). End product synthesis limitation has been induced most often by establishing a high rate of photosynthesis under high light and high $p(CO_2)$, then switching to non-photorespiratory conditions. This is often accomplished by reducing the $p(O_2)$ but can also be accomplished by increasing the $p(CO_2)$. Often photosynthesis is not stimulated by the inhibition of photorespiration and this is interpreted as feedback on the rate of photosynthesis by end product formation, primarily starch and sucrose synthesis. This limitation has been called the feedback limitation of photosynthesis (Sharkey 1990). When leaves are switched to feedback conditions, the phosphate level in the stroma falls (Sharkey and Vanderveer 1989) and the ATP/ADP ratio drops (Sharkey et al. 1986a).

In this report we address several unanswered questions about the feedback from end product synthesis on photosynthetic electron transport. First, is feedback induced by switching to low $p(O_2)$ the same as feedback induced by switching to high $p(CO_2)$, which has the same effect of suppressing photorespiration (Sharkey 1988)? Second, what changes occur in chlorophyll fluorescence quenching upon switching to feedback induced by O_2 versus feedback induced by CO_2? Third, is it possible to determine the mechanism of the feedback on electron transport? Finally, does feedback for extended periods result in a long-term reduction in the electron transport capacity of a leaf. We addressed these questions with two series of experiments which we report here.

Materials and methods

Protocol

Two series of experiments were carried out. In the first series, feedback was induced by switching from 500 μbar CO_2, 210 mbar O_2, and 1000 μmol $m^{-2} s^{-1}$ PFD to either 14 mbar O_2 (O_2-induced feedback) or 1600 μbar CO_2 (CO_2-induced feedback). In effect we put leaves just barely into feedback limited conditions, then reduced the rate of photorespiration by switching gas conditions to increase the degree of feedback suffered by the leaves.

Two controls were used. Non-feedback controls were switched from 350 μbar CO_2 and 14 mbar O_2 to 350 μbar CO_2 and 210 mbar O_2 at 1000 μmol $m^{-2} s^{-1}$. In effect the leaves were held in what we suspected were non-feedback conditions, then the rate of photorespiration was increased by changing gas conditions. If photosynthesis was reduced, we could be sure that photosynthesis was not feedback limited. Low light controls were held in 100 μmol photons $m^{-2} s^{-1}$. Samples were taken for enzyme analysis and violaxanthin/zeaxanthin measurements 20 min after steady-state photosynthesis was established following the switch to feedback or non-feedback conditions or after 20 min in low light.

In the second series of experiments single leaves were enclosed in the gas-exchange cuvette and dark-adapted for 20 min. After determination of F_o, the PFD was increased until photosynthesis was light saturated at 350 μbar $p(CO_2)$. This occurred at different light inten-

sities, depending upon the leaf. Maintaining light at saturating intensity, the $p(CO_2)$ in the air was increased to 500 μbar then changed from 500 to 1200 μbar or more. In all cases increasing the $p(CO_2)$ from 500 to 1200 μbar did not stimulate the rate of CO_2 assimilation. Two to three data points were collected while photosynthesis was CO_2-saturated. Photosynthesis with 20 mbar $p(O_2)$ in the air was measured at ambient and at saturating $p(CO_2)$.

The treatment was carried out exposing leaves to just-saturating light intensity and 1500 μbar $p(CO_2)$ for 4 h. Controls were maintained 4 h at the same saturating light intensity but in ambient $p(CO_2)$ (350 μbar). After the treatment, plants were dark-adapted for 20 min and F_o and F_m post-treatment were measured. The light response and CO_2 response were then remeasured. All measurements were made on at least 3 different plants.

Two leaves of *Phaseolus vulgaris* were maintained in the cuvette in ambient $p(CO_2)$ and in dark conditions for 24 h after the treatment. At the end of this recovery, photosynthesis and fluorescence parameters were measured at ambient $p(CO_2)$ and at the light intensity saturating photosynthesis.

Gas exchange measurements

Gas exchange for the first (short-term) series of experiments was measured as described in Vassey and Sharkey (1989). The rate of RuBP utilization was calculated according to von Caemmerer and Farquhar (1981) and Sharkey (1988) using values for the ratio of photorespiration to photosynthesis given in Brooks and Farquhar (1985) and assuming the $p(CO_2)$ in the chloroplast was 60% of ambient and the rate of non-photorespiratory respirations was 0.5 μmol $m^{-2} s^{-1}$. The PFD was 1000 μmol $m^{-2} s^{-1}$ for all experiments except that labelled low light, which was done under 100 μmol photons $m^{-2} s^{-1}$ PFD.

For the second series (long-term) of experiments we used the system described by Loreto and Sharkey (1990) with the following exceptions. The aluminum cuvette in which leaves were enclosed had a 130 cm^2 glass window in the top side. The cuvette was ventilated by two fans to reduce boundary layer resistances. The $p(CO_2)$ before and after the cuvette was measured with a LiCor 6262 infrared gas analyzer. Leaf temperature was maintained at 23 °C ± 0.5 °C. A 2.5-kW xenon-arc lamp was used as the light source. Light intensity at the leaf surface was varied by interposing neutral density screens. The apparent quantum yield of photosynthesis was calculated by dividing the photosynthetic rate by the light intensity at which it was obtained as in Cornic and Briantais (1991) or in Sharkey et al. (1988). The equations of von Caemmerer and Farquhar (1981) were used to calculate the photosynthetic parameters.

Fluorescence measurements

In the description of the fluorescence measurements, we adopted the nomenclature proposed by van Kooten and Snel (1990). Chlorophyll fluorescence was measured with a Heinz Walz PAM 101 fluorometer equipped with a polyfurcated light guide (Schreiber et al. 1986). Leaves were maintained in total darkness for 20 min before the initial fluorescence (F_o) was measured. The leaves were then illuminated for 1 s with a flash of 3500 μmol $m^{-2} s^{-1}$ using a Schott LK1500 light source. After the fluorescence had recovered to the original level, a second flash of 5000 μmol $m^{-2} s^{-1}$ was given. The maximum fluorescence is a function of the inverse of the flash intensity and so extrapolation of the peak height versus the inverse of the flash intensity to 0 gives the peak height at infinite flash intensity (Markgraf and Berry 1990). The value recorded was considered to be the fluorescence intensity with all the PS II centers closed and in the non-energized state (F_m). The leaves were then illuminated with an actinic light source supplying 1000 μmol photons $m^{-2} s^{-1}$. After determining the fluorescence intensity at steady-state (F_s), two flashes were again given and the peak height at infinite PFD was estimated (the actinic light source was kept on during these high intensity pulses). Under these conditions, the fluorescence observed was that with all the PS II reaction centers closed and in an energized state (F'_m). After measuring F'_m, all lights were switched off and replaced by a 3 s illumination with a KL1500 light source with an RG9 filter

(passing > 740 nm) in place. This caused PS I to oxidize all PS II and was necessary to obtain an accurate measurement of fluorescence intensity with the reaction centers open in the energized state (F'_o). The empirical fluorescence quenching parameters q_P and q_N were calculated as described by Weis and Berry (1987). Considering the new nomenclature, these calculations were as follows:

$$q_P = 1 - (F_s - F'_o)/(F'_o) \text{ and}$$

$$q_N = 1 - (F'_m - F'_o) .$$

These parameters may not correspond exactly to photochemical and non-photochemical processes (Havaux et al. 1991) but are still useful indicators for comparing photochemical and non-photochemical deactivation of PS II.

The quantum yield of electron transport was determined as in Genty et al. (1989). Electron transport rate was calculated from $\Delta F/F$ ($\Delta F/F = (F_m - F_s)/F_m$) and gas-exchange measurements as in Harley et al. (1992).

PS I redox state

PS I redox state was estimated by measuring the activation of NADP-dependent malate dehydrogenase (NADP-MDH) (Scheibe and Stitt 1988). The activity of NADP-MDH was measured in a crude extract of freeze-clamped leaves, the extract was then reduced by bringing the concentration of dithiothreitol (DTT) to 130 mM for 1 h the activity of the reduced enzyme assayed. Measured rates of NADP-MDH activity were corrected for the limited ability of cytosolic NAD-MDH to utilize NADP (Scheibe and Stitt 1988). The extraction medium was 50 mM Bicine pH 7.8, 5 mM $MgCl_2$, 5 mM DTT, 1 mM EDTA, and 1.5% polyvinylpolypyrollidone. One ml extraction medium was used for 5.5 cm^2 leaf material. The assay was conducted in one ml of 100 mM tris-HCl pH 8, 2 mM oxaloacetate, 1 mM EDTA, 0.2 mM NADPH, and 50 μl extract. The change in absorbance was followed in a dual wavelength filter photometer (Sigma ZFP 22) at 334–405 nm. For NAD-MDH activity, the NADPH was replaced by NADH.

Pigment extraction and separation

After reaching steady-state photosynthesis under the initial gas conditions, some leaves were freeze-clamped for assays of NADP-dependent malate dehydrogenase activity. A similar set of leaves was treated in the same manner 20 min after reaching steady-state photosynthesis under the new gas compositions. Freeze-clamped leaves were extracted in acetone (5.5 cm^2 per 2 ml). A 10 μl aliquot was chromatographed on a C18 reversed phase HPLC column (5 μm Ultrasphere 4.6 × 250 mm). The separation was accomplished with a two-solvent system, solvent A was acetonitrile : MeOH (3 : 1), solvent B was water (Humbeck et al. 1989). Initial flow rate was 0.9 ml min^{-1}, the initial composition was 10% B which was reduced to 3.5% over 25 min. The flow rate was then changed to 2 ml min^{-1} and the composition changed to 100% A over 7 min. Elution was then isocratic for 5 min to clean the column. Peaks were identified by retention times and absorption spectra (Humbeck et al. 1989). Satisfactory separation of lutein and zeaxanthin could not be achieved and so we tried not to separate them and report only lutein plus zeaxanthin and assume that the amount of lutein remained constant (Demmig et al. 1987).

Plant material

Plants of *Phaseolus vulgaris* L. var. Linden were grown in a growth chamber in 4 liter pots containing a soil : peat : perlite : rice hull (3 : 3 : 3 : 2) mix. Plants were grown under a 12 h photoperiod with 24/17 °C day/night temperature, 60% RH with a photon flux density of 500 μmol $m^{-2} s^{-1}$. The plants were fertilized 5 times per week with Hoagland's solution B (Hoagland and Arnon 1939). The plants were four to six weeks old at the time of the measurements. For the long-term measurements we used *Phaseolus vulgaris* L. var. Linden and *Xanthium strumarium* L. planted in a commercial potting mixture (Metro-mix 350 W.R. Grace Co.). Plants were grown in two growth chambers (Conviron E15) under 16-h daylength and air temperature of 23/16 °C (day/night). Light intensity at the canopy level varied with plant age and height between 700 and 1100 μmol $m^{-2} s^{-1}$. Plants

were watered daily with half strength Hoagland's solution. Terminal, fully mature, light exposed leaves only were used during the experiments.

Results

Short-term experiments

When $p(CO_2)$ was 500 μbar and $p(O_2)$ was 210 mbar, neither reducing the $p(O_2)$ to 14 mbar nor increasing the $p(CO_2)$ to 1600 μbar substantially increased the rate of assimilation, even though photorespiration would have been suppressed in both cases (Table 1). The reduction in the rate of RuBP use upon changing the gas composition to feedback conditions was greater for the O_2-induced feedback than for the CO_2-induced feedback (Table 1).

Under all conditions, quenching of chlorophyll *a* fluorescence was high, ranging from 0.88 at a $p(CO_2)$ of 500 μbar and $p(O_2)$ of 210 mbar to 0.95 under low light. At high light, q_P ranged between 0.41 and 0.59 and q_N varied from 0.72 to 0.85. At low light q_N was zero. There was no distinct pattern among the absolute values of fluorescence quenching and the composition of the air to which the leaves were exposed. However, there were consistent changes in the components of chlorophyll fluorescence quenching upon changing the $p(O_2)$ or $p(CO_2)$ (Table 2). On switching from non-photorespiratory to photorespiratory conditions (non-feedback) the decline in assimilation was accompanied by a decrease in q_N and an increase in q_P, consistent with the increase in the use of RuBP. Feedback induced by low $p(O_2)$ resulted in a decline in q_P as RuBP use declined, and an increase in q_N. Feedback induced by very high $p(CO_2)$ was similarly accompanied by an increase in q_N but there was almost no effect on q_P.

NADP-MDH was fully activated under all conditions at 1000 μmol photons $m^{-2} s^{-1}$ (Table 3), indicating that feedback-limited photosynthesis did not change the degree of reduction of PS I. At 100 μmol photons $m^{-2} s^{-1}$, NADP-MDH was largely inactivated indicating that the

Table 2. Changes (Δ) in chlorophyll fluorescence quenching parameters in response to the changes in gas composition given in Table 1. All non-zero numbers are significantly different from zero at the 5% level of confidence except Δq_N of the CO_2-induced feedback which is significant at the 7% level. The quenching parameters are not additive so that $\Delta q_P + \Delta q_N \neq \Delta q$

	Δq	Δq_P	Δq_N
Non-feedback	0.00	0.14	−0.06
O_2-induced feedback	0.01	−0.14	0.08
CO_2-induced feedback	0.01	0.01	0.03

Table 3. NADP-dependent malate dehydrogenase activation following non-feedback, feedback, and low light treatments. Activation was calculated as initial activity divided by activity in DTT treated extract multiplied by 100. Values followed by different letters are significantly different at the 5% level of confidence as tested by the Tukey multiple range test

	Activation
Condition	%
Non-feedback	99[a]
O_2-induced feedback	101[a]
CO_2-induced feedback	105[a]
Low light	22[b]

Table 1. Gas partial pressures used in experiments reported here and photosynthetic CO_2 assimilation (A). The PFD was 1000 μmol $m^{-2} s^{-1}$ and leaf temperature was 25 °C. The CO_2 values are the partial pressures in the air outside the leaves. Initial conditions were maintained for 20 min or longer and final conditions were maintained for an additional 20 min. Each value is the average of 5 leaves. The rate of RuBP usage, R, was calculated from the rate of photosynthesis using equations in Sharkey (1988) assuming that the $p(CO_2)$ in the chloroplast was 60% of ambient and that the rate of mitochondrial respiration was 1 μmol $m^{-2} s^{-1}$. The CO_2 and O_2 experiments were done different weeks and the plants had different rates of photosynthesis

	Initial condition				Final condition			
	CO_2	O_2	A	R	CO_2	O_2	A	R
	μbar	mbar	μmol $m^{-2} s^{-1}$		μbar	mbar	μmol $m^{-2} s^{-1}$	
Non-feedback	350	14	16.4 ± 1.9	17.6	350	210	13.5 ± 1.6	24.7
O_2-induced feedback	500	210	14.0 ± 1.6	21.8	500	14	13.9 ± 1.6	14.8
CO_2-induced feedback	500	210	18.6 ± 1.3	28.7	1600	210	19.1 ± 1.0	22.4
Low light	350	210	3.0 ± 0.4	6.2				

method does detect when PS I is not fully reduced. Light intensity appeared to be the major factor affecting the redox state of PS I.

Twenty minutes after leaves had reached steady-state photosynthesis under the final conditions given in Table 1, they were freeze-clamped and assayed for pigment composition. The status of the xanthophyll cycle was estimated as the ratio of lutein + zeaxanthin to violaxanthin (Table 4). High light induced the conversion of violaxanthin to zeaxanthin, and this conversion was enhanced under feedback-limited conditions. The increase in q_N under these conditions may be related to the increase in zeaxanthin.

Table 4. Lutein (L) + zeaxanthin (Z) as a proportion of violaxanthin (V). Values followed by different letters are significantly different at the 5% level of confidence as tested by the Tukey multiple range test

	(L + Z)/V
Condition	
Non-feedback	7.2[a]
O_2-induced feedback	11.7[b]
CO_2-induced feedback	9.8[b]
Low light	3.6[c]

Long-term experiments

After leaves had been held in 1500 μbar CO_2 and saturating light for 4 h, the rate of photosynthesis was reduced at nearly all light levels (Fig. 1). Control plants maintained for 4 h in saturating light but at ambient $p(CO_2)$ did not show any change of photosynthesis as a result of the treatment (Fig. 1). Plants maintained for 4 h in 1500 μbar CO_2 but low light also showed no effect (data not shown).

The response of photosynthesis to CO_2 was

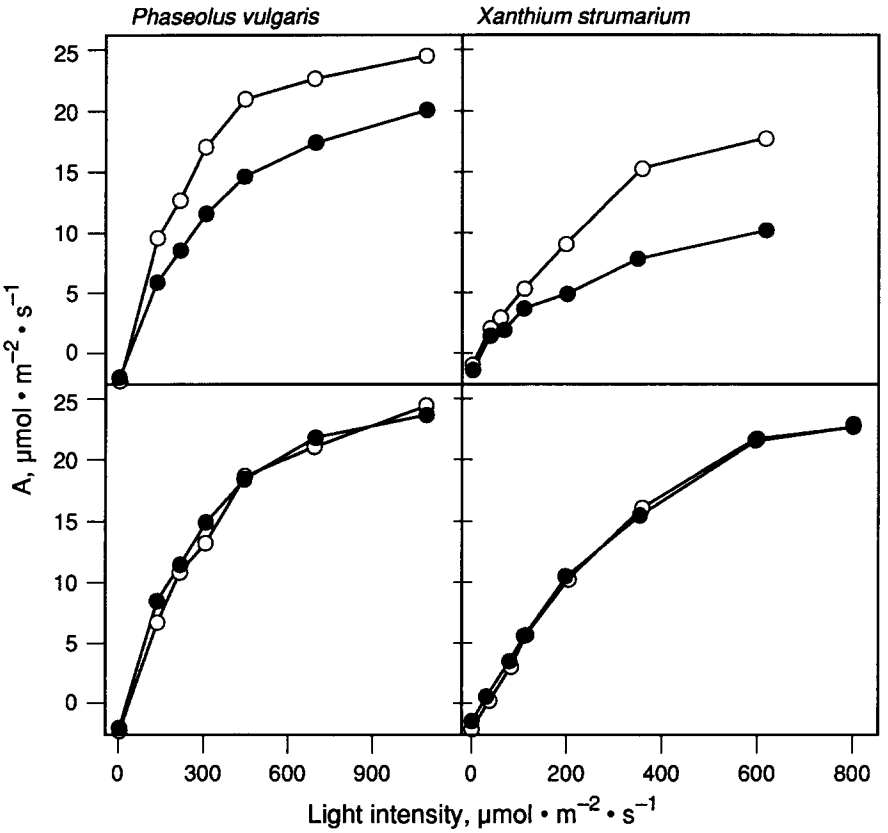

Fig. 1. Light response of photosynthesis (A) of *Phaseolus vulgaris* and *Xanthium strumarium*. Open circles = light response before the treatment; closed circles = light response after the treatment. During the treatment, leaves were maintained 4 h at 1500 μbar CO_2 partial pressure and at saturating light intensity. Other conditions are described in the text. The top 2 panels show data from plants held at 1500 μbar and just saturating PFD. While the lower panels show data from leaves held at the same light but only 350 μbar CO_2.

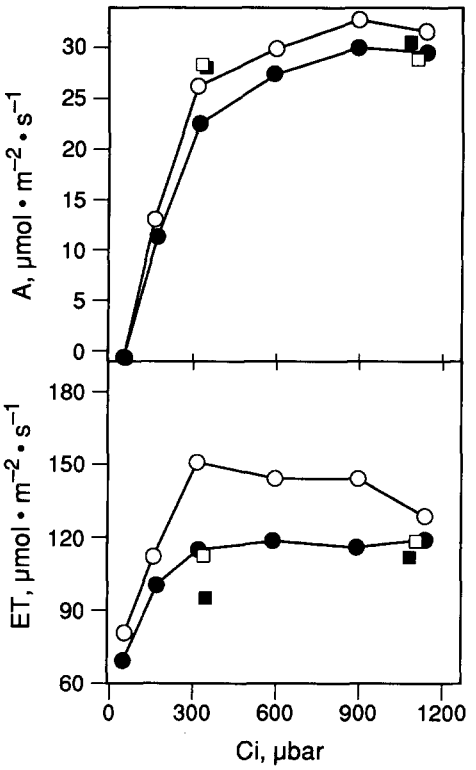

Fig. 2. Photosynthesis (A) and photosynthetic electron transport (ET) rates as a function of intercellular $p(CO_2)$ (C_i). Open circles = CO_2 response before the treatment. Closed circles = light response after the treatment. The squares are data obtained at low $p(O_2)$. During the treatment, leaves were maintained 4 h at 1500 μbar CO_2 partial pressure and at saturating light intensity. Electron transport rates were calculated from chlorophyll fluorescence data.

only slightly modified by the treatment in high $p(CO_2)$ (Fig. 2). Photosynthesis was insensitive to $p(O_2)$ before the treatment but, when assayed at 300 μbar, some O_2 sensitivity was observed following 4 h at high $p(CO_2)$ (Fig. 2). The electron transport rate, calculated by fluorescence and gas-exchange parameters, slowly declined at $p(CO_2)$ higher than ambient before treatment while it was lower and steady over the range of $p(CO_2)$ higher than ambient after the treatment (Fig. 2).

The initial fluorescence, F_o, increased after the treatment ($P < 0.05$), while F_m decreased (data not shown). As a consequence, a strong reduction of the ratio between variable fluorescence and F_m was observed following the treatment. Similarly, the quantum yield of electron transport, $\Delta F/F$, and the apparent quantum yield of

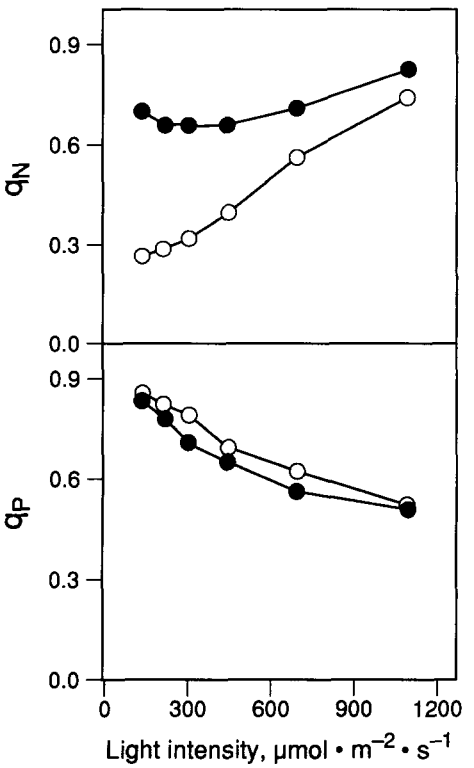

Fig. 3. Non-photochemical, q_N, and photochemical, q_P quenching of chlorophyll fluorescence in a leaf of *Phaseolus vulgaris* L. Before (open circles) and after (closed circles) the treatment.

Table 5. Changes in photosynthetic rate (A) and fluorescence parameters following a 4-h treatment at 15 μbar of CO_2 partial pressure and at a light intensity saturating photosynthesis (1000 μmol m^{-2} s^{-1}). Recovery from the treatment occurred in the dark and at ambient CO_2

Parameter	Before	1 h recovery	24 h recovery
A (μmol m^{-2} s^{-1})	13.5	10.4	13.8
F_o (arbitrary units)	12	13	12
F_m (arbitrary units)	54	40	47
F_v/F_m (unitless)	0.78	0.67	0.74
q_N (unitless)	0.59	0.77	0.64

photosynthesis were reduced following the treatment (data not shown).

The non-photochemical quenching of fluorescence, q_N, dramatically increased after the treatment. The increase was especially noticeable at low light intensity (Fig. 3). On the other hand, q_P was similar before and after the treatment (Fig. 3). No changes were observed in any of the chlorophyll fluorescence parameters in control plants (data not shown).

After 24-h recovery, the photosynthetic rate and F_o also recovered to the prestress levels. However, F_m, the ratio of F_v/F_m, and the non-photochemical quenching of fluorescence did not fully recover (Table 5).

Discussion

At moderately high $p(CO_2)$ (500 μbar), both a decrease in $p(O_2)$ and an increase in $p(CO_2)$ had little stimulatory effect on net CO_2 assimilation, even though these conditions reduce photorespiration. The lack of response of photosynthesis to these two gases has been interpreted to indicate that photosynthesis is limited by the rate of triose phosphate utilization (Sharkey 1990). The rate of carbon fixation and reduction, and of photosynthetic electron transport, must be regulated to keep pace with end-product utilization. Decarbamylation of ribulose-1,5-bisphosphate carboxylase has been demonstrated under both O_2- and CO_2-induced feedback (Sharkey et al. 1986a and Sage et al. 1988, respectively). Electron transport has been shown to be lower under low $p(O_2)$ than under normal $p(O_2)$, and this was associated with a higher q_N under reduced oxygen (Sharkey et al. 1988). The results presented here confirm that under O_2-induced feedback there is an increase in non-radiative energy dissipation, in keeping with a down-regulation of photosynthetic electron transport under these conditions. A similar reduction in the rate of RuBP utilization and an increase in q_N during CO_2-induced feedback suggest a similar down-regulation of electron transport under these conditions as well.

In these experiments q_N increased regardless of the mechanism by which feedback was imposed; however, photochemical quenching decreased only when feedback was induced by switching to low $p(O_2)$, not when feedback was induced by high $p(CO_2)$. The difference between CO_2- and O_2-induced feedback could be simply a difference in the degree of feedback induced (Table 1), or could reflect a direct effect of O_2 on chlorophyll fluorescence-quenching mechanisms (Schreiber and Neubauer 1990). The increase in non-photochemical quenching is generally regarded as a regulatory change (Horton 1989, Schreiber and Neubauer 1990).

Two possible mechanisms of the feedback imposed in these experiments on electron transport have been proposed. First, low stromal phosphate levels during feedback-limited photosynthesis (Sharkey and Vanderveer 1989) could inhibit the coupling factor, leading to large transmembrane pH gradients which restrict electron flow (Kobayashi et al. 1979). This type of regulation has been reviewed by Horton (1989) and Schreiber and Neubauer (1990). Second, limited ATP supply under feedback limitation (Sharkey et al. 1986b) could reduce the availability of the electron acceptor in the photosynthetic carbon reduction cycle (Rao et al. 1986, Furbank et al. 1987, Laisk et al. 1991). Under both O_2- and CO_2-induced feedback conditions, NADP-dependent malate dehydrogenase remained fully reduced. In this case the photosynthetic control was not great enough to cause PS I to become oxidized as has been seen in other experiments (Quick et al. 1989). Since the enzyme was fully reduced as far as we could measure before switching to feedback conditions, it was not possible to determine if reduction of PS I contributed to the inhibition of electron transport under feedback conditions.

One of the mechanisms contributing to non-photochemical dissipation of light energy involves the formation of zeaxanthin from violaxanthin (Demmig et al. 1987, Demmig-Adams, 1990). In the experiments reported here there were higher levels of zeaxanthin under high than low light. On switching to feedback limited conditions where there was a reduction in electron flow and an increase in q_N, there was a further conversion of violaxanthin to zeaxanthin indicating a regulatory response to the feedback resulting in enhanced non-photochemical dissipation of light energy (Noctor et al. 1991).

Despite the regulatory changes reducing PS II activity, 4 h of feedback conditions caused a persistent reduction in the capacity of the leaves for photosynthesis (Fig. 1). After 24 h nearly all of the effects of feedback conditions were alleviated. It is difficult to interpret this persistent reduction in capacity as either adaptive or maladaptive for the plant.

The rate of electron transport in leaves before the 4-h treatment was highest at 300 μbar C_i and declined with increasing C_i. Following the 4-h treatment at higher CO_2, the rate of electron transport was constant with increasing C_i above 300 μbar (at the level which had occurred at the highest measured C_i before the treatment). We interpret these results to indicate that the photosynthetic electron transport capacity, unused during feedback, was lost over 4 h. This interpretation is consistent with the low O_2 results as well (Fig. 2). The interpretation that electron transport capacity in excess of the needs of carbon metabolism is lost through light-dependent mechanisms is not new (Horton 1989). However, this phenomenon has never before been demonstrated by *increasing* the CO_2 level around a leaf.

The results in Fig. 2 indicate that feedback-limited photosynthesis will rarely be found under natural conditions. Whenever feedback might occur, the capacity for electron transport will be reduced in a relatively short time so that electron transport capacity will appear to be limiting even though the capacity for starch and sucrose synthesis originally set the maximum rate of photosynthesis.

In conclusion, we believe these results indicate that feedback from starch and sucrose synthesis on photosynthetic electron transport increases non-photochemical quenching, in part by causing violaxanthin conversion to zeaxanthin. Following 4 h of feedback, a persistent reduction in the capacity for electron transport can be demonstrated.

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