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Relationship between Photosynthetic Electron Transport and Stromal Enzyme Activity in Pea Leaves[1]

Toward an Understanding of the Nature of Photosynthetic Control

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ABSTRACT

The responses of the quantum efficiencies of photosystem (PS) II and PSI measured *in vivo* simultaneously with estimations of the activities and activation states of NADP-malate dehydrogenase, chloroplast fructose-1,6-bisphosphatase, and ribulose-1,5-bisphosphate carboxylase were used to study the relationship between electron transport and carbon metabolism. The effects of varying irradiance and CO_2 partial pressure on the relationship between the quantum efficiencies of PSI and II, and the activity of these enzymes shows that the interrelationships vary according to the limitations placed on the system. The relationship between the quantum efficiencies of PSII and PSI was linear in most situations. In response to increasing irradiance, the activity of all three enzymes increased. In the case of NADP-malate dehydrogenase this increase was well correlated with the estimated flux of electrons through PSI and PSII. The other two enzymes showed a more complex relationship with the estimated flux of electrons through both photosystems. These relationships are consistent with the known interactions between these stromal enzymes and the thylakoids. The response to varying CO_2 partial pressure is more complex. The efficiencies of PSI and II declined with decreasing CO_2 partial pressure and the activity of each enzyme varied uniquely. However, there are clear correlations between the activities of the enzymes and the flux of electrons through the photosystems. In contrast to the data obtained under conditions of varying irradiance, there is clear evidence of photosynthetic control of electron transport when the CO_2 concentration is varied.

A knowledge of the relationships between the activities of PSII and PSI, the redox state of the stroma and the activation states of the light-regulated enzymes of the Benson-Calvin cycle is necessary, if not fundamental, for the understanding of photosynthetic electron transport and photosynthetic control. It is clear that electron transport can only operate efficiently when the supply of NADP, ADP, and P_i is nonlimiting and the composite parameter of the redox and phosphorylation potentials, termed assimilatory power or assimilatory force, is low (9). Assimilatory force is kept low when reduction and assimilation of CO_2 by the Benson-Calvin cycle is rapid. This can only occur when the CO_2 is in adequate supply and the light-modulated enzymes of the Benson-Calvin cycle are activated (9). Light modulation of NADP-MDH occurs principally by reversible thiol reduction *via* the ferredoxin-thioredoxin system (3). (A complete list of abbreviations used in this paper can be found in Table I.) These redox-sensitive components respond to both the supply of electrons from PSI and also to their utilization in metabolism. For this reason it has been suggested that the activation state of NADP-MDH is a useful "metabolic indicator" of the redox state of the stroma (24). In addition, since direct measurement of the NADP/NADPH system must be viewed with extreme caution the measurement of the activation state of NADP-MDH may provide a more accurate physiological method of measurement of the relevant reduction state of the stroma. The thiol-modulated enzymes of the Benson-Calvin cycle, such as fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase, show a more complicated response to prevailing conditions in the stroma because of cooperative activation by reductant, pH, and substrate (13, 29, 30). It has been suggested that FBP and thioredoxin act sequentially to activate FBPase (12, 13). That substrate is required for activation *in vivo* has been demonstrated via work with isolated intact chloroplasts (13) where, in contrast to NADP-MDH, which showed significant light activation in the presence or absence of oxygen, FBPase was inactive in anaerobic conditions. Activation of the latter enzyme was stimulated in this situation by dihydroxyacetone phosphate which enters the chloroplast via the phosphate translocator and is converted into the substrate FBP by the reactions of the Benson-Calvin cycle (13).

The relationship between the quantum efficiencies of PSI

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Table I. *Abbreviations Used in This Paper*

NADP-MDH	= NADP-malate dehydrogenase
ϕ_I	= quantum efficiency of PSI
ϕ_{II}	= quantum efficiency of PSII
ϕ_{exc}	= quantum efficiency of photochemical quenching by oxidized reaction centres of PSII
J_I	= product of ϕ_I and irradiance
J_{II}	= product of ϕ_{II} and irradiance
q_Q	= coefficient for the photochemical quenching of Chl *a* fluorescence
Fo	= level of modulated Chl *a* fluorescence during a dark interval following a brief irradiation by far-red light to oxidize any Q_A
Fm	= level of modulated Chl *a* fluorescence during a saturating pulse of irradiance
Fs	= level of modulated Chl *a* fluorescence at steady state
FBP	= fructose-1,6-bisphosphate
FBPase	= fructose-1,6-bisphosphatase
P_Q	= plastoquinone
Q_A	= the primary electron acceptor to PSII
RuBP carboxylase	= ribulose-1,5-bisphosphate carboxylase

and II, and the quantum efficiency of CO_2 fixation has been described (5, 6, 28). These results have shown that there is a tight coupling between the quantum efficiencies of both photosystems, and that when photorespiration is suppressed there is a good correlation between the efficiency of either photosystem and the quantum yield of CO_2 fixation. However, the relationship between the quantum efficiencies of the photosystems and the activation states of stromal enzymes is largely unexplored.

Previous observations suggest that though the reduction state of the acceptor sides of both photosystems are not simply related, there is an overall positive correlation between them (24). It also appears that in air the acceptor pool of PSI is not over-reduced, except temporarily, for example during sudden increases of irradiance (7, 8, 28). The enzymes measured in the present study give information on the availability of reducing equivalents in the stroma (via the activation state of NADP-MDH) and the capacity for catalysis by the Benson-Calvin cycle (FBPase and RuBP carboxylase). Estimations of enzyme activity were made simultaneously with those of the efficiencies of PSII and PSI to provide new insight into the coordinate control of thylakoid function and carbon metabolism.

MATERIALS AND METHODS

Plant Material

All measurements were made on young, fully developed pea leaves (*Pisum sativum*) variety "Finale." Plants were grown hydroponically in pots in a glasshouse under natural light during the months of February and March 1989.

Biophysical Measurements

Chl fluorescence and light-induced absorbance changes at 820 nm (ΔA_{820}), due to P-700 oxidation or reduction, were measured within a leaf area of 1.25 cm^2, defined by a circular mask as described previously (7, 8). The ΔA_{820} corresponding to complete oxidation of P-700 in the area irradiated was obtained by first irradiating the leaf in air with 660 nm radiation at 1000 μmol $m^{-2} \cdot s^{-1}$ photosynthetically active quantum flux (PAQF) for 200 to 300 s to activate the Benson-Calvin cycle and allow regeneration of the NADP pool. The leaf was then irradiated with saturating far-red light obtained by filtering the radiation output from a 200 W Quartz Halogen bulb with a Schott RG715, and Balzers NIR and red dichroic DT filters. The resulting absorbance change was taken to correspond to complete oxidation of the P-700 pool.

Individual leaves were enclosed in the photometry equipment and exposed to one of a range of irradiances in air, or to a constant irradiance (735 μmol $m^{-2} \cdot s^{-1}$) in a gaseous phase consisting of a CO_2 concentration of between 28 and 400 pm in a 2% O_2 and N_2 background. The irradiance was obtained from an array of light emitting diodes (H2K peak emission 660 nm [Stanley, Tokyo, Japan]) and was interrupted for 10 s every 120 s to allow the ΔA_{820} and Fo to be determined. In all treatments each leaf was maintained at a constant irradiance and in a stable gaseous phase until the ΔA_{820} had stabilized. In this instance, the ΔA_{820} measurement was chosen as a monitor because of its good signal to noise ratio and its simplicity of application under the conditions of these experiments, which generally resulted in a substantial oxidation of P-700 and therefore a relatively large value of ΔA_{820}. Once the ΔA_{820} had stabilized measurements of Chl *a* fluorescence (Fo, Fss, Fsat) and the steady-state rapid light induced absorbance change at 820 nm were made (6). The portion of the lamina that had been irradiated was immediately frozen and cut from the leaf using a solid brass cutter chilled to liquid nitrogen temperatures. This operation required the interruption of the irradiation of the leaf but the leaf was frozen within 5 s of this interruption. We estimate that less than 10% of the activity measured at any time is lost during these extraction procedures.

The ϕ_I, the redox state of PSII (q_Q), the ϕ_{exc} (that is the

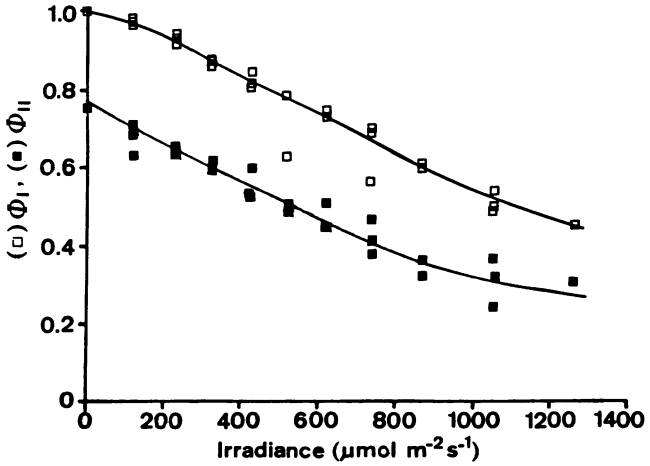

Figure 1. Relationships between ϕ_I (□), ϕ_{II} (■), and irradiance for pea leaves in air (20% O_2, 350 ppm CO_2, approximately 80%, N_2).

photochemical efficiency of open PSII centers), and the ϕ_{II} were calculated as described previously (4, 6).

Enzyme Assays

NADP-MDH, FBPase, and RuBP carboxylase activities were measured in extracts of leaf discs in which metabolism had been stopped by freezing followed by immersion in liquid N_2. The leaves were pulverised in liquid N_2 and the leaf powder was resuspended either in 0.1 M Tricine-KOH buffer (pH 8.0) containing 1 mM dithiothreitol, 10 mM $MgCl_2$, 1 mM EDTA, and 0.1% Triton X-100 or, alternatively, the extraction buffer described by Scheibe and Stitt (24). The measured activition states of the enzymes were similar following extraction in either buffer. Activities were assayed immediately upon thawing. NADP-malate dehydrogenase was measured essentially as described in Scheibe *et al.* (23) by following the oxidation of NADP in a reaction mixture (1 mL) containing leaf extract (equivalent to 5 μg Chl), 0.2 mM NADPH, 0.5 mM oxaloacetate, 1 mM EDTA, 10 mM $MgCl_2$, and 0.1 M Tricine-KOH buffer (pH 8.0). Maximum activation was achieved by incubation of the extract with 100 mM dithiothreitol in 0.1 M Tricine-KOH buffer (pH 8.0) for 20 min at 25°C prior to assay.

The assay for FBPase was adapted from that of Leegood *et al.* (13). Activity was measured by the increase in absorbance at 340 nm in a reaction mixture (1 mL) containing 100 mM Tricine-KOH buffer (pH 8.0), 10 mM $MgCl_2$, 10 mM dithiothreitol, 0.2 mM NADP, 0.5 mM fructose-1,6-bisphosphate, and glucose phosphate isomerase (10 units mL^{-3}) and glucose 6-phosphate dehydrogenase (2.5 units mL^{-3}). Maximum activity was measured following incubation of the extract with 100 mM dithiothreitol, 2 mM fructose-1,6-bisphosphate, 10 mM $MgCl_2$, and 0.1 M Tricine-KOH buffer (pH 8.0) for 10 min at 25°C prior to assay. RuBP carboxylase activity was measured as described by Parry *et al.* (17).

RESULTS

Response of the ϕ_I and ϕ_{II} to Increasing Irradiance

Increasing irradiance in a gaseous phase comprising 350 ppm CO_2, 20% O_2, and balance N_2 caused a decline in the efficiencies of both photosystems (Fig. 1). In the case of the efficiency of ϕ_I the decline is only marked above 200 μmol $m^{-2} \cdot s^{-1}$. The data for ϕ_{II} show a monophasic decline with increasing irradiance. A comparison of the efficiencies of the photosystems (ϕ_{II} and ϕ_I, Fig. 2) shows a strong linear relationship. The relationship between ϕ_I and the excitation transfer efficiency of PSII (ϕ_{exc}) also shows a strong linear correlation. As ϕ_I declines q_Q also declines; in previous studies (5, 6) this relationship has been found to be curvilinear, however, in this instance the degree of scatter makes it impossible to determine the detail of the relationship between ϕ_I and q_Q. At values of ϕ_I close to 1.0 an abrupt decline of both q_Q and ϕ_{II} is implied because even at the lowest irradiance employed (120 μmol $m^{-2} \cdot s^{-1}$) q_Q and ϕ_{II} are lower than the values recorded for the dark adapted state.

Response of the Activities of NADP-MDH, FBPase, and RuBP Carboxylase to Increasing Irradiance

In darkness the activities of the thiol-modulated enzymes NADP-MDH and stromal FBPase were low (1–10% maximal activity). The activation state of FBPase rose sharply with increasing irradiance to a light intensity of approximately 400 μmol $m^{-2} \cdot s^{-1}$ after which the activation state was relatively constant with increasing irradiance. In contrast, the activities of RuBP carboxylase (Fig. 3) and NADP-MDH continued to increase with increasing irradiance. At 400 μmol $m^{-2} \cdot s^{-1}$ NADP-MDH was at approximately 20% maximum activation and FBPase was at approximately 80% of maximum activation under these conditions. RuBP carboxylase activity

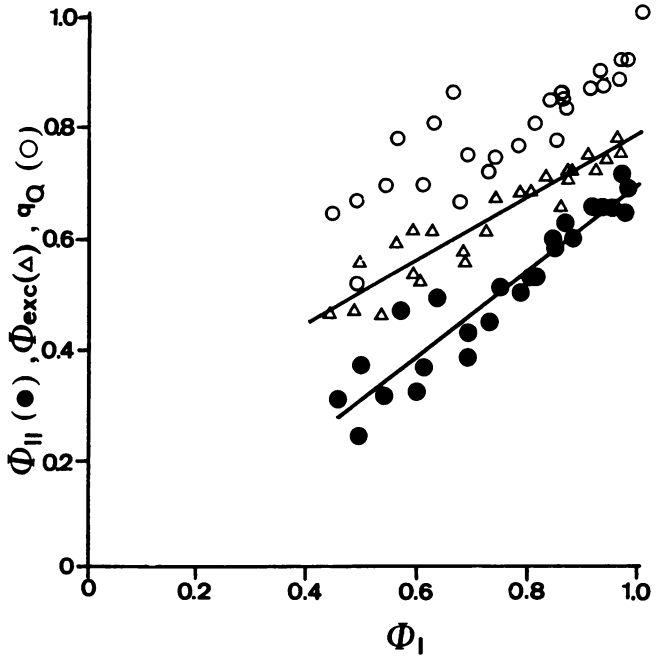

Figure 2. Interrelationships between ϕ_I and ϕ_{II} (●), ϕ_{exc} (△), and q_Q (○), in pea leaves in air over a range of irradiances. The square of the correlation coefficient between ϕ_I and ϕ_{II} is 0.90, between ϕ_{II} and ϕ_{exc} is also 0.90, and between ϕ_I and q_Q (not including the dark-adapted value for q_Q and ϕ_I) is 0.78.

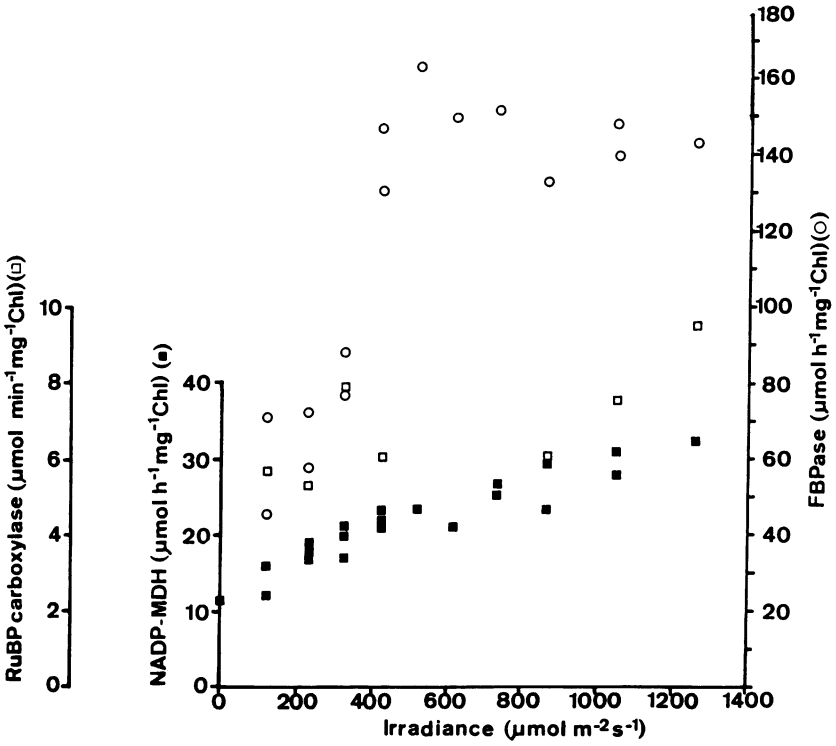

Figure 3. Irradiance dependence of FBPase (○), NADP-malate dehydrogenase (■), and RuBP carboxylase (□) from pea leaves in air. The maximum activity of NADP-MDH measured in extracts for similar leaves was 123 μmol h^{-1} mg^{-1} Chl and the maximum activity for RuBP carboxylase in this instance was 12 μmol min^{-1} mg^{-1} Chl.

increased gradually with increasing light intensity from approximately 44% activation at 100 μmol $m^{-2}\cdot s^{-1}$ to 75% activation at 1000 μmol $m^{-2}\cdot s^{-1}$.

The flux of reducing equivalents through PSI (J_I) is a function of the product of ϕ_I and irradiance. A similar argument applies to the product of ϕ_{II} and irradiance. The relationship between the activation state of NADP-MDH and J_I (Fig. 4) and J_{II} (Fig. 5) is linear. Even in complete darkness the enzyme is not totally inactive implying the existence of a pool of NADPH in the dark-adapted state. The linear relationship between J_I and J_{II} and NADP-MDH activation suggests that with the increasing flux of electrons through PSI and PSII the level of NADPH increases in proportion.

The relationship between J_{II} and the activation of FBPase (Fig. 6) is similar to that between NADP-MDH and J_I and J_{II}. With increasing values of J_{II} the activation of FBPase increases. However, in this instance complete activation of the enzyme occurs leading to a saturation of FBPase activity at the higher values of J_{II} (the data for J_I are similar [not shown]).

The activation of RuBP carboxylase, when related to J_{II} (Fig. 7), shows an increase with increasing J_{II} though this correlation is very weak.

Response of ϕ_{II} and ϕ_{II} to Decreasing CO_2 Partial Pressure

Under conditions of constant irradiance (750 μmol $m^{-2}\cdot s^{-1}$) and varying CO_2 concentrations (25–400 ppm) in a gas mixture which suppresses photorespiration the efficiencies of both photosystems declined with decreasing CO_2 partial pressure (Fig. 8). Whereas ϕ_{II} decreased to approximately zero at 0 ppm CO_2 (by extrapolation), ϕ_I displays more complex behavior. From 400 to 100 ppm CO_2 ϕ_I declined with decreasing CO_2 concentration, then at 100 ppm there is a divergence in the data (Fig. 8). Below 100 ppm some samples showed a continuation in the decline in ϕ_I that occurred above 100 ppm. Other samples indicate an apparent increase in ϕ_I with decreasing CO_2 concentration. A comparison of ϕ_{II} (Fig. 9) with ϕ_I suggests that for most samples the relationship between the efficiencies of both photosystems is linear. The exceptional samples are the three that do not lie on the principal line of correlation between ϕ_I and CO_2 partial pressure (Fig. 8). The

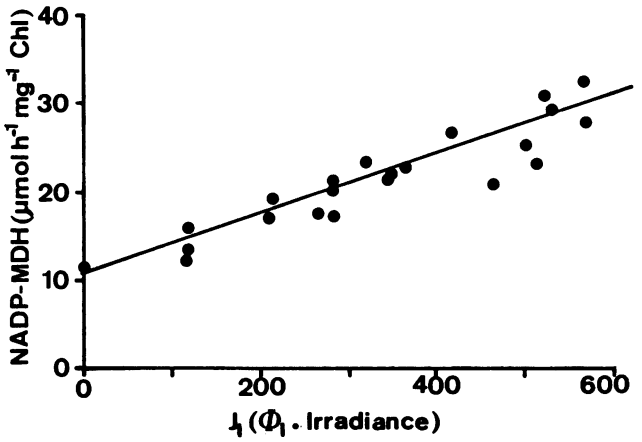

Figure 4. Relationship between J_I (the product of ϕ_I and irradiance) and the activity of NADP-MDH extracted from pea leaves subjected to a range of irradiances in air. The square of the correlation coefficient between J_I and NADP-MDH activity is 0.82.

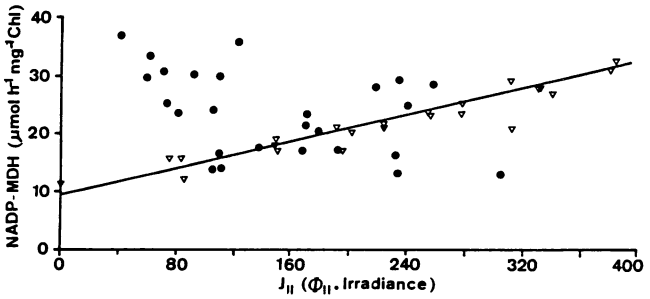

Figure 5. Relationship between NADP-malate dehydrogenase activity and J_{II} (the product of ϕ_{II} and irradiance) for pea leaves subjected to a range of irradiances in air (▽), or varying CO_2 concentrations in 2% O_2 in N_2 at constant irradiance (735 μmol m^{-2} s^{-1}) (●). The solid line is a regression line for the data obtained at varying irradiance.

linear relationship between ϕ_I and ϕ_{II} (Fig. 9) is qualitatively similar to that recorded in response to a range of irradiances (Fig. 2). The regression line through the data (excluding the three exceptional samples, see above) is, however, quantitatively different to that obtained from the irradiance experiment and implies that once ϕ_{II} is zero, ϕ_I will be about 0.27. This relationship between ϕ_{exc} and ϕ_I is similar to that obtained during the irradiance experiment (data not shown). At the highest values of ϕ_I recorded in this experiment (approximately 0.6) the values obtained for q_Q (approximately 0.8) overlap with those obtained during the irradiance experiment (Fig. 1). However, in the CO_2 experiment, as ϕ_I decreased the value of q_Q declined more sharply than during the irradiance experiment. It is this marked fall in q_Q that accounts for the relatively steeper fall in ϕ_{II} relative to ϕ_I in the experiment with varying CO_2 relative to the experiment with varying irradiance.

Effects of Varying CO_2 Concentration on the Activation of NADP-MDH, FBPase, and RuBP Carboxylase

The relationships between the activities of NADP-MDH and FBPase, and CO_2 concentration are shown in Figure 10, and the relationship between the activity of RuBP carboxylase and CO_2 concentration is shown in Figure 11. It is clear that because of scatter it is difficult to draw any conclusions concerning the dependency of the activation of any of these enzymes on CO_2 concentration. However, when considered in relation to an index of thylakoid electron flux, these data become more understandable as discussed below.

Relationship between Enzyme Activation States and J_{II} Under Conditions of Varying CO_2 Partial Pressure

The activation state of NADP-MDH, at low values of J_{II}, increased with decreasing J_{II} (Fig. 5). At high values of J_{II} (above about 140), the degree of scatter in the data prevented any conclusions regarding the detail of the relationship between J_{II} and NADP-MDH activation.

The activation of FBPase under a range CO_2 concentrations and constant irradiance increases with decreasing values of J_{II} below a value of about 140 (Fig. 6). With values of J_{II} above 140 the FBPase activation state increases with increasing values of J_{II}. The relationship between J_{II} and FBPase activation in the latter case is similar to that displayed between J_{II} and FBPase activation over a range of irradiances and constant CO_2 concentration (Fig. 6).

RuBP carboxylase activation shows a slight increase with increasing J_{II} (Fig. 7) over a range of CO_2 concentrations and constant irradiance. The levels of RuBP carboxylase activation, in terms of J_{II}, are higher under conditions of constant irradiance and varying CO_2 partial pressure than they are under conditions of varying irradiance and a gaseous phase comprising of 350 ppm CO_2, 21% O_2, and balance N_2.

To summarize with varying CO_2 concentrations the relationships between J_{II} and the activation of FBPase, and to a lesser extent NADP-MDH, at high values of J_{II} were similar to those obtained by changing the irradiance (under conditions where photorespiration can occur). At low values of J_{II} (corresponding to low CO_2 concentrations) the activities of both FBPase and NADP-MDH increased with decreasing J_{II} suggesting that a partial breakdown of the balance between the stroma and the thylakoids is offset by photosynthetic control of electron transport.

DISCUSSION

The responses of ϕ_I and ϕ_{II} to varying irradiance, and the relationships found between ϕ_{II}, ϕ_{exc}, and q_Q, with ϕ_I over a range of irradiances are similar to those reported by Harbinson *et al.* (6) and Genty *et al.* (5). The data show clearly the roles of both q_Q and ϕ_{exc} in determining ϕ_{II} (11) such that the fluxes of electrons through both photosystem are balanced. PSII also shows a fall in quantum efficiency at high values of ϕ_I without any corresponding fall in either ϕ_{exc} or ϕ_I. This fall in ϕ_{II} appears to be due to a drop in q_Q. Given the sequential arrangement of PSI and II in the electron transport chain it would have been expected that ϕ_I and ϕ_{II} would have reached

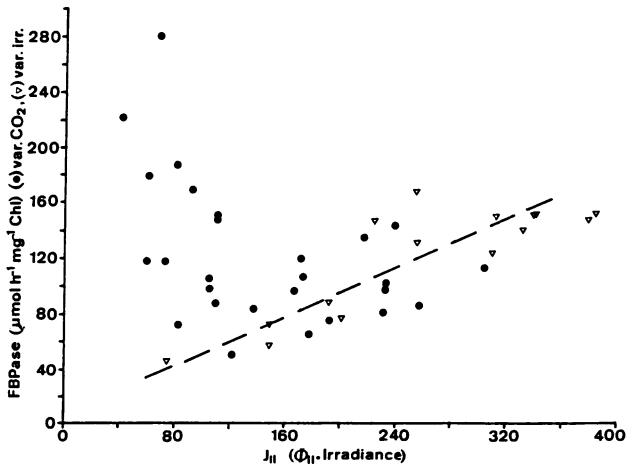

Figure 6. Relationship between FBPase and J_{II} (the product of ϕ_{II} and irradiance) from pea leaves subjected to varying irradiance in air (▽), or varying CO_2 concentration in 2% O_2 in N_2 at constant irradiance (735 μmol m^{-2} s^{-1}) (●). The solid line indicates a fit (by eye) to the data obtained at varying irradiance.

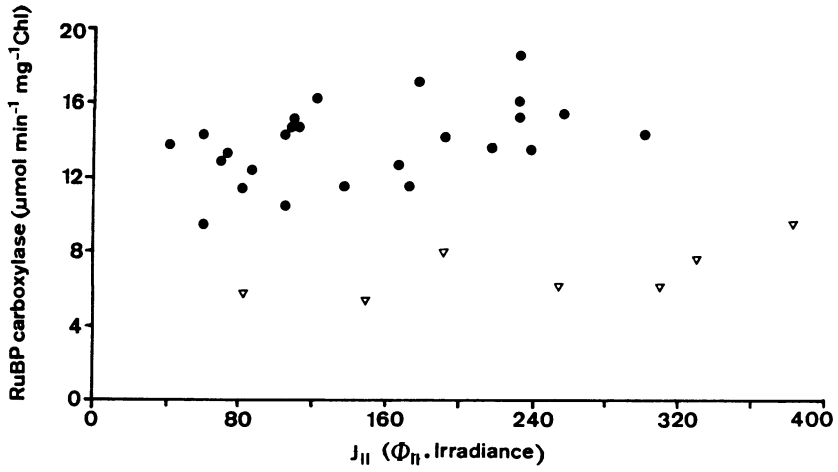

Figure 7. Relationship between RuBP carboxylase activity and J_{II} (the product of ϕ_{II} and irradiance) from pea leaves subjected to a range of irradiances in air (●) or to varying CO_2 concentration in 2% O_2 balance N_2 at an irradiance of 735 μmol m^{-2} s^{-1} (▽).

zero concurrently; however, this is not the case. This anomaly is more marked in the case of the data obtained over a range of CO_2 concentrations. In the latter case, the relationship between ϕ_{exc} and ϕ_I is similar to that obtained over a range of irradiances showing an underlying consistency in the material between the two types of experiment. The more marked fall in q_Q relative to ϕ_I in the experiment where CO_2 concentration was varied caused ϕ_{II} to decline to zero when ϕ_I was still 0.30. The failure of ϕ_I and ϕ_{II} to decline to zero concurrently cannot be explained in terms of an imprecision (caused by leaf optics) affecting the measuring systems for the two photosystems differently. Such an error would be expected to influence the linearity of the relationship between ϕ_I and ϕ_{II} only and not the intercept with the ordinate or abscissa. This short fall could be caused either by the presence of a cyclic flux of reducing equivalents around PSI or to a consistent failure to allow for the presence of PSI fluorescence in the measurement of Chl fluorescence. Both proposed mechanisms for cyclic electron flow (16) would result in competition between the cyclic path and the linear path for electron flow to plastoquinone. Therefore cyclic flow would cause a drop in q_Q and a fall in ϕ_{II} relative to ϕ_I. PSI fluorescence occurs in parallel with PSII fluorescence (see ref. 10) though its magnitude is relatively constant under conditions where PSII fluorescence yield changes considerably. By ignoring the small flux of PSI fluorescence and attributing all Chl fluorescence to PSII, an error is introduced which has a greater effect on Fo than either Fs or Fm, and therefore ϕ_{II} will be slightly underestimated. A cyclic flux of electrons around PSI could also explain the more rapid fall of ϕ_{II} against ϕ_I caused by decreasing CO_2 partial pressures, compared to the fall of ϕ_{II} produced by increased irradiance. Under conditions of constant irradiance and temperature, decreases in ϕ_{II} can be produced by an increase in the resistance for electron flow between the two photosystems. An increase in resistance will result in an increase in the pool of P-700$^+$ and normally a fall in both q_Q and ϕ_{exc} such that ϕ_{II} declines. An increase in the resistance for the electron flow between the two photosystems can be brought about by a fall in the intrathylakoid pH (27). The maintenance of a greater intrathylakoid proton concentration would require either a change in the H^+/e^- ratio for linear electron flow or in an increase in the cyclic flux around PSI, or both. An increase in the rate of proton pumping relative to linear electron transport is necessary because as the intrathylakoid proton concentration is increased the rate of passive proton leakage through the thylakoid membrane will also increase. The existence of an active cyclic path around PSI would also allow the retention of a relatively high ϕ_I at low CO_2 concentrations in the absence of significant photorespiration. Low CO_2 concentrations under these conditions would be expected to severely restrict noncyclic electron flow due to a failure to regenerate NADP. The efficiency of PSII declines toward zero as CO_2 partial pressures are reduced as would be expected given its predominant involvement in linear electron flow. However, until cyclic electron flow *in vivo* is unambig-

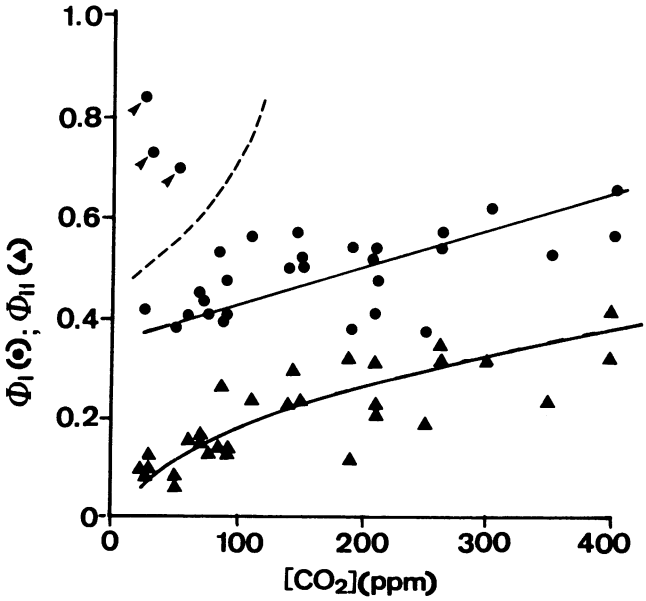

Figure 8. Relationship between CO_2 concentration in a background of 2% O_2 balance N_2 and ϕ_I (●) and ϕ_{II} (▲) for pea leaves at a constant irradiance (735 μmol m^{-2} s^{-1}). The data for ϕ_I marked with an arrowhead (∋) may be in error due to overreduction of the PSI acceptor pool.

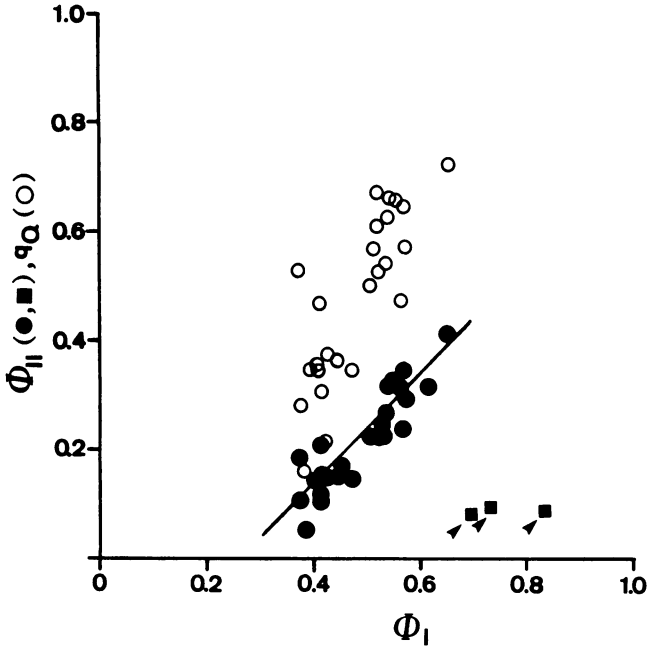

Figure 9. Relationship between ϕ_{II} (●, ■), q_Q (○), and ϕ_I for pea leaves subjected to a range of CO_2 concentrations in 2% O_2 balance N_2 and an irradiance of 735 μmol m^{-2} s^{-1}). The data indicated by (■) represent those values of ϕ_{II} for which the corresponding estimate of ϕ_I might be incorrect due to restriction of PSI oxidation on the acceptor side.

uously demonstrated, its inference from changes in ϕ_I relative to ϕ_{II} must be considered as speculative.

The three aberrant samples with values of ϕ_I higher than would be expected may be due to overreduction of PSI acceptor pool. These samples are associated with both low q_Q (0.214–0.304) and high values of NADP-MDH activation (29.5–33.6 μmol h^{-1} mg^{-1} Chl). If cyclic electron flow is occurring as well as linear electron flow, then both the PQ pool and the NADP pool must be considered as limitations for the flux of electrons from PSI. If overreduction of the PSI acceptor pool limits P-700 oxidation, an estimate of ϕ_I based on the relative size of the P-700$^+$ pool will be inaccurate.

Under conditions of varying irradiance the relationships between the estimated fluxes through both photosystems (J_I and J_{II}) and the activation state of the enzymes NADP-MDH and FBPase is simple. Both enzymes become more active as the flux of electrons increases. The light activation of NADP-MDH occurs principally via the modulation of thiol groups on the enzyme (23). Thus, the activation state of this enzyme can be used as a metabolic indicator of the physiologically relevant redox-state of the chloroplast stroma. Since NADP-MDH activity increases as the flux of reducing equivalents through both photosystems increases, this implies a progressive increase in the steady state pool of NADPH in the stroma as the flux of electrons from the thylakoids increases. Scheibe and Stitt (24) have discussed the possible physiological significance of NADP-MDH activation. It is clear from the data presented here that with an increasing flux of reductant through both photosystems the potential for transport of reducing power from the stroma to the cytosol via the malate-oxaloacetate shuttle will increase. This will have important consequences for the coordination of the synthesis of ATP and NADPH by the thylakoids.

The equilibrium of the thiol-mediated reduction of FBPase lies in favor of the oxidized form of the enzyme (26). The substrate, FBP, activates the enzyme and simultaneously shifts the overall equilibrium in favor of the reduction of FBPase, thus stabilizing it against oxidative inactivation (12). Nonetheless, the relationship between the activation state of FBPase and the fluxes through the photosystems (J_I and J_{II}) is linear over the range of irradiances used. However, at the highest irradiances the maximum activation state of FBPase is reached. The difference between the response of FBPase and NADP-MDH to increasing J_I and J_{II} is a reflection of the dual activation of FBPase by substrate and thiol reduction. NADP barely reached 30% of maximum activation even at 1000 μmol $m^{-2} \cdot s^{-1}$. This low activation state of NADP-MDH suggests that the stroma remains in a relatively oxidized state even at 1000 μmol $m^{-2} \cdot s^{-1}$

Previous measurements have shown that the half-time of P700$^+$ reduction (7) is constant with increasing irradiance. Therefore, the resistance for electron flow between PSI and II is normally not subject to a change in response to increasing irradiance. This is contrary to the commonly accepted model for photosynthetic control which proposes that with increasing irradiance the Benson-Calvin cycle becomes progressively limiting with respect to photosynthesis. This would restrict the rate of electron flow through the thylakoid electron trans-

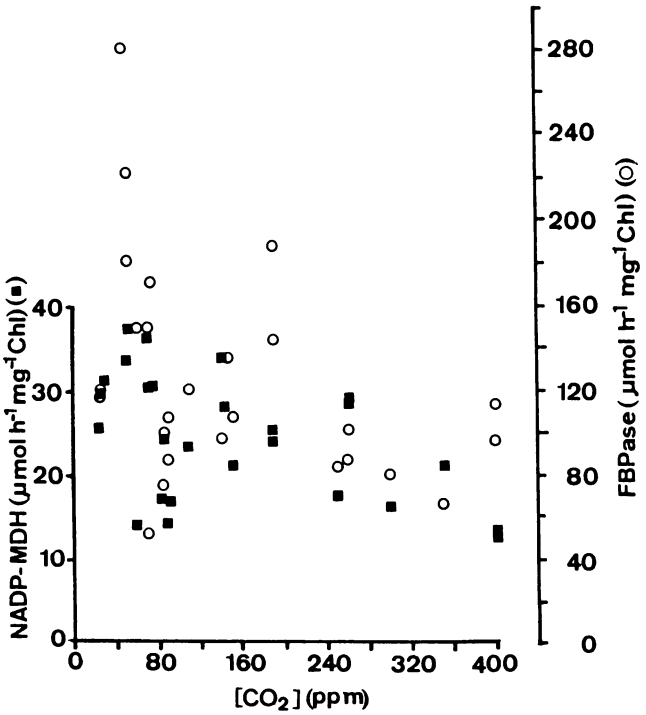

Figure 10. Relationship between CO_2 concentration (in a 2% O_2 balance N_2 background) and the activities of NADP-MDH (■), and FBPase (○) extracted from pea leaves under a constant irradiance (735 μmol m^{-2} s^{-1}).

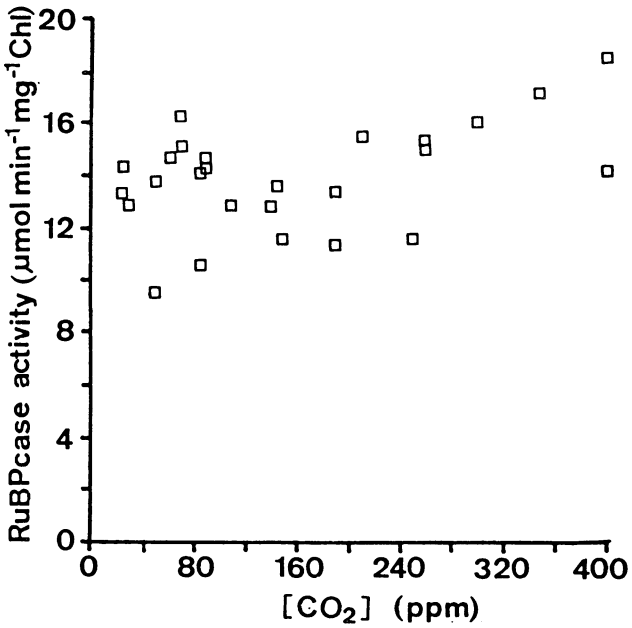

Figure 11. Relationship between CO_2 concentration, in a 2% O_2 balance N_2 background, and the activity of RuBP carboxylase extracted from pea leaves under constant irradiance. The maximum activity of the enzyme in these experiments was 18 μmol min^{-1} mg^{-1} Chl.

port chain via a decrease in intrathylakoid pH which reduces the rate of reaction between PQH_2 and the Reiske FeS complex of the Cyt *b6/f* complex (1). The data presented here show that only small increases in the activation of NADP-MDH occur with increasing irradiance even though the peak irradiance employed would be almost saturing for photosynthesis in a typical pea leaf. As these measurements reflect the steady state condition, they suggest that increased activity of the Benson-Calvin cycle with increasing irradiance can occur with only small increases in the stromal NADPH pool and without any detectable restriction of PSI acceptor activity. This also occurs without any change in resistance for electron flow between the two photosystems.

These two observations together show a remarkable degree of coordination of source and sink activities between the thylakoid and stroma even under conditions where light is not limiting photosynthesis. This does not suggest the absence of photosynthetic control but reflects the subtlety of control that holds redox-state relatively constant. Benson-Calvin cycle activity is a very strong sink for reducing equivalents and when carboxylation of ribulose bisphosphate is rapid the activation state of NADP-MDH is limited. In physiological conditions the steady state activation of NADP-MDH is always low unless the rate of carbon assimilation is severely impaired as occurs at low temperatures (24). With decreasing CO_2 partial pressures the quantum efficiencies of both PSI and II decrease. Under conditions of constant irradiance this implies that there is regulation of electron flow between the photosystems. Photosynthetic control of electron transport occurs. There is also the suggestion of an increase in cyclic electron flow around PSI with decreasing CO_2 pressure. Even though flux through PSII declines with decreasing CO_2 concentrations, at very low values of J_{II} (which correspond to very low CO_2 partial pressures) the increase in NADP-MDH activity with decreasing flux clearly shows that there is a slippage in stromal redox control. The activation state of NADP-MDH did not exceed 30% of maximum even at low CO_2 concentrations (28 ppm). This strongly suggests that there is an efficient regulation of both noncyclic and cyclic electron flow in these circumstances that limits the increase in the degree of reduction of components in the stroma.

RuBP carboxylase activation was nearly 80% at high irradiance. The activation state decreased with decreasing irradiance in line with previous observations (14, 18, 19, 21). However, *Pisum sativum* does not show the strong inhibition of ribulose bisphosphate carboxylase activity in darkness associated with the production of the tight-binding inhibitor, carboxyarbinatol 1-phosphate (25). The small decreases in rublose bisphosphate carboxylase at low irradiance in these experiments also suggest the absence of an inhibitor. However, the activation state is probably maintained via the 'activase' system (15) which requires ATP (20–22). Changes in the activation state of the ribulose bisphosphate carboxylase may, therefore, respond to changes in the ATP/ADP ratio of the stroma. However, changes in RuBP carboxylase activation state can be observed in leaves in the absence of any large change in adenylate concentrations (2). There are, therefore, undoubtedly other factors involved in the regulation of RuBP carboxylase activation *in vivo* that, thus far, remain unresolved (21, 22). We have no explanation for the difference between the relationship between J_{II} and ribulose-1,5-bisphosphate activity under conditions of varying irradiance in air, and varying CO_2 concentrations in 2% O_2 in N_2. Of enzymes measured in this study, those regulated by mechanisms involving thiol modulation (NADP-MDH FBPase), marked changes in activity with changes in J_I or J_{II}. This is not true of ribulose bisphosphate carboxylase, an enzyme that is not influenced by the thioredoxin system but primarily responds to ATP content.

CONCLUSIONS

Under conditions of varying irradiance (where photorespiration can occur), the flux through PSII to NADP is determined by irradiance and the quantum efficiencies of PSII and PSI. Under these conditions the activities of both NADP-MDH and FBPase are closely correlated with the estimated flux through PSII and PSI.

Previous work (7) showed no change in the $t_{½}$ for P-700$^+$ reduction over a range or irradiances (*i.e.* no evidence for a change in degree of photosynthetic control of electron transport). This study shows only a small change in the activation of NADP-MDH with increasing irradiance. Together these two observations suggest that the capacities of the thylakoids and the stroma are exceptionally well coordinated under the conditions of these experiments.

With varying CO_2 partial pressure, constant irradiance and low O_2 concentration (to suppress photorespiration) large changes in both ϕ_I and ϕ_{II} occur which suggests that photosynthetic control of electron transport is occurring under these

conditions. As CO_2 concentration decreased flux through linear electron transport also decreased. This may have been accompanied by an increase in cyclic flux around PSI. With varying CO_2 concentrations the relationships between J_{II} and the activation of FBPase (and to a lesser extent NADP-MDH at high values of J_{II} were similar to these obtained by changing the irradiance (under conditions where photorespiration can occur). At low values of J_{II} (corresponding to low CO_2 concentrations) the activities of both FBPase and NADP-MDH increased with decreasing J_{II}, suggesting that a partial breakdown of the balance between the stroma and the thylakoids is offset by photosynthetic control of electron transport.

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