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Response of photosynthetic electron transport and carbon metabolism to a sudden decrease of irradiance in the saturating or the limiting range

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(1) A moderate (2–4-fold) decrease in the light intensity leads to a transient inhibition of photosynthesis in saturating CO_2. The reasons for this inhibition were investigated by measuring photochemical and nonphotochemical chlorophyll fluorescence quenching, activation of NADP-malate dehydrogenase, $NADP^+$ and adenine nucleotides, phosphorylated intermediates, fructose 2,6-bisphosphate, and activation of sucrose phosphate synthase. (2) The inhibition is due to a restriction of glycerate 3-*P* reduction. This shows there is a delay until electron transport adjusts to a lower photon flux. (3) During transients between limiting light intensities, PGA reduction is restricted due to a shortfall of ATP. It is suggested that changes of energy quenching may not reveal how the thylakoid proton gradient is behaving during this transient. (4) During transients down from supersaturating light intensities, there is a temporary shortfall of NADPH. Simultaneously, there is a transient overreduction of Q_A. The recovery of photosynthesis matches the relaxation of energy quenching. The temporary inhibition may be related to a slow reversal of ΔpH control of electron transport, or of energy dissipation. (5) Sucrose phosphate synthase and fructose 2,6-bisphosphate require about 5 min to adjust to the lower light intensity. This does not contribute in a major way to the temporary inhibition of photosynthesis, because sucrose synthesis will be rapidly restricted by the decrease in triose phosphate which results from the inhibition of glycerate 3-phosphate reduction.

Introduction

Plants carry out photosynthesis at a wide range of light intensities. At low light, there will be a premium on mechanisms which optimise utilisation of the available light, while at high light mechanisms, allowing an orderly dissipation of excess energy will be important. The utilisation of light will also have to be coordinated with the fluxes around the Calvin cycle, and the rate of endproduct synthesis. The response to a sudden decrease of the light intensity should provide a useful experimental system for studying these interactions.

Abbreviations: Fru-2,6-P_2, fructose 2,6-bisphosphate; Fru-6-*P*, fructose 6-phosphate; Glc-6-*P*, glucose 6-phosphate; hexose-*P*, the sum of glucose 6-phosphate, fructose 6-phosphate and glucose 1-phosphate; PGA, glycerate 3-phosphate; P_i, inorganic phosphate; Q_A, the acceptor for Photosystem II; qNP, nonphotochemical quench; Ru-1,5-P_2, ribulose 1,5-bisphosphate; SPS, sucrose-phosphate synthase; Chl, chlorophyll.

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Decreasing the light intensity leads to a temporary inhibition of photosynthesis [1–5]. Vines et al. [2,3] showed this temporary inhibition was largest in conditions when rapid photorespiration occurs, and suggested it reflects a depletion of the photorespiratory pools. Subsequently, Prinsley et al. [4,5] showed that a transient inhibition is still found at 2% O_2 when the light is suddenly decreased from saturating to limiting intensities. They proposed there is a delay until the rate of endproduct synthesis adjusts to the new lowered rate of CO_2 fixation and that this leads to a temporary depletion of metabolites and inhibition of Ru-1,5-P_2 regeneration [5]. However, the temporary decrease of assimilatory power after lowering the light intensity [4] suggests further factors in electron transport also contribute to this transient. The precise contribution of electron transport and endproduct synthesis remains unclear, and the regulatory mechanisms involved in these delayed responses have not been directly investigated.

If photosynthesis is temporarily inhibited by an excessive rate of endproduct synthesis, we would expect the recovery to be correlated with the down-regulation

of sucrose synthesis. The rate of sucrose synthesis is modulated via changes of the Fru-2,6-P_2 level [6,7] and sucrose-phosphate synthase (SPS) activation [7,8]. We have therefore compared the recovery of O_2 evolution with the relaxation of Fru-2,6-P_2 levels and SPS activation of their new values. To provide information about the regulation of redox status, energisation and energy dissipation during a transient to lower light intensities we also investigated photochemical and nonphotochemical chlorophyll fluorescence quenching [9], adenine nucleotide and $NADP^+$ levels, and activation of NADP-malate dehydrogenase [10]. Our experiments were carried out in two fundamentally different conditions, namely, (a) transients between limiting light intensities where there is a linear relation between the light intensity and the steady rate of photosynthesis and (b) transients between saturating or near-saturating light intensities. In this way, we hoped to distinguish between mechanisms which are involved in optimising and balancing fluxes at limiting light, and mechanisms which are involved in control of energy dissipation at high light intensities.

Materials and Methods

Spinach (*Spinacia oleracea* L. var U.S. Hybrid 424) and barley (*Hordeum vulgare* c.v. Roland) were grown as in Ref. 8. Leaf discs (diameter 10–12 mm) were punched from spinach leaves and stored in a humidified box until use. Each sample contained four leaf discs (equivalent to 150–200 μg Chl) from a randomised collection of discs. Barley leaf segments (2 cm long) were cut from the primary leaves of whole plants held in low light, and were used immediately. Each sample contained four segments (equivalent to 120–150 μg Chl) from four different plants.

O_2 evolution was measured in a leaf disc O_2 electrode (Hansatech, Kings Lynn) [11]. Saturating CO_2 was delivered from 400 μl 2 M bicarbonate buffer (pH 9.5) containing carbonic anhydrase (2 U/ml). Chlorophyll fluorescence [9] was measured using a PAM chlorophyll fluorescence measuring system (Walz, Effeltrich, F.R.G.) as in Refs. 10 and 11). Saturating pulses were given every 20 s; after lowering the actinic light intensity, the first pulse was given 5 s later. All values of photochemical quenching were related to the F_0 value obtained when the leaf was darkened at that light intensity. The values for nonphotochemical quenching are related to the initial value of F_m unless stated otherwise. It should be noted that a nonphotochemical quench of 0.4–0.5 is already attained in low light, only relaxes slowly, and is insensitive to low concentrations of uncouplers. This component can be ascribed to state-transitions. The pH-dependent energy quench only develops at near-saturating and saturating light intensities. (Quick, P. and Stitt, M., unpublished data).

To collect material for assay of metabolites or enzyme activation, leaf discs or segments were illuminated in defined conditions in the O_2 electrode and their O_2 evolution and chlorophyll fluorescence quenching measured. The O_2 electrode was then opened, and the leaf material transferred to liquid N_2 and quenched [11], ensuring illumination was continued at the same intensity as in the O_2 electrode by using a projector and appropriate filters. Samples were extracted and assayed for Fru-2,6-P_2 and phosphorylated metabolites as in Ref. 11, for ATP, ADP and AMP as in Ref. 12, for NADP malate dehydrogenase as in Ref. 10 and for SPS as in Ref. 8, using 2 mM Fru-6-*P*, 10 mM Glc-6-*P*, 3 mM UDPGlc, and 5 mM P_i to provide selective conditions for the assay of the kinetically active SPS. $NADP^+$ was assayed in the $HClO_4$ extracts used for the adenine nucleotides, adding 1.6 mM 6-phosphogluconate, 100 mM Tris-HCl (pH 7.6), 5 mM MgCl, and starting the reaction with 0.1 U/ml 6-phosphogluconate dehydrogenase.

Results

Dependence on light intensity

Fig. 1 shows the transients of O_2 evolution when the light intensity is progressively halved in saturating CO_2. There was a large transient inhibition of O_2 evolution when light was reduced in the super-saturating range, and the recovery is accompanied by oscillations in the rate of O_2 evolution. At lower light intensities, there was only a small transient inhibition of photosynthesis when the light intensity was halved and no oscillations were found. Similar results were obtained in spinach (data not shown).

Transients in the range where light is limiting

In Fig. 2, spinach leaves at 15 °C were illuminated at 570 $\mu mol \cdot m^{-2} \cdot s^{-1}$ which supports about 80% of the light-saturated rate of photosynthesis. Lowering the light 4-fold to 144 $\mu mol \cdot m^{-2} \cdot s^{-1}$ led to a 4-fold decrease of the steady rate of O_2 evolution, but this rate was only

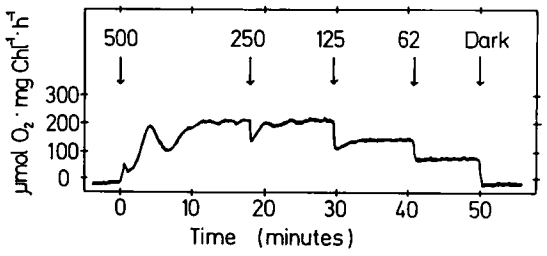

Fig. 1. Transients in O_2 evolution and chlorophyll fluorescence quenching during a stepwise halving of the light intensity in saturating CO_2. Barley leaf segments were illuminated at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the light was then lowered to 250, 125, 62 and zero $\mu mol \cdot m^{-2} \cdot s^{-1}$.

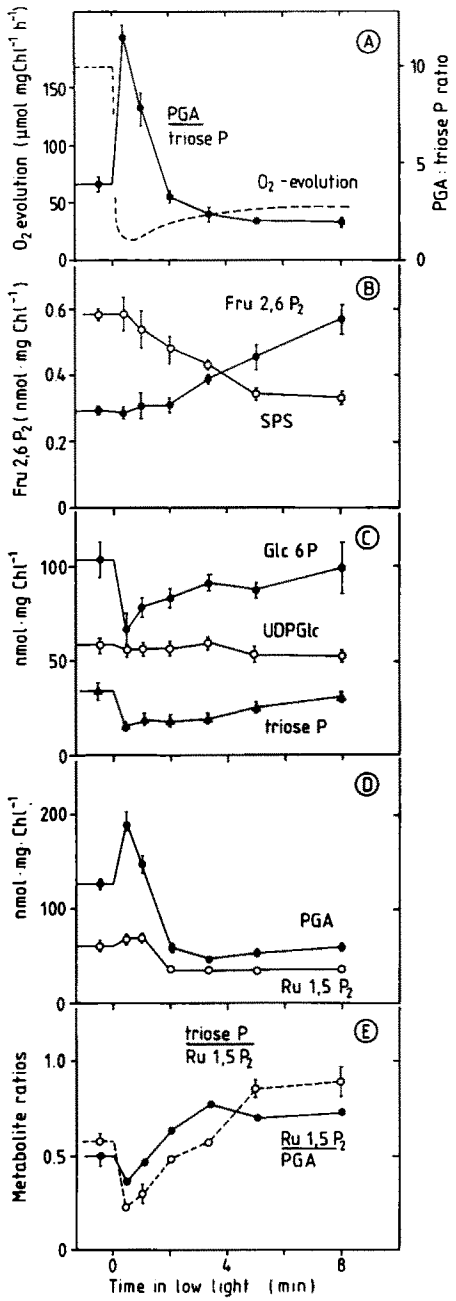

Fig. 2. Alterations of O_2 evolution, SPS activation, Fru-2,6-P_2 and metabolites after lowering the light in the limiting range. Spinach leaf discs in saturating CO_2 were illuminated for 10 min at 570 μmol · m^{-2} · s^{-1} at 15 °C, and the light was then reduced to 144 μmol · m^{-2} · s^{-1}. (A) O_2 evolution (– – –) and PGA/triose-*P* ratio (●———●). (B) Fru-2,6-P_2 (●) and SPS (○) activation. (C) Hexose-*P* (●) and triose-*P* (▲). (D) PGA (●) and Ru-1,5-P_2 (○). (E) Metabolite ratios for triose-*P*/Ru-1,5-P_2 (○------○) and Ru-1,5-P_2/PGA (●———●). Each point represents the mean ± S.E. of three separate samples.

achieved after about 4 min (Fig. 2A). The adjustment of Fru-2,6-P_2 and SPS was even slower (Fig. 2B). For example, during the first 2 min there was already a marked recovery of O_2 evolution, but Fru-2,6-P_2 hardly changed. After 3 min, about 80% of the deficit in O_2 evolution had been recovered, while Fru-2,6-P_2 and SPS had only adjusted half way towards their final value.

The transient inhibition of photosynthesis was not accompanied by a general depletion of metabolites (Fig. 2C and D). Immediately after lowering the light, Glc-6-*P* and triose-*P* fell (Fig. 2C), but PGA and Ru-1,5-P_2 increased (Fig. 2D). Later, as O_2 evolution recovered, the levels of hexose-*P* and triose-*P* rose, while PGA and Ru-1,5-P_2 decreased. A general increase of all the metabolites was only found during the very last phase (3–5 min) of the recovery. The way in which control shifts between PGA reduction, Ru-1,5-P_2 regeneration and carboxylation is revealed by plotting the substrate/product ratios for each of these processes (Fig. 2E). The PGA/triose-*P* ratio (PGA reduction) rises sharply when the light intensity is decreased, and then declines as photosynthesis recovers. In contrast, the triose-*P*/Ru-1,5-P_2 (Ru-1,5-P_2 regeneration) and Ru-1,5-P_2/PGA (Ru-1,5-P_2 carboxylation) ratios decline when the light is lowered, and rise as O_2 evolution recovers. Thus, a temporary restriction of PGA reduction is the major factor leading to the transient inhibition of photosynthesis.

A similar experiment was carried out at 30 °C with barley, lowering the light intensity from 100 to 12 μmol · m^{-2} · s^{-1}. Sucrose synthesis is selectively increased at higher temperatures and the steady rate of photosynthesis may already be restricted by low metabolites in these conditions [13]; this should therefore provide a particularly favourable system to detect any inhibition of O_2 evolution due to an overshoot of sucrose synthesis. There was a transient inhibition of photosynthesis after lowering the light intensity, but it was associated with an increase in Ru-1,5-P_2, and an increased PGA/triose-*P* ratio (data not shown). Thus, the inhibition is again due to a restriction of PGA reduction, rather than an inhibition of Ru-1,5-P_2 regeneration by low levels of metabolites. As in Fig. 2, the rate of O_2 evolution also recovered long before Fru-2,6-P_2 had relaxed to its new value (data not shown).

To investigate why PGA reduction was being restricted, we measured adenine nucleotides and $NADP^+$ during a transient from 250 to 60 μmol · m^{-2} · s^{-1} at 15 °C in spinach (Fig. 3A–C). For comparison, metabolites were also measured during a transition to darkness, to reveal how far the energy levels collapse after preventing all ATP and NADPH synthesis by the thylakoids (Fig. 3D–F). Immediately after the light intensity is decreased, the ATP/ADP ratio falls, and AMP rises (Fig. 3C). Although these changes are smaller than those occurring in the dark (Fig. 3F), they suggest there is a very considerable restriction on ATP synthesis. There is no evidence for a shortage of NADPH after lowering the light; $NADP^+$ even fell slightly (Fig. 3B), in marked contrast to the rapid doubling of the $NADP^+$ when the light was turned out (Fig. 3E). These transient

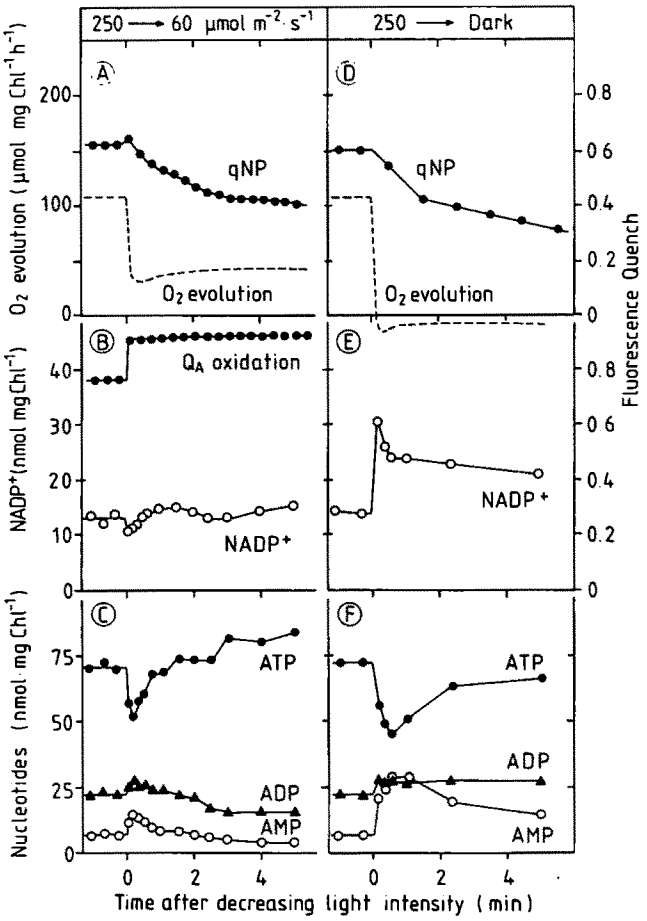

Fig. 3. Changes of O_2 evolution, photochemical and nonphotochemical chlorophyll fluorescence quenching, adenine nucleotides and NADP after lowering the light intensity in the limiting range. Spinach leaf discs were illuminated for 10 min at 15 °C with 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$, before lowering the light to 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$. (A) O_2 evolution (– – –) and nonphotochemical quenching (●). (B) $NADP^+$ (○) and Q_A oxidation (●). (C) ATP (●), ADP (▲) and AMP (○). In a parallel experiment, spinach leaf discs were illuminated at 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and then darkened. (D) O_2 evolution (– – –) and nonphotochemical quenching (●). (E) $NADP^+$ (○). (F) ATP (●), ADP (▲) and AMP (○).

changes of $NADP^+$ and adenine nucleotides were gradually reversed over the next 4 min. The ATP/ADP ratio rose 3-fold, reaching a higher value than at the original light intensity and AMP decreased 4-fold (Fig. 3C). This recovery of the adenine nucleotide energy status was accompanied by a 50% increase of $NADP^+$ (Fig. 3B).

The transient changes of NADP were compared with photochemical chlorophyll fluorescence quenching, which provides information about the redox state of Q_A, the acceptor for Photosystem II, Ref. 9. Q_A typically becomes more oxidised at lower light intensities [14]. This was also the case in most of our experiments. However, Q_A was always slightly more reduced just after the light had been lowered than it was after several minutes in low light (Fig. 3B). Thus the acceptor sides of Photosystems I and II are both transiently overreduced until the leaf adjusts to the lower light intensity.

The changes of the adenine nucleotides were compared with non-photochemical quenching, which provides a measure of thylakoid energisation [9]. Non-photochemical quenching relaxed very slowly after decreasing the light, or darkening. Close inspection of the changes after darkening reveals two components in the relaxation; a minor component which is completed within 1.5 min, and a slower component which decays almost linearly for the remainder of the experiment. We will show elsewhere (Quick, P. and Stitt, M., unpublished data) that the faster component is related to the ΔpH-dependent energy quenching [15] and the slower component is due to state transitions, as in protoplasts [16]. The relaxation after lowering the light to 60 $\mu mol \cdot m^{-1} \cdot s^{-1}$, therefore, suggests energy quenching relaxes to negligible values at this low light intensity, but that this occurs slowly, requiring about 2.5 min. This means that the rapid collapse of the ATP/ADP ratio is not accompanied by a comparable rapid decrease of energy quenching.

Transients in the range where light is saturating

A large decrease in the light intensity in the saturating range was obtained by illuminating barley leaves at 15 °C and 1400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and then lowering the light to 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This led to a transient 50% inhibition of photosynthesis which reversed after about 1 min but triggered a series of small oscillations of O_2 evolution (Fig. 4A). Triose-*P* decreased (Fig. 4B) immediately after decreasing the light, and PGA increased (Fig. 4C). These changes were reversed as O_2 evolution recovered. Ru-1,5-P_2 rose slightly after lowering the light, and then fell and, for the remainder of the transient, changed in a reciprocal manner to PGA (Fig. 4C). The PGA/triose-*P* ratio changes reciprocally to O_2 evolution (Fig. 4A), and the triose-*P*/Ru-1,5-P_2 and Ru-1,5-P_2/PGA ratios change in parallel to O_2 evolution (Fig. 4D). Clearly, the temporary restriction of photosynthesis can be ascribed to a restriction on PGA reduction.

Fig. 5 investigates the reason for this restriction. The ATP/ADP ratio remains unaltered or even rose slightly (Fig. 5A) and AMP also remained constant (data not shown). Instead, $NADP^+$ increases, showing there is a transient overoxidation of the acceptor side of Photosystem I. The response to a decrease from supersaturating light intensities was also investigated in spinach (Fig. 6). Again, a decrease within the range where light remains saturating (1400–700 $\mu mol \cdot m^{-2} \cdot s^{-1}$) led to a transient inhibition of O_2 evolution (Fig. 6A) and an increase of $NADP^+$ (Fig. 6B), but there was no measurable change of the ATP/ADP ratio or AMP (Fig. 6C).

Lowering the light from 1400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to slightly (350 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or strongly (90 $\mu mol \cdot m^{-2} \cdot s^{-1}$) limiting intensities leads to an even larger

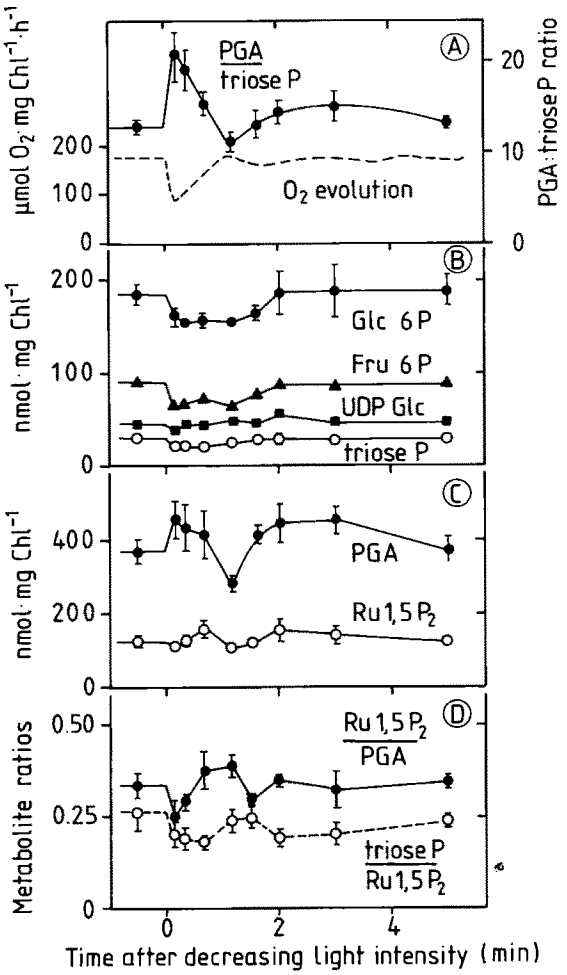

Fig. 4. Response of O_2 evolution, non-photochemical quenching, Q_A oxidation, and metabolites after lowering the light within the saturating range. Barley leaves in saturating CO_2 at 15 °C were illuminated for 12 min at 1400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ before decreasing the light intensity to 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. (A) O_2 evolution (- - -) and PGA/triose-*P* ratio (●). (B) Glc-6-*P* (●), Fru-6-*P* (▲) UDPGlc (■) and triose-*P* (○). (C) Ru-1,5-P_2 (○) and PGA (●). (D) Metabolite ratios for triose-*P*/Ru-1,5-P_2 (○------○) and Ru-1,5-P_2/PGA (●———●).

transient increase of $NADP^+$ (Fig. 6E and H), but now there is also a transient decrease of the ATP/ADP ratio and AMP increases (Fig. 6F and I), especially at 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Comparison with the changes after darkening (Fig. 6K and L) suggests lowering the light to limiting intensities leads to a very substantial transient restriction of NADP reduction and ATP synthesis.

The NADPH and ATP supply then readjust, and rapid O_2 evolution is reestablished at the lower light intensity. After a transition down to another saturating light intensity, the recovery of rapid O_2 evolution is accompanied by a 30% decrease of $NADP^+$ (Fig. 5B and 6B), showing the NADPH supply has recovered. The ATP/ADP ratio remains unchanged (Fig. 5C) or even declines slightly (Fig. 6C). After a transition from a saturating down to a limiting light intensity, the recovery of photosynthesis is associated with a recovery of the NADPH supply (Fig. 6E and H). There is also an increase of the ATP/ADP ratio and AMP declines (Fig. 6F and I), showing the adenine nucleotide energy status increases gradually after transfer to limiting light.

These transients of $NADP^+$ and adenine nucleotide levels were compared with chlorophyll fluorescence quenching. Decreasing the light always led to oxidation of Q_A within 5 s. Q_A became even more oxidised as O_2 evolution recovered at the low light intensity (Figs. 5B and 6B, E and H). This implies the acceptor side of Photosystem II is transiently overreduced after lowering the light, compared to the steady state value in low light. It also implies that the redox state of Q_A and $NADP^+$ change in opposite directions during the recovery of O_2 evolution. The recovery of O_2 evolution and the reciprocal change of $NADP^+$ and Q_A were closely matched to the relaxation of nonphotochemical quenching (Figs. 5A and 6A, D, G and J).

Nonphotochemical quenching decays slowly in the dark and at least two components can be distinguished as already seen during transitions from low light (Fig. 3D). Comparison with the faster component after darkening (Fig. 6J) suggests that energy quenching decays completely at 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 6G), and by about one half and a quarter at 350 (Fig. 6D) and 700 (Fig. 6A) $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. However,

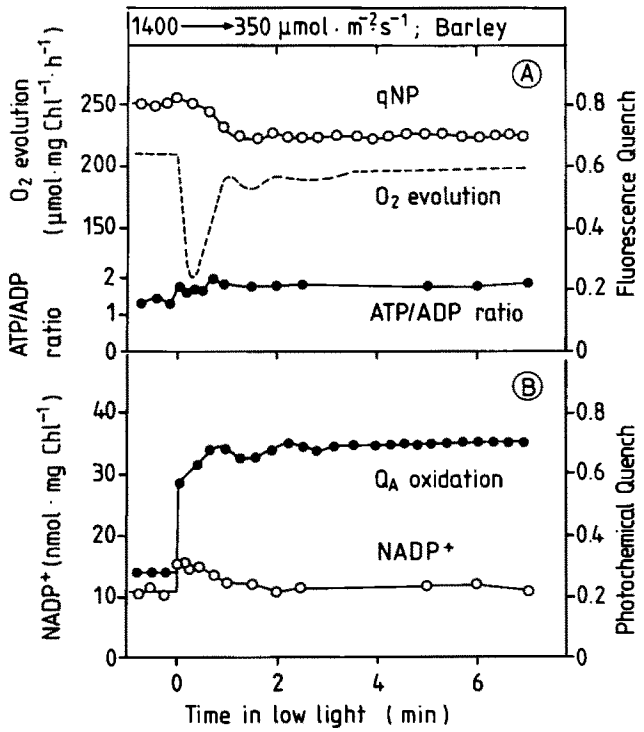

Fig. 5. Alterations of O_2 evolution, chlorophyll fluorescence quenching, adenine nucleotides and $NADP^+$ after a decrease of the light intensity within the saturating range. Barley leaves at 15 °C in saturating CO_2 were illuminated for 12 min at 1400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and the light intensity then lowered to 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. (A) O_2 evolution (- - -), nonphotochemical quenching (○———○) and the ATP/ADP ratio (●———●). (B) Q_A oxidation (●) and $NADP^+$ (○).

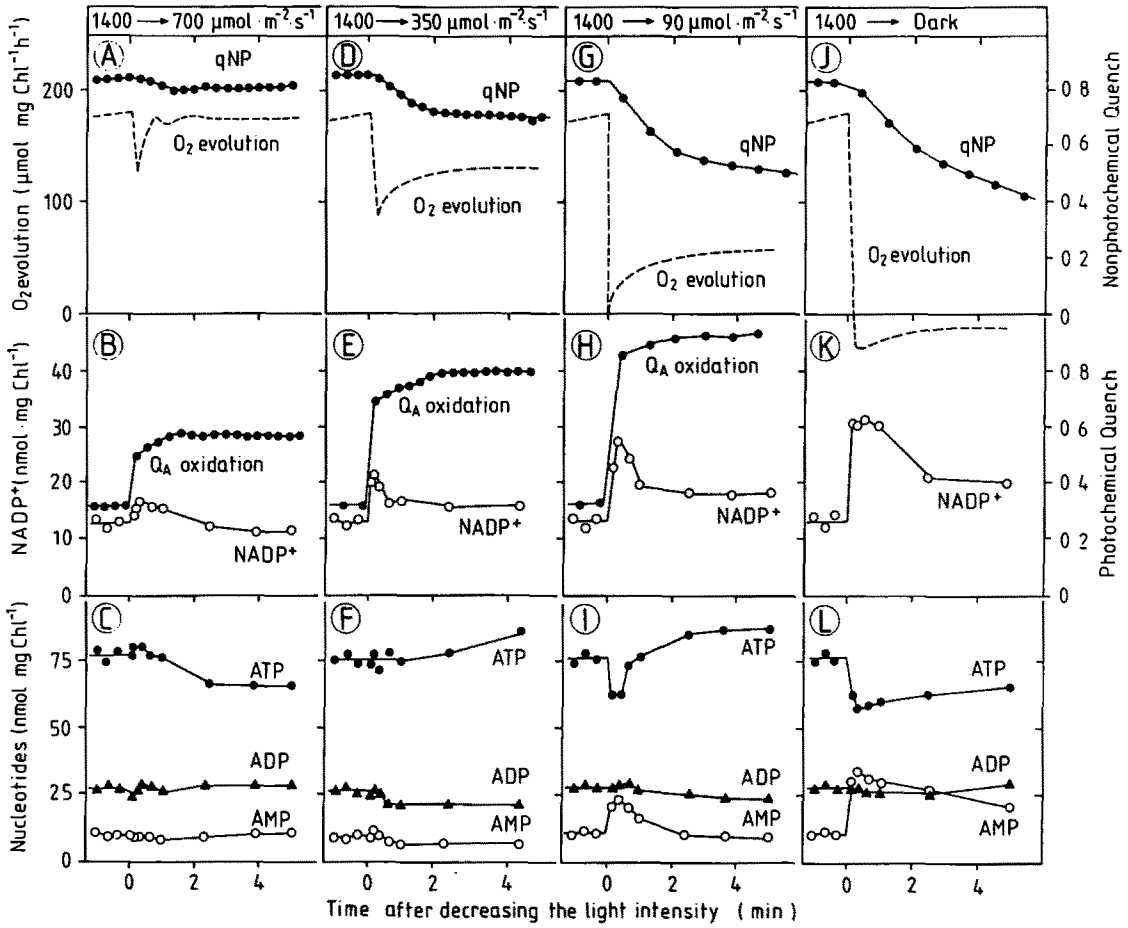

Fig. 6. Comparison of the response of O_2 evolution, chlorophyll fluorescence quenching, adenine nucleotides and $NADP^+$ after lowering the light intensity to a different extent from a supersaturating level. Spinach leaf discs at 15 °C in saturating light were illuminated for 10 min at 1400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ before lowering the light to 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (A, B and C), 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (D, E and F) 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (G, H and T) or darkness (K, L and M). The change of O_2 evolution (– – –), nonphotochemical quenching (qNP, ●) photochemical quenching (Q_A oxidation, ●) $NADP^+$ (○), ATP (●), ADP (▲) and AMP (○) were measured. Each point represents a single sample containing 4 spinach leaf discs.

this decay requires several minutes. This means the rapid collapse of the ATP/ADP ratio after decreasing the light intensity is not accompanied by a comparable decrease of energy quenching. Further, the gradual recovery of the ATP/ADP ratio at 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 6I) occurs as the energy quench is decreasing to negligible values. Since the rate of O_2 evolution also increases (Fig. 6G), this implies ATP sythesis is occurring at a higher rate, and in the face of a higher ATP/ADP ratio, but with lower energy quenching.

Fig. 7 summarises the relation between the steady state $NADP^+$ levels, Q_A oxidation and the ATP/ADP ratios in saturating CO_2. $NADP^+$ decreases until light becomes saturating, and then remains constant, suggesting that NADPH rises as light is increased in the limiting range, but does not increase further once light has become saturating. This resembles the light response for activation of NADP-MDH, which has been used as a 'metabolic indicator' for the redox status of the stromal NADPH system [10]. As previously reported [17], the ATP/ADP ratio decreases as the light intensity is increased in the limiting range. For comparison, Fig. 7 also shows the values immediately after reducing the light to these intensities after 10 min preillumination at supersaturating intensities (1400 $\mu mol \cdot m^{-2} \cdot s^{-1}$). NADP is transiently more oxidised, the ATP/ADP ratio is lower (except at 750 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and Q_A is more reduced than in the steady state.

Activation of NADP-malate dehydrogenase

NADP-malate dehydrogenase activation was measured to provide further evidence for a transient overoxidation of the acceptor side of Photosystem I after decreasing the light. The light activation of NADP-malate dehydrogenase via thioredoxin is strongly modulated by $NADP^+$ [18] allowing it to be used as a metaoblic marker for the stromal NADP redox state [10]. In Fig. 8A, the leaves were darkened to reveal how rapidly NADP-malate dehydrogenase is inactivated when electron transport is completely stopped. For

comparison, in Fig. 8B the light intensity was lowered from 1400 to 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (comparable to Fig. 6D–F). NADP-malate dehydrogenase activity decreased to a minimum after 30 s at the new light intensity. Since NADP-malate dehydrogenase deactivated at the same rate after darkening, it is likely that the changes of enzyme activity underestimate the extent to which the stromal NADPH system becomes transiently overoxidised after lowering the light intensity. Activation then increased again by 50% as O_2 evolution recovered. During this recovery, the reduction state of the acceptor of Photosystem II (as revealed by Q_A reduction) and Photosystem I (as revealed by NADP-MDH activation) change in an opposite direction. This shift towards increased reduction of Photosystem I is accompanied by a decrease of the nonphotochemical quenching. A similar transient overoxidation of NADP-malate dehydrogenase was also seen during a transient from 1400 to 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in barley (data not shown).

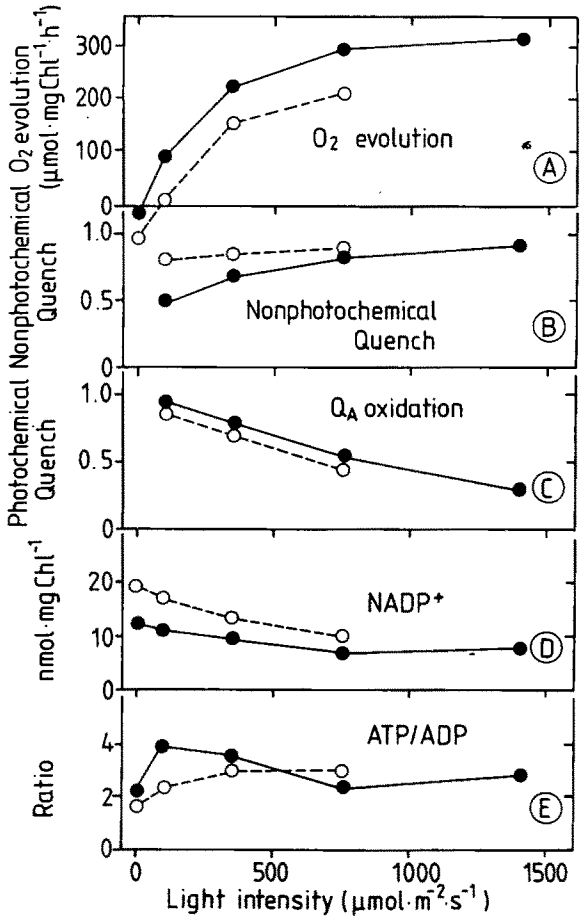

Fig. 7. O_2 evolution, nonphotochemical quenching, Q_A oxidation, ATP/ADP ratios, and NADP levels, at various light intensities: comparison of values at steady state (●——●) and values immediately after lowering the light from 1400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to a lower intensity (o------o). The results are summarised from Fig. 7. (A) O_2 evolution. (B) Nonphotochemical quench. (C) Q_A oxidation. (D) $NADP^+$. (E) ATP/ADP ratio.

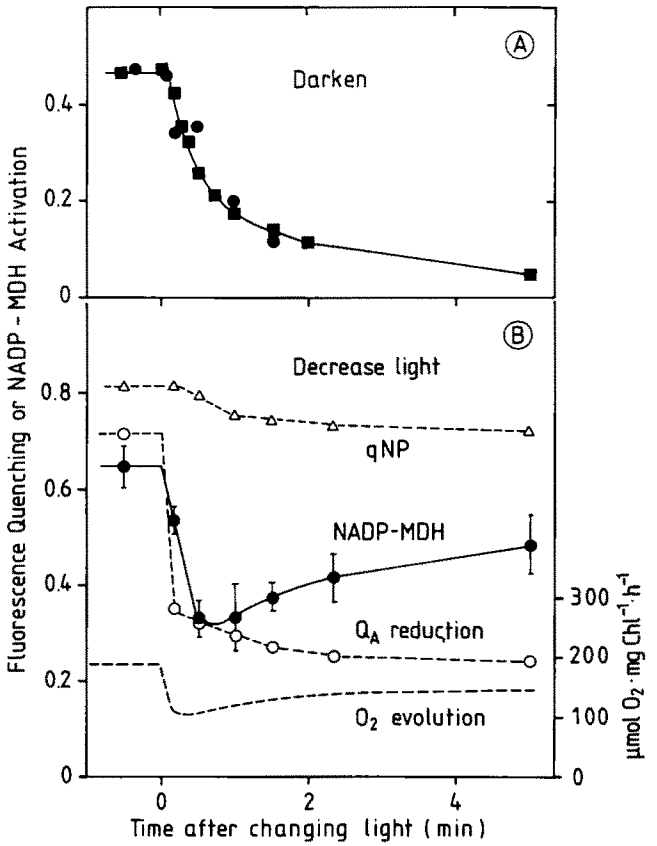

Fig. 8. Comparison of O_2 evolution, nonphotochemical quenching, Q_A reduction and activation of NADP-malate dehydrogenase after a transient to a lower light intensity. (A) From 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to darkness, showing two separate experiments (●, ■). (B) From 1400 to 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The results are the mean ± S.E. of four separate samples, each containing four spinach leaf discs.

Discussion

Control of sucrose synthesis

The regulatory mechanisms in the cytosol respond quite slowly to a sudden decrease of the light intensity. Deactivation of SPS and readjustment of Fru-2,6-P_2 both need over 5 min at 15 °C. A similar slow response of SPS is found during induction [10,11,19], suggesting the 'coarse' control of this enzyme may be quite slow. Much faster changes of Fru-2,6-P_2 occur during induction [10,11]. The delayed response after lowering the light is due to the behaviour of metabolites such as Fru-6-*P* and PGA which modulate Fru-6-*P* 2-kinase [6,7]. The increase of PGA and decline of Fru-6-*P* after lowering the light intensity will temporarily inhibit Fru-6-*P* 2-kinase. Fru 2,6-P_2 only starts to rise as these changes are reversed by the gradual inactivation of SPS and (see below) the adjustment of electron transport.

However, this delayed adjustment of sucrose synthesis is not the main reason for the transient inhibition of

photosynthesis after lowering the light. Firstly, this inhibition is largest at high light intensities, when little or no change of cytosolic fluxes is needed. During transitions between different limiting intensities there is only a small transient inhibition, even though a relatively large adjustment of the flux to endproducts will be needed. Secondly, detailed examination of Figs. 2 and 3 shows a major part of the transient inhibition of O_2 evolution is reversed before Fru-2,6-P_2 levels or SPS activation have changed. Thirdly, a general increase of metabolite levels only occurs during the last phase of the recovery. Fourthly, in all of these experiments, there is to a transient increase of Ru-1,5-P_2 and PGA after lowering the light. This is incompatible with the idea that photosynthesis is inhibited because the Calvin cycle metabolite pools have been depleted to the point where Ru-1,5-P_2 regeneration becomes limiting.

Shortfall of ATP and NADPH

The transient inhibition of photosynthesis is accompanied by an increased PGA/triose-*P* ratio, and the recovery from this inhibition is accompanied by a decrease of the PGA/triose-*P* ratio. Clearly, photosynthesis is inhibited by a transient restriction on PGA reduction. The reason for this restriction varies, depending on the kind of light transition. If the light is well above saturating and the intensity is decreased to another intensity which is still saturating, there is an interruption of the NADPH supply. If light is lowered from saturating to limiting intensities, there is a shortfall in the supply of NADPH and ATP. If the light is already limiting, a further decrease leads to an interruption of the ATP supply.

In the earlier experiments of Prinsley et al. [4,5], the light intensity was decreased from well-above saturating to well-below limiting. Although this very large change of the light intensity also led to a decrease of Ru-1,5-P_2, in all their experiments the PGA/triose-*P* ratio increased transiently, showing delivery of energy from the thylakoid was the primary factor restricting photosynthesis. It should be noted that restriction of PGA reduction will lead to a decrease in triose-*P* and an even larger decrease in Fru-1,6-P_2 [20]. Since the cytosolic Fru-1,6-*P*ase has a sigmoidal substrate saturation curve [20], its activity will be restricted even though Fru-2,6-P_2 does not increase immediately. A strong restriction on PGA reduction would also restrict Ru-1,5-P_2 regeneration, irrespective of how endproduct synthesis is being regulated.

Control of ATP synthesis

Why are ATP or NADPH synthesis transiently inhibited after lowering the light? The temporary inhibition of ATP synthesis when the light is lowered to limiting intensities suggests the thylakoid pH gradient has suddenly decreased. This would inhibit the ATP synthase and may even lead to a transient hydrolysis of ATP [21] as already proposed for isolated chloroplasts [22]. However, the ATP/ADP ratio decreases before any appreciable change of energy quenching could be detected. Our explanation, therefore, requires the assumption that energy quenching does not provide a reliable measure of the proton motive force during transients. This assumption is supported by studies showing nonphotochemical quenching develops more slowly than 9-amino acridine fluorescence quenching after illuminating isolated chloroplasts (Ref. 23, and see also discussion in Ref. 9). A similar lag is seen when chloroplasts are darkened (Quick, P., Horton, P. and Stitt, M., unpublished data).

These results also emphasize that photosynthesis is driven by an increased supply of reducing equivalents as the light intensity is increased in conditions of saturating CO_2, rather than an increased ATP/ADP ratio (see also Ref. 17). Two independent lines of evidence show that the availability of reducing equivalents increases as the light intensity is increased; $NADP^+$ decreases (Fig. 8), and the activation of NADP-malate dehydrogenase increases [10]. These conclusions differ from those obtained in isolated chloroplasts which contain high NADPH levels [24] and a highly activated NADP-malate dehydrogenase [10] even in low light. This discrepancy may be due to it being difficult to maintain stromal alkalisation and metabolite levels at low light intensities in isolated chloroplasts. The results for leaves in saturating CO_2 also contrast with leaves in ambient or low CO_2, where the NADPH/NADP ratio decreases at higher light intensities [25] and P-700 becomes more oxidised [26]. This difference may reflect the effect of increased energisation on electron transport at ambient CO_2 (see below).

Control of NADPH generation

When the light is reduced from a saturating intensity there is a transient increase of NADP, and inactivation of NADP-malate dehydrogenase, showing the acceptor side of Photosystem I is transiently overoxidised at the new light intensity. In contrast Q_A, the acceptor for Photosystem II is initially overreduced at the new light intensity. Thus, photosynthesis is inhibited by a transient overoxidation of Photosystem I even though there are surplus reducing equivalents available at Photosystem II. This reciprocal behaviour suggests there is either a temporary restriction on electron transport between the two photosystems, and/or a transient restriction of photochemistry at Photosystem I.

Weis et al. [26] have shown there are similar reciprocal changes of steady state Q_A reduction and A_{820} as the light intensity becomes saturating, which are accompanied by increased nonphotochemical quenching. They have suggested that ΔpH exerts a strong control on the rate of electron transport from Photosystem II to Pho-

tosystem I in vivo. The slow relaxation of the nonphotochemical quench is consistent with a slow decay of the thylakoid ΔpH after lowering the light (Figs. 5 and 6). However (see above), the kinetics of nonphotochemical quenching may not reflect the rate at which the thylakoid ΔpH changes during rapid transients. It is also curious that a transient overreduction of Q_A is found during a transient from saturating to limiting intensities, even though th ATP/ADP ratio also collapses. An explanation purely in terms of a direct effect of ΔpH on electron transport would therefore require that declining ΔpH inhibits ATP synthesis before it relieves the restriction on linear electron flow.

It may also be important to consider that most of the incident light is dissipated at saturating intensities. When the light intensity is suddenly decreased, there will be a transient inhibition of photosynthesis until energy dissipation is reduced to a level which is appropriate to the new photon flux. It is intriguing that the recovery of O_2 evolution corresponds closely to the relaxation of the nonphotochemical quenching. This provides a measure for thermal energy dissipation at Photosystem II [15] and will probably also decrease excitation at Photosystem I by reducing overspill [15].

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