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Oscillations in photosynthesis are initiated and supported by imbalances in the supply of ATP and NADPH to the Calvin cycle

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Abstract. Oscillations in the rate of photosynthesis of sunflower (*Helianthus annuus* L.) leaves were induced by subjecting leaves, whose photosynthetic apparatus had been activated, to a sudden transition from darkness or low light to high-intensity illumination, or by transfering them in the light from air to an atmosphere containing saturating CO_2. It was found that at the first maximum, light- and CO_2-saturated photosynthesis can be much faster than steady-state photosynthesis. Both Q_A in the reaction center of PS II and P_{700} in the reaction center of PS I of the chloroplast electron-transport chain were more oxidized during the maxima of photosynthesis than during the minima. Maxima of P_{700} oxidation slightly preceded maxima in photosynthesis. During a transition from low to high irradiance, the assimilatory force F_A, which was calculated from ratios of dihydroxyacetone phosphate to phosphoglycerate under the assumption that the reactions catalyzed by NADP-dependent glyceraldehydephosphate dehydrogenase, phosphoglycerate kinase and triosephosphate isomerase are close to equilibrium, oscillated in parallel with photosynthesis. However, only one of its components, the calculated phosphorylation potential $(ATP)/(ADP)(P_i)$, paralleled photosynthesis, whereas calculated NADPH/NADP ratios exhibited antiparallel behaviour. When photosynthetic oscillations were initiated by a transition from low to high CO_2, the assimilatory force F_A declined, was very low at the first minimum of photosynthesis and increased as photosynthesis rose to its second maximum. The observations indicate that the minima in photosynthesis are caused by lack of ATP. This leads to overreduction of the electron-transport chain which is indicated by the reduction of P_{700}. During photosynthetic oscillations the chloroplast thylakoid system is unable to adjust the supply of ATP and NADPH rapidly to demand at the stoichiometric relationship required by the carbon-reduction cycle.

Key words: Assimilatory force – Calvin cycle – Electron flow (cyclic) – *Helianthus* (photosynthesis) – Oxygen reduction – Phosphorylation potential – Photosynthesis (oscillations)

Introduction

During recent years, oscillations in photosynthesis have commanded considerable interest (Ogawa 1982; Walker et al. 1983; Sivak et al. 1985; Sivak and Walker 1987; Scheibe and Stitt 1988; Stitt et al. 1988), because during the oscillations maximum rates of photosynthesis have been observed to be considerably in excess of maximum steady-state rates of light- and CO_2-saturated photosynthesis. It has been concluded, that the potential of leaves for fast photosynthesis is not fully utilized even under conditions earlier believed to produce the highest photosynthetic yields. In spite of considerable research efforts, no agreement exists as to the nature of the limitation placed on maximum steady-state photosynthesis. We have reassessed the problem and report experiments which indicate that oscillations are caused by imbalances in the availability of ATP and NADPH for carbon reduction. Our estimates of changes of chloroplast phosphate further indicate that lack of phosphate may not be directly responsible for the decrease of photosynthesis at the minima of oscillations (see Stitt 1986; Walker and Osmond 1986; Laisk and Walker 1986; Sivak and Walker 1987).

Material and methods

Sunflower plants (*Helianthus annuus* L.) were grown in a growth cabinet in soil supplied with minerals in 16/8 h and 28/22° C day/night cycles. The light intensity during the daytime was 60 nmol · cm^{-2} · s^{-1}. Fully developed upper leaves of three- to four-week-old plants were used for the experiments. They remained attached to the plants during the gas exchange and optical measurements, and were enclosed in a sandwich-type cuvette (Oja 1983).

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Abbreviations: PGA = 3-phosphoglycerate; DHAP = dihydroxyacetone phosphate; P_{700} = electron-donor pigment in the reaction center of PS I; Q_A = quinone acceptor in the reaction center of PS II

The upper side of a leaf was smeared with a paste pepared by heating 10% potato starch in water and attached to a thermostated window of the cuvette. Only the lower side was exposed to the gas stream. Illumination was provided to the upper leaf side. White light from a Xenon lamp was filtered through a heat filter to remove far-red radiation. The CO_2 solubilization in the leaf during the presence of high CO_2 concentrations was measured by switching the cuvette to CO_2-free air and recording the amount of rapidly released CO_2 (Oja et al. 1986). Chlorophyll fluorescence and P_{700} photooxidation were recorded during the gas-exchange measurements by the pulse modulation fluorometer of Schreiber (Schreiber et al. 1986; Schreiber et al. 1988).

For metabolite measurements, the upper side of the leaf was not pasted to the glass but held at a distance of three mm from it. For rapid freeze-stopping of metabolism, ethanol cooled down to −80° C was rapidly poured into the space between glass and upper epidermis of the leaf, while gas-exchange measurement through the lower epidermis was continued. Filling the space with ethanol took about 1 s but stopping metabolism of a leaf element in actual contact with ethanol occurred within 0.1 s under constant irradiance and CO_2 concentration. The frozen leaf was ground with liquid nitrogen and the leaf powder was permitted to thaw in 4 ml cold 4.5% perchloric acid. After 10 min at 4° C, samples were centrifuged for a few minutes at 1000 · *g*, tris(hydroxymethyl)aminomethane was added to a total concentration of about 90 mM, and resulting solutions were neutralized with 5 M K_2CO_3. After removing $KClO_4$ the clear solutions were frozen and kept in liquid nitrogen. For the measurements of 3-phosphoglycerate (PGA) and dihydroxyacetone phosphate (DHAP), 10 mg charcoal was added per ml freshly thawed solution to remove interfering material. Both metabolites were determined enzymically (Michal 1984; Racker 1984). The NADP was determined by enzymic cycling (Takahama et al. 1981), and ATP and ADP by the luciferin/luciferase method (Wulff and Döppen 1985).

Results

Recording photosynthetic parameters during oscillations of photosynthesis in leaves. Figure 1 shows simultaneous recordings of the relative reduction of P_{700}, the donor of electrons in PS I, and of CO_2 uptake by a leaf of *Helianthus annuus* in a dark/light transition. On illumination, charge separation in the reaction center of PS I caused the oxidation of P_{700}. A maximum in P_{700} oxidation preceded a maximum in CO_2 uptake. It was followed by a minimum which, by about 10 s, preceded a minimum in CO_2 uptake. Several oscillations followed, with P_{700} oxidation and reduction always preceding, by up to 14 s, maxima and minima in photosynthesis. Under the conditions of the experiment, the final steady-state oxidation of P_{700} was about 80% of the oxidation observed in saturating 720-nm light which supports very little electron donation from PS II to PS I.

The initial peak in CO_2 uptake shown in Fig. 2 during the transition from air containing 350 μl · l^{-1} CO_2 to air with 2000 μl · l^{-1} CO_2 is due to solubilization of CO_2 in the chloroplast stroma and to rapid consumption of ribulose-1,5-bisphosphate (RuBP) at the high CO_2 concentration. However, the second peak at about 20 s from the transition is related to carbon reduction. Photosynthetic CO_2 uptake then declined to a minimum, rose again and stabilized via a series of oscillations. The maximum rate of photosynthesis during the first peak was about 30% higher than the final steady-state rate. As in the dark/light experiment of Fig. 1, the oscillations in photosynthesis were preceded by oscillations in the oxidation state of P_{700}, with minima occurring about 15 s before minima in CO_2 uptake.

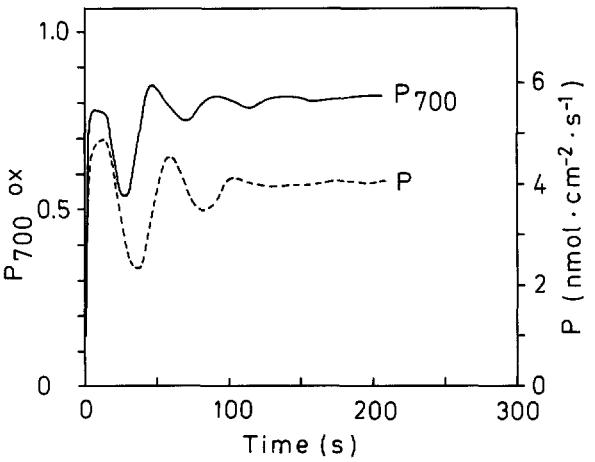

Fig. 1. Simultaneous recording of photosynthetic CO_2 uptake *(dashed line)* and the state of photooxidation of P_{700} *(solid line)* in a leaf of Helianthus annuus. The leaf was illuminated at zero time with white light (162 nmol photons · cm^{-2} · s^{-1}) in air containing 2000 μl · l^{-1} CO_2. It had been preilluminated before under the same conditions, and illumination had been interrupted by a short dark interval (36 s). *Left ordinate: 1* = P_{700} oxidation in saturating 720 nm light; *0* = redox state of P_{700} in darkness. *Right ordinate:* CO_2 uptake in nmol · cm^{-2} · s^{-1}

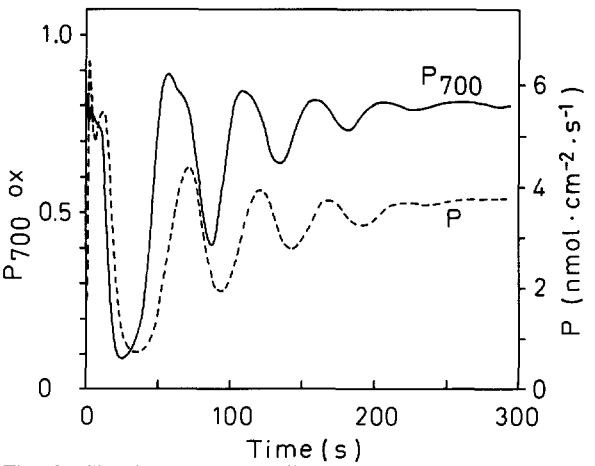

Fig. 2. Simultaneous recording of photosynthetic CO_2 uptake *(dashed line)* and state of photooxidation of P_{700} *(solid line)* in a leaf of *H. annuus*. The leaf was first illuminated in air with white light (162 nmol photons · cm^{-2} · s^{-1}). At zero time, air with 340 μl · l^{-1} CO_2 was replaced by air containing 2000 μl · l^{-1} CO_2. Uptake of CO_2 from this atmosphere was recorded. For explanation of the ordinates, see Fig. 1

Levels of PGA and DHAP during oscillations in photosynthesis. Levels of PGA and DHAP were measured in two experiments which were similar to that shown in Fig. 1, except that oscillations were initiated not by a dark/light transition but by a transition from low to high light. Each experimental point is taken from an individual segment of one and the same leaf whose gas exchange was mon-

Table 1. Metabolite levels measured in *Helianthus annuus* leaves after a transition from low to high irradiance illumination (Expts. 1, 2) and from limiting to saturating CO_2 (Expt. 3). Calculations are shown of the assimilatory force F_A, of NADPH/NADP and ATP/ADP ratios, of phosphorylation potentials and of free phosphate. For assumptions see text

Expt. 1, low/high light transition (6.5 → 101 nmol · cm^{-2} · s^{-1})

	low	increasing	max	min	max	min	steady
Measured values							
Photosynthesis, nmol · cm^{-2} · s^{-1}	0.28	1.8	5.7	2.8	4.1	2.9	4.3
NADP, nmol · cm^{-2}	1.53	1.18	1.89	1.67	2.14	1.28	1.39
PGA, nmol · cm^{-2}	10.7	1.25	15.1	28.9	23.7	31.9	17.2
DHAP, nmol · cm^{-2}	0.82	0.46	2.55	1.64	2.05	2.15	2.32
ATP, nmol · cm^{-2}	1.31	2.17	1.83	1.99	3.59	2.36	3.26
ADP, nmol · cm^{-2}	2.15	1.29	1.37	1.21	0.61	2.20	–
Calculated values							
F_A, M^{-1}	47	209	104	35	53	42	84
NADPH/NADP:	0.63	1.12	0.32	0.50	0.17	0.95	0.80
Phosphorylation potential, ATP/(ADP · P_i), M^{-1}	74	186	322	70	315	44	105
ATP/ADP	0.61	1.68	1.34	1.64	5.89	1.07	–
P_i, mM	8.2	9.0	4.16	23.4	18.7	23.0	–

Expt. 2, low/high light transition (20 → 104 nmol · cm^{-2} · s^{-1})

	low	–	max	min	steady
Measured values					
Photosynthesis, nmol · cm^{-2} · s^{-1}	1.29	–	6.2	3.6	4.9
NADP, nmol · cm^{-2}	1.89	–	2.3	1.67	1.99
PGA, nmol · cm^{-2}	16.3	–	16.2	32.6	24.7
DHAP, nmol · cm^{-2}	1.35	–	4.15	3.94	4.04
ATP, nmol · cm^{-2}	4.07	–	4.73	3.85	3.56
ADP, nmol · cm^{-2}	1.63	–	0.97	1.85	2.15
Calculated values					
F_A, M^{-1}	51	–	158	75	101
NADPH/NADP	0.32	–	0.09	0.5	0.26
Phosphorylation potential, ATP/(ADP · P_i), M^{-1}	158	–	1813	151	395
ATP/ADP	2.5	–	4.88	2.09	1.66
P_i, mM	15.8	–	2.7	13.8	4.2

Expt. 3, low/high CO_2 transition (340 → 2000 µl CO_2 · l^{-1})

	low	max	min	steady
Measured values				
Photosynthesis, nmol · cm^{-2} · s^{-1}	3.56	4.60	3.27	4.37
PGA, nmol · cm^{-2}	14.36	25.2	42.4	29.7
DHAP, nmol · cm^{-2}	0.809	0.63	0.68	0.95
Calculated values				
F_A, M^{-1}	35	15	10	20

itored. At the maxima and minima of photosynthesis metabolism was freeze-stopped. The frozen leaf material was prepared for metabolite analysis as described in *Material and methods*. A third experiment was performed in which CO_2 was suddenly increased at a constant photon fluence rate (103 nmol · cm^{-2} · s^{-1}) from a limiting to a saturating concentration.

Results are given in Table 1. On illumination, the level of PGA decreased transiently owing to its rapid reduction (shown only in one of the experiments, but see also Santarius and Heber 1965), but since the photosynthetic carbon cycle had been maintained active during the low-light period, its reduction product DHAP was rapidly converted into RuBP whose carboxylation caused PGA levels to increase. During the first maximum of CO_2 uptake, PGA was not yet at its maximum. Rather, it

reached its maximum level during the first minimum of CO_2 uptake, decreased as photosynthesis accelerated again and increased towards the second minimum of CO_2 uptake. Like PGA, DHAP also increased considerably during the transition from low to high light. However, in contrast to PGA, it reached its maximum level during the first maximum of CO_2 uptake, decreased somewhat as photosynthesis fell to a minimum and then increased again. In two different experiments with similar oscillation responses and similar rates of maximum steady-state photosynthesis, its maximum level differed by a factor of almost two. In the experiment with the higher DHAP level, the irradiance had been higher prior to the light jump.

When photosynthetic oscillations were initiated by a transition from low to high CO_2, PGA increased as expected from a decrease in carboxylation resistance (Table 1). It reached a maximum when photosynthesis fell to a minimum and decreased at the second maximum of photosynthesis while DHAP increased. Changes in PGA and DHAP during photosynthetic oscillations were measured at a higher time resolution than in our experiments by Furbank and Foyer (1986) and Stitt et al. (1988). Our observations agree with theirs.

pH-shifts in the chloroplast stroma during oscillations, as measured by CO_2 solubility. 3-Phosphoglycerate is formed by an acid-producing reaction

$$RuBP^{4-} + CO_2 \longrightarrow 2PGA^{3-} + 2H^+. \qquad \text{Eq. (1)}$$

In order to obtain information about pH shifts in the stroma during photosynthetic oscillations, changes in the solubilization of CO_2 induced in the light by a transition from low to high CO_2 were measured. At different oscillatory states, the leaf was switched to a CO_2-free atmosphere. This caused the rapid release of dissolved CO_2 from the leaf. Chloroplasts contain carbonic anhydrase which releases CO_2 from the stroma according to the reaction

$$HCO_3^- \Longleftrightarrow CO_2 + OH^-. \qquad \text{Eq. (20)}$$

Because the light was not turned off during the release experiments, the results represent the CO_2 capacity of the leaf in the presence of a transthylakoid proton gradient. As most of the dissolved CO_2 was released within a few seconds, RuBP could not accumulate to any extent and there was no substantial reassimilation of the dissolved CO_2.

Results of the measurement of released CO_2 are shown in Fig. 3 as changes in the CO_2 capacity L of a leaf; L represents the thickness of a CO_2-saturated water layer which contains as much dissolved CO_2 as the leaf contains $CO_2 + HCO_3^-$. After switching the leaf from low to high CO_2, the CO_2 capacity of the leaf was low. It did not change much while PGA accumulated, but increased as photosynthesis approached a second maximum, and decreased again after the maximum. The pH values in the chloroplast stroma were calculated from the CO_2 capacity of the leaf using the Henderson-Hasselbalch equation

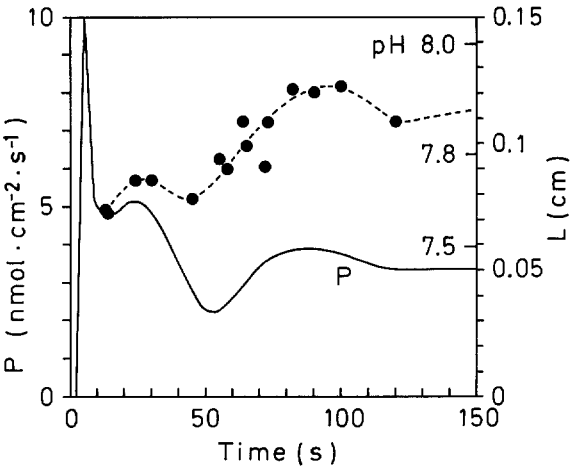

Fig. 3. Simultaneous recording of photosynthetic CO_2 uptake *(solid line, left ordinate)* and CO_2-capacity *(dashed line, right ordinate)* in a leaf of *H. annuus*. Photosynthetic oscillations were induced during iflumination with 160 nmol photons · cm^{-2} · s^{-1} white light by increasing the CO_2 concentration of the gas stream passing over the leaf from about 300 µl · l^{-1} to 2000 µl · l^{-1}. For measuring the CO_2 capacity L (thickness of a CO_2-saturated water layer containing as much CO_2 as the leaf contains $CO_2 + HCO_3^-$), the leaf was rapidly switched to CO_2-free air. The CO_2 released into the CO_2-free atmosphere was measured. From the amount of the released CO_2, pH values of the chloroplast stroma were calculated according to Oja et al. (1986)

and a pK of the system CO_2/HCO_3^- of 6.1 (Yokota and Kitaoka 1985) assuming that the volume of the chloroplast stroma was 1 µl · cm^{-2} leaf area. Maximum changes in pH were 0.3 units in the experiment of Fig. 3 and 0.2 units in a similar experiment (not shown). The maximum increase in PGA during the transition from low to high CO_2 (Table 1) was 28 nmol · cm^{-2} leaf area. Assuming that a considerable part of it was compartmented in chloroplasts and is dissociated completely, it should have caused a decrease in pH amounting to almost one pH unit (buffer capacity of the chloroplast stroma is close to about 35 mM · pH $unit^{-1}$ at physiological pH values; Pfanz and Heber 1986). Measured smaller changes in pH indicate that part of the PGA had entered the cytosol and-or the pH of the stroma had decreased sufficiently towards the pK of PGA that the carboxylic acid was not completely dissociated. Also, the leaf possesses a powerful pH-stat mechanism which can damp pH oscillations (Kurkidjian and Guern 1989; Wagner et al. 1990).

Assimilatory force, phosphorylation potentials, phosphate levels and redox states of the chloroplast NADP system during oscillations of photosynthesis after a transition from low to high light. The assimilatory force F_A is de fined as the product of the phosphorylation potential (ATP)/(ADP)(P_i) and the redox ratio (NADPH)/(NADP) (Heber et al. 1986b). It is very difficult to determine by measurements of adenylates, phosphate and pyridine nucleotides, but a useful approximation to its magnitude can be obtained from the measurements of PGA and DHAP. There is reason to assume (Dietz and Heber 1984; Usuda 1988) that the reactions catalyzed by phos-

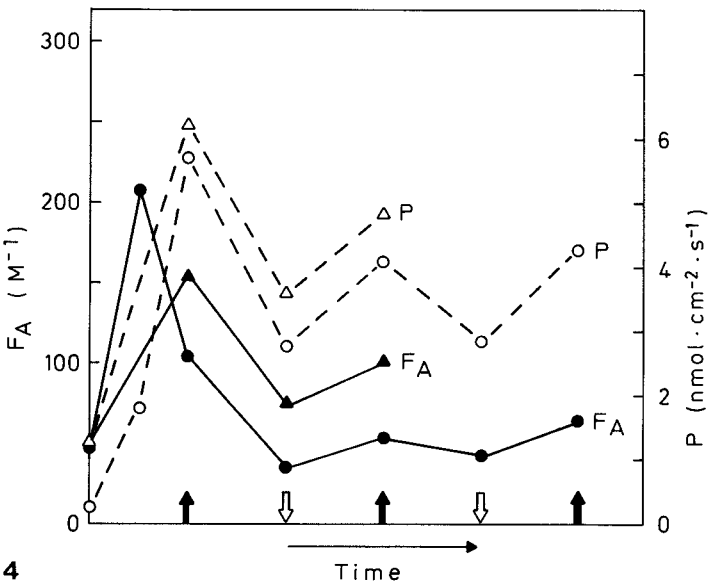

Fig. 4. Assimilatory force F_A (M^{-1}, *closed symbols*) and rates of photosynthesis (nmol · cm^{-2} · s^{-1}, *open symbols*) in the low/high light transition experiments 1 and 2 of Table 1

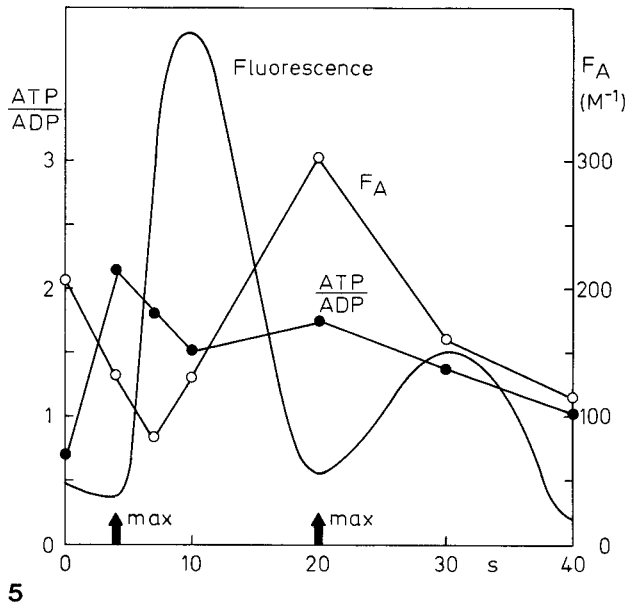

Fig. 5. Chlorophyll fluorescence, assimilatory force F_A and ATP/ADP ratios in a spinach *(Spinacia oleracea)* leaf. Photosynthetic oscillations were initiated by a transition from air to 5% CO_2 in air. The intensity of red light was 200 W · m^{-2}. Maxima of photosynthesis are indicated by *arrows*. Data were taken and recalculated from Furbank and Foyer (1986)

phoglycerate kinase, NADP-dependent glyceraldehydephosphate dehydrogenase and triosephosphate isomerase are sufficienfly close to equilibrium during many conditions of photosynthesis to justify calculation of F_A as

$$F_A = \frac{(\text{DHAP})\ 9.8 \cdot 10^{-6}}{(\text{PGA})\ (H^+)} = \frac{(\text{ATP})}{(\text{ADP})(P_i)} \frac{(\text{NADPH})}{(\text{NADP}^+)}. \quad \text{Eq. (3)}$$

In previous work (Siebke et al. 1990), (H^+) was taken as $1.58 \cdot 10^{-8}$ M, i.e. pH 7.8, whereas $9.8 \cdot 10^{-6}$ is taken as the equilibrium constant of the reaction

$$\text{PGA} + \text{ATP} + \text{NADPH} + H^+ \Longleftrightarrow \text{DHAP} + \text{ADP} + P_i + \text{NADP}^+ . \quad \text{Eq. (4)}$$

Although pH is not constant during the oscillations, we have chosen to use Eq. 3 as in previous publications (Heber et al. 1986b; Dietz and Heber 1989; Siebke et al. 1990). The arguments developed further below would actually have been strengthened, had we chosen to consider pH changes as they are shown in Fig. 3.

Results of calculating F_A are shown in Table 1 and Fig. 4. Also a set of data was taken from Fig. 1 in Furbank and Foyer (1986), who investigated photosynthetic oscillations in spinach leaves, and calculations are shown together with fluorescence data in Fig. 5. Depending on whether photosynthetic oscillations were initiated by a transition from low to high light (Fig. 4) or from low to high CO_2 (Table 1, Fig. 5), results differ in detail, but not in the essential observations. In the former case, high F_A coincided with maxima in photosynthesis, whereas in the latter case the decrease in carboxylation resistance caused by the increase in CO_2 first produced a decrease in F_A. This decrease was reversed only at the second maximum of photosynthesis (minimum of fluorescence in Fig. 5).

The cytosolic NADP system is separated from the chloroplast system, and it is usually largely reduced (Heber and Santarius 1965). Therefore, NADPH measurements in leaves include in addition to some chloroplast NADPH a large proportion of cytosolic NADPH, whereas NADP measurements reflect mainly the status of chloroplast NADP. Because NADPH measurements in leaves do not give meaningful information on the level of chloroplast NADPH, the assumption was made that the chloroplast NADP system contains a total of 2.5 nmol · cm^{-2} NADPH plus NADP. Under these assumptions, calculations of NADPH/NADP ratios are possible from NADP measurements (Table 1).

The calculations show that the chloroplast NADP system was more oxidized at the maxima of photosynthesis than at the minima. This agrees with measurements of the activity of the NADP-malate dehydrogenase which is active when NADPH/NADP ratios are high and inactive when they are low (Scheibe and Stitt 1988). It also agrees with the observation that P_{700} is more oxidized at the maxima than at the minima of oscillations (Figs. 1 and 2). However, it should be noted that maxima in P_{700} photooxidation precede maxima in photosynthesis and that similar relations hold for the minima. It is possible, therefore, that a slightly different timing of the collection of leaf samples for NADP measurements would have shown greater amplitudes in the NADPH/NADP ratios than obtained by sampling at the maxima and minima of photosynthesis (Table 1).

The assimilatory force F_A has the two components, phosphorylation potential and redox ratio NADPH/NADP, if changes in the proton concentration can be

neglected (Eq. 3). Information on redox ratios permits calculation of phosphorylation potentials by Eq. 3 (see Table 1). At the maxima of photosynthesis, phosphorylation potentials are high and at minima they are low. It is important to note that the differences in the phosphorylation potentials between the maxima and minima of photosynthesis would actually have been larger than shown in Table 1, had we considered the information on pH shifts during the oscillations which are demonstrated in Fig. 3. If, for instance, the stroma pH is 7.6 at the minima of photosynthesis and not 7.8 as assumed in the calculations, phosphorylation potentials would be 40% lower at the minima than shown in Table 1.

Adenosine triphosphat and ADP were measured during the transient from low to high light, and ATP/ADP ratios were calculated. Together with the phosphorylation potentials, they permit calculation of phosphate levels. It can be seen that estimated phosphate levels are lower in the maxima of photosynthesis than in the minima. However, it should be noted that the adenylate measurements include total leaf adenylates, not only chloroplast adenylates. Chloroplast and cytosolic adenylate systems communicate with one another (Heber and Santarius 1970; Yin et al. 1990), but cytosolic adenylates are in a higher state of phosphorylation than chloroplast adenylates. Thus, chloroplast phosphate levels may be lower than listed in Table 1.

Discussion

Oscillations may be considered a reflection of important regulatory processes in photosynthesis. This explains the attention this phenomenon has received. To our knowledge, oscillations in P_{700} photooxidation were first reported by the Sheffield photosynthesis group (Walker et al. 1988). Earlier attempts to elucidate causes of oscillations in photosynthesis had included measurements of chlorophyll fluorescence (Ogawa 1982; Walker et al. 1983), light scattering and the 518-nm electrochromic shift (Sivak et al. 1985). The saturation pulse analysis of chlorophyll fluorescence (Bradbury and Baker 1981; Schreiber et al. 1986) gives information on the state of reduction of a bound quinone, Q_A, in the reaction center of PS II and about membrane energization. Relative variable fluorescence which indicates the reduction of Q_A oscillates antiparallel to photosynthesis, whereas oscillations of q_E, a quenching parameter which characterizes membrane energization, slightly precede the photosynthetic oscillations.

Table 2 summarizes information derived from the simultaneous measurements of optical parameters with oscillations of photosynthesis in leaves. It can be seen that all parameters indicating energization of the photosynthetic apparatus are maximum just prior to maxima in photosynthesis. The acceptor side of both reaction centers of photosynthesis is oxidized when photosynthesis approaches maximum, and it is reduced showing accumulation of Q_A^- and leading to the reduction of photooxidized P_{700} when photosynthesis is close to minimum.

Table 2. Relationship between the kinetics of chlorophyll fluorescence, light scattering, P_{518}, P_{700} and oscillations in photosynthesis of leaves

	Rate of CO_2 uptake	
	Maximum	Minimum
Nonphotochemical or energy-dependent fluorescence quenching, Q_E	max[a]	min[a]
Light scattering	max[a]	min[a]
Electrochromic shift P518	max[a]	min[a]
P_{700} reduction, PS I	min[a]	max[a]
Q_A reduction, PS II	min	max

[a] Preceding changes in CO_2 uptake with a small phase shift; see Sivak et al. (1985), Walker et al. (1983), Walker et al. (1988)

As has been shown by Weis et al. (1990), the acceptor side of PS I is normally largely oxidized during photosynthesis at high irradiance, and the energy of the surplus of photons reaching PS I is converted into heat proportionally to the relative oxidation of P_{700}. Only under conditions which favour the accumulation of electrons on the acceptor side of PS I does oxidation of P_{700} decrease. Therefore, increased reduction of P_{700} during the minima of photosynthesis as shown in Figs. 1 and 2 is an indicator of over-reduction at the acceptor side of PS I.

Substrate measurements similar to ours and with similar results have been published before by Furbank and Foyer (1986) and by Stitt et al. (1988). We base our explanation of oscillations also on their work. Our contribution to the problem is the assimilatory-force analysis.

The coincidence between high assimilatory force, F_A, and high photosynthesis is informative. Flux of carbon in photosynthesis must be directly related to a driving force and inversely related to a flux resistance. Obviously, the primary driving force is light but products of light reactions are ATP and NADPH. They drive flux of carbon in the Calvin cycle. Because the carboxylation resistance decreases when the supply of CO_2 is suddenly increased at constant light, it is not unexpected that F_A first decreases in the low/high CO_2 transition (Table 1). Also, it is not unexpected that F_A increases when photosynthetic oscillations are initiated by an increase in irradiance. The light reactions are now simply capable of providing more ATP and NADPH. However, it is important that F_A is high at maximum carbon flux and low when photosynthesis is at its minimum. This shows that the minima in photosynthesis cannot be caused directly by restrictions in the carbon reactions of photosynthesis. In such a case, F_A would have to be at a maximum when carbon flux is at its minimum. It must be concluded that the minima in photosynthesis are caused by the inability of the chloroplast electron-transport chain to supply either enough ATP or NADPH.

Chloroplast NADPH is the mirror image of NADP. After a sudden transition from low- to high-irradiance light NADP first decreased transiently and then increased again. It was actually higher at the first maximum of photosynthesis under high light than under low light. The NADP was low again when photosynthesis was at

its minimum. Therefore, electron pressure is high and cannot be limiting in minima of oscillations. Our experimental data indicate that imbalances between the production of NADPH and ATP play a considerable role in photosynthetic oscillations. When NADPH accumulates at the minima of photosynthesis, phosphorylation potentials are low. They are high at the maxima of photosynthesis, when NADPH/NADP ratios are low.

In the following, we discuss our data on the basis of an interplay between linear electron flow to CO_2 and to other electron acceptors and cyclic electron transport. The work particularly of Arnon and his colleagues has shown that cyclic electron transport can function only if the electron-transport chain is appropriately poised (Arnon and Chain 1975, 1979). Details of the regulation of cyclic electron transport are unknown (Moss and Bendall 1984), but it is known that it cannot occur under conditions of over-oxidation and of over-reduction. Ogawa (1982) has already suggested that oscillations are generated when the electron-transport chain comes out of correct poising.

Over-oxidation is characterized by oxidized P_{700}, oxidized ferredoxin and oxidized NADP. In this situation, NADP is the preferred electron acceptor, whereas under conditions of over-reduction (reduced intersystem electron carriers) electrons are difficult to donate into the cyclic electron-transport pathway although they are available on the reducing side of PS I. Transient over-reduction is demonstrated in the experiments of Figs. 1 and 2 which show that P_{700} becomes essentially reduced during the first minimum in a cycle of photosynthetic oscillations. There is ample evidence about high reduction of Q_A and, consequently, plastoquinone in the same situation (Table 2). Evidence for high oxidation in the maxima of the oscillations can be derived from the behaviour of P_{700} (Figs. 1, 2) and the data of Table 1 which show oxidation of the chloroplast NADP system.

The mode of coupling between ATP synthesis and photosynthetic electron transport in leaves is still an enigma. It appears that at least at high photosynthetic rates linear electron flow to CO_2 supports insufficient ATP production for CO_2 assimilation. Additional ATP production by alternative electron flows to nitrite, oxaloacetate (NADP-malate dehydrogenase) or by cyclic electron flow to plastoquinone is necessary. If ATP synthesis and NADPH production must be adjusted to one another, any state of photosynthesis is a compromise between the regulatory processes determining electron flow to CO_2 and to those alternative acceptors. To permit a high rate of electron flow to CO_2, the NADP system must be largely oxidized, because it is known that NADPH allosterically inhibits ferredoxin-NADP reductase (Lechtenberg et al. 1990). At the same time, ferredoxin and-or NADP must be sufficiently reduced to support alternative electron flows to nitrite, oxaloacetate and oxygen and-or the cyclic electron flow (which depends not only on the redox state of ferredoxin but also on that of plastoquinone). Therefore, the flux resistance of the ferredoxin-NADP reductase must be adjusted so as to maintain a redox situation which simultaneously satisfies the requirements of linear flow to CO_2, linear flows to nitrite, oxygen and oxaloacetate and-or to the cyclic electron pathway. At a sudden transition from a low to a high rate of photosynthesis, a new compromise must be reached between the contrasting requirements of fast linear electron flow to CO_2 and the corresponding ATP production which is needed to satisfy the ATP requirements of carbon assimilation. This compromise is reached via a series of oscillations: when a leaf photosynthesizing in air under high irradiance is suddenly supplied with saturating CO_2, PGA is rapidly generated by the carboxylation of ribulose bisphosphate. Its reduction leads to the oxidation of the chloroplast NADP system. Fast coupled electron flow then produces the first maximum of thylakoid energization and the first maximum of photosynthesis. Similarly, the transition from darkness or low to high-irradiance illumination results after a transient reduction of the chloroplast NADP system in its oxidation while the rapid generation of PGA by the preactivated photosynthetic apparatus makes fast electron flow possible. This also generates a maximum in photosynthesis. However, the oxidation of the NADP system diminishes electron flow into the alternative and cyclic pathways, decreasing ATP synthesis and leading to ATP deficiency. In this situation, photosynthesis decreases towards a minimum and NADPH accumulates. Increased reduction of the NADP system now favours cyclic electron flow (which is still hindered by the partial overreduction of the intersystem electron carriers) and, especially, alternative linear flows to nitrite and oxaloacetate. Direct oxidation of the PSI acceptor side by oxygen in a Mehler reaction is also possible (Schreiber and Neubauer 1990), although oscillations can be observed also in complete absence of oxygen in the ambient gas. Additional linear and cyclic photophosphorylation reduces and finally abolishes the ATP deficiency. In consequence, photosynthesis accelerates. This sequence of events repeats itself until the oscillations of photosynthesis are damped out.

It is important to note that maxima in light scattering or energy-dependent fluorescence quenching and maxima in 518-nm absorption have been reported to precede maxima in photosynthesis (Sivak et al. 1985). Apparently, thylakoid energization was already declining and reduction of the NADP system increasing when photosynthesis approached a maximum. This situation illustrates that photosynthesis can be driven either by the redox component of the assimilatory force or by the phosphorylation potential. Simultaneously, control of electron flow either by the transthylakoid proton gradient or by the ferredoxin-NADP reductase explains why steady-state photosynthesis, although light- and CO_2-saturated, is slower than maximum photosynthesis during oscillations.

Attempts to simulate oscillations as explained above using a mechanistic computer model of synthesis have so far been unsuccessful. An imbalance between linear and cyclic electron transport has in the model led to an irreversible inhibition of photosynthesis which is characterized by overreduction of electron carriers and a collapse of the proton gradient (Laisk and Eichelmann 1989). To permit recovery of photosynthesis in the com-

puter model, it was necessary to change the parameters manually by introducing an ATP-producing reaction. Apparently, an important control reaction leading to over-reduction and permitting recovery from it was still not considered (Heineke et al. 1989).

In previous work on photosynthetic oscillations it has been concluded that temporary phosphate deficiency is responsible for photosynthetic oscillations (Laisk and Walker 1986). It is based, among others, on the observation that feeding of mannose to leaves, which results in the sequestration of inorganic phosphate as mannose phosphate, causes photosynthesis to oscillate already at low CO_2 concentrations (Harris et al. 1983). The estimated concentration of phosphate (Table 1) is lower at the peaks of photosynthesis than at the troughs. This may be considered as evidence against the idea of straightforward phosphate limitation in minima of photosynthesis. On the other hand, the estimates are based on assumptions, from which the most important is the assumption of thermodynamic equilibrium of PGA kinase and glyceraldehydephosphate dehydrogenase reactions. If photosynthesis is limited by PGA kinase because of a low ATP level, the reaction sequence will no longer be at equilibrium.

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