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Photoinhibition of photosynthesis under anaerobic conditions studied with leaves and chloroplasts of *Spinacia oleracea* L.

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Abstract. The role of oxygen in the photoinactivation of the photosynthetic apparatus of *Spinacia oleracea* L. was investigated. Moderate irradiation (1200 μmol photons m^{-2} s^{-1}) of spinach leaves in an atmosphere of pure nitrogen caused strong inhibition of subsequently measured net CO_2 assimilation, whereas considerably less photoinhibition was observed in the presence of low partial pressures (10–20 mbar) of O_2. The decrease in activity caused by anaerobiosis in the light was not based on stomatal closure; the decline of assimilation represents a photoinhibition, as activity was not impaired by low irradiation (80 μmol photos m^{-2} s^{-1}). In contrast, gassing with pure N_2 in the dark caused strong inhibition. Electron-transport rates and chlorophyll-fluorescence data of thylakoids isolated from photoinhibited leaves indicated damage to the electron-transport system, in particular to photosystem II reaction centers. In vitro, photoinhibition in isolated thylakoid membranes was also strongly promoted by anaerobiosis. Photoinhibition of electron-transport rates under anaerobic conditions was characterized by a pronounced increase in the initial fluorescence level, F_0, of chlorophyll-fluorescence induction, in contrast to photoinhibition under aerobic conditions. The results are discussed in terms of two mechanisms of photoinhibition, one that is suppressed and a second that is promoted by oxygen.

Key words: Anaerobiosis – Chlorophyll *a* fluorescence – Photoinhibition – Photosynthesis (CO_2 assimilation, electron transport) – *Spinacia* (photoinhibition) – Thylakoids, isolated.

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Abbreviations: Chl = chlorophyll; DCMU = 3-(3', 4'-dichlorophenyl)-1,1-dimethylurea; PSI, II = photosystem I, II

Introduction

Excess light energy causes damage to the photosynthetic apparatus of green plants. Such damage becomes manifest as inhibition of various photosynthetic reactions. The phenomenon of photoinhibition has been recently reviewed by Powles (1984). There is wide agreement that a primary but not the only site of damage is the reaction center of photosystem (PS) II. The molecular mechanism of photoinhibition is largely unknown. Possibly, radical reactions are involved in it. In particular, reactive species of oxygen may be formed when photochemical side reactions are enhanced by surplus excitation of photosynthetic pigments. According to a recent study of photoinhibition in isolated thylakoid membranes (Barényi and Krause 1985), reaction systems that scavenge reactive products of O_2, significantly diminish but do not totally prevent photoinhibition of electron transport. This indicates the involvement of oxygen. However, there are conflicting reports regarding the role of oxygen in photoinhibition. In experiments with isolated spinach thylakoids, Trebst (1962) demonstrated a photoinhibition of PSII activity under anaerobic conditions, which was prevented by the presence of O_2 at low partial pressures (about 4 mbar). Promotion of photoinhibition by anaerobiosis was also reported by Satoh and Fork (1982a, b). They observed inactivation of PSII, and in a slower reaction also of PSI, in intact chloroplasts of *Bryopsis corticulans*. This inhibition occurred in the absence of O_2 in low light that under aerobic conditions did not cause damage. In contrast, Satoh (1970) and Powles and Björkman (1982) reported that in isolated thylakoids photoinhibition of PSII was essentially O_2-independent, whereas photoinactivation of PSI re-

quired the presence of oxygen. Likewise, Morris et al. (1982) found that removal of O_2 did not significantly affect photoinhibition of whole-chain electron transport in *Chenopodium quinoa* thylakoids. From a chlorophyll-fluorescence study with spinach thylakoids, Satoh (1971) concluded that two different mechanisms were responsible for PSII photoinhibition in the presence and absence of O_2. Increased levels of O_2 in the suspension medium promoted photoinhibition of CO_2-dependent O_2 evolution by intact chloroplasts and cells of spinach (Krause et al. 1978).

Photosynthetic and photorespiratory carbon metabolism in intact leaves occuring in normal air is supposed to protect from photoinhibition via utilization of photochemical energy. Such protection has recently been demonstrated in CO_2-fixing intact chloroplasts (Barényi and Krause 1985). In leaves, photoinhibition of photosynthesis readily occurs upon illumination in a CO_2-free atmosphere with low partial pressure (10–20 mbar) of oxygen (for references, see Powles 1984). Then, the activities of the carbon-reduction and carbon-oxidation cycles are both severely restricted, which leads to an energy surplus. In CO_2-free air (210 mbar O_2), less or no photoinhibition is usually observed; this protecting effect of high O_2 levels has been attributed to photorespiratory energy dissipation. In apparent contradiction to this hypothesis, leaves of *Sinapsis alba* exhibited little photoinhibition at 40 mbar O_2 (Cornic 1978), when the rate of photorespiration should still be low. However, the extent of photoinhibition strongly increased in Cornic's experiments, when the O_2 partial pressure was lowered to 1 mbar.

To clarify the role of O_2, we have investigated the photoinhibition occurring in attached spinach leaves upon illumination with a moderate photon fluence rate under aerobic and anaerobic conditions. We found that anaerobiosis strongly promotes photoinhibition. This result is corroborated by experiments carried out in vitro with isolated thylakoid membranes.

Material and methods

Plant material. Plants of *Spinacia oleracea* L., cultivar Yates, were grown in a partially shaded greenhouse (maximum midday photon flux density was about 1200 μmol photons m^{-2} s^{-1}) in a hydroponic culture with Hoagland's plant culture medium. Four- to six-week-old plants were used for the experiments.

Gas-exchange technique. Rates of net CO_2 assimilation and of transpiration were determined in a gas-exchange system (Wong 1979), modified as described by von Caemmerer and Farquhar (1981). A single leaf (area 40–65 cm^2) attached to the plant was placed into a glass and aluminum leaf chamber, the roots being immersed in air-saturated nutrient solution. Within the chamber, a fan circulated air past the leaf. The air temperature in the chamber was maintained by circulating water through the water jacket of the chamber from an external temperature-controlled water bath. The leaf temperature was measured with a copper-constantan thermocouple attached to the abaxial surface of the leaf and was kept at 25 °C.

Air was passed through the chamber at a rate of 8–10 l min^{-1} monitored with a Brooks mass-flow meter, model 5810 (Brooks Instrument Div., Emerson Electric Co., Hatfield, Pa., USA). Various gas mixtures were obtained by mixing CO_2, CO_2-free air and nitrogen using three Tylan mass-flow controllers, model FC 260 (Tylan Corp., Carson, Cal., USA). Illumination was provided by a water-cooled, high-pressure, xenon arc lamp (Osram, München, FRG; XBF 2500 W), the UV and infra-red components being removed with a Schott 115 filter (Schott and Gen., Mainz, FRG). Irradiance was varied by changing the distance of the lamp from the leaf and by interposing copper screens. Rates of CO_2 assimilation (A) and of transpiration (E), leaf conductance to water vapor (G), and intercellular partial pressure of CO_2 (P_i) were calculated from measured data according to Wong et al. (1978); P_i was varied by changing the ambient partial pressure of CO_2. The area of one side of the leaf only was considered in the calculations.

Photoinhibitory treatment of leaves consisted of 2 h irradiation with 1200 μmol photons m^{-2} s^{-1} white light. The gas phase was composed as specified in Results.

Isolation of thylakoid membranes from single spinach leaves. One leaf (midrib removed) was homogenized with a Sorvall Omnimix for 10 s in 20 ml of solution A, pH 6.1 (Jensen and Bassham 1966) containing in addition 0.4% bovine serum albumin, 0.05% cystein, and 2 mM sodium ascorbate. The homogenate was filtered through nylon mesh (20 μM) and centrifuged for 1 min at 2000 *g*. The pellet was suspended in 10 ml of $MgCl_2$ solution (5 mM), and 10 ml of a double-strength medium were added to give final concentrations of 0.33 M sorbitol, 5 mM $MgCl_2$, 1 mM KH_2PO_4, 5 mM NaCl, 0.4% bovine serum albumin, and 40 mM N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid (Hepes), pH 7.6 (NaOH). This suspension was centrifuged for 5 min at 2000 *g* and resuspended in 0.05 ml of the same medium (without serum albumin). Chlorophyll (Chl) was determined according to Arnon (1949). The suspension was stored at 0 °C and for measurements was diluted to 10 μg Chl ml^{-1}. Samples of 2 ml were used.

Whole-chain electron-transport rates of these chloroplasts were measured at 25 °C in the presence of 25 μM methylviologen, 1 mM NaN_3, and 2.5 mM NH_4Cl upon illumination with 660 μmol photons m^{-2} s^{-1} (about 70% saturating) blue light (filter 9782, Corning, New York, USA). Oxygen uptake was determined with a Clark-type electrode (Delieu and Walker 1972).

Chlorophyll-fluorescence induction at 25 °C in the thylakoid suspension with or without 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU) was detected at 683 nm (filters IL 683 and RG 645 of Schott & Gen. and Calflex C from Balzers, Liechtenstein) by means of glass-fiber optics leading the emitted light from the top of the suspension to a photodiode. The diode was connected to a transient recorder (Model TCC-1000, Riken Denshi Co., Japan). A part of the glass-fiber bundle was used to provide the sample with blue actinic light (see above). Irradiance at the surface of the suspension was 400 μmol photons m^{-2} s^{-1}. For recording in the absence of DCMU,

samples were preilluminated for 1 min with far-red light (4 μmol photons m^{-1} s^{-1}, filters IL 720 and RG 695, Schott) followed by 30 s dark time prior to onset of actinic blue irradiation.

Corrected chlorophyll-fluorescence spectra at 77 K were measured with a Perkin-Elmer (Norwalk, Conn., USA) MPF-44B Fluorescence Spectrophotometer with Model 150 Xenon Power Supply and attached DCSU-2 unit and X-Y recorder. An OG 530 filter (Schott) was interposed between sample and emission detector. Samples contained 5.6 μg Chl ml^{-1}.

Photoinhibition of thylakoid reactions in vitro. Chloroplasts were isolated according to Jensen and Bassham's (1966) procedure as modified by Heber (1973). After the second centrifugation step, the envelopes were osmotically broken (5 mM $MgCl_2$) and the thylakoids stored in solution B (pH 6.7) to which 5 mM $MgCl_2$ had been added. Photoinhibitory treatment and measurements were carried out in a medium of 0.33 M sorbitol, 5 mM $MgCl_2$, 1 mM KH_2PO_4, 5 mM NaCl, and 40 mM Hepes, pH 7.6 (NaOH). Oxygen was removed from the medium by addition of 10 mM glucose, 0.1 mg ml^{-1} glucose oxidase (EC 1.1.3.4), and 1000 units ml^{-1} catalase (EC 1.11.1.6). Defined low levels of O_2 were adjusted using limiting amounts of glucose. For aerobic conditions (air-saturated medium), glucose was omitted. Photoinhibition was caused by irradiation of 2-ml samples (containing 100 μg Chl) with 3000 μmol photons m^{-2} s^{-1} white light at 20 °C. Subsequent to pretreatment, the thylakoid membranes were sedimented (3 min, 2000 *g*) and resuspended in the above medium (air saturated; without the glucose-oxidase system). Chlorophyll-fluorescence induction at 686 nm was recorded (see Krause et al. 1982) and uncoupled electron transport from water to ferricyanide (0.6 mM $K_3[Fe(CN)_6]$, 10 mM NH_4Cl) measured at 20 °C.

Enzymes were purchased from Boehringer (Ingelheim, FRG).

Results

Photoinhibition in vivo. Enhancement of photoinhibition under anaerobic as compared with aerobic conditions is demonstrated in Figs. 1–4. Spinach

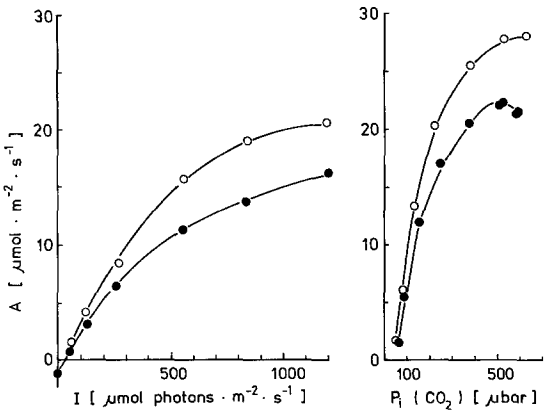

Fig. 1. Net CO_2 assimilation, *A*, of a spinach leaf as function of irradiance, *I*, and intercellular CO_2 partial pressure, P_i (CO_2); measurements before (○) and after (●) 2 h irradiation with 1200 μmol photons m^{-2} s^{-1} white light in a CO_2-free atmosphere containing 10 mbar O_2 in N_2. The *A–I* curves were measured in air containing 340 μbar CO_2; for *A–*P_i curves, irradiance was 1200 μmol photons m^{-2} s^{-1} white light. The leaf temperature was 25 °C

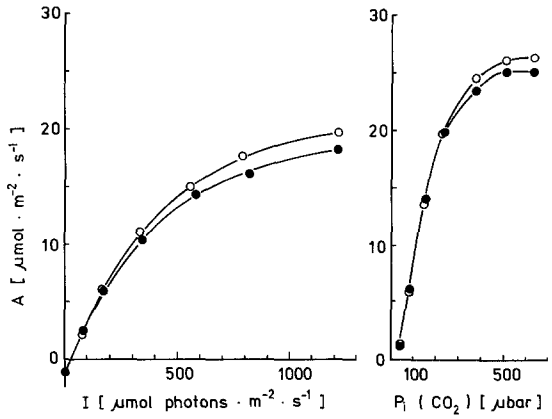

Fig. 2. Net CO_2 assimilation, *A*, of a spinach leaf as function of irradiance, *I*, and intercellular CO_2 partial pressure, P_i (CO_2); measurements before (○) and after (●) 2 h irradiation with 1200 μmol photons m^{-2} s^{-1} white light in air (340 μbar CO_2). Other conditions as for Figure 1

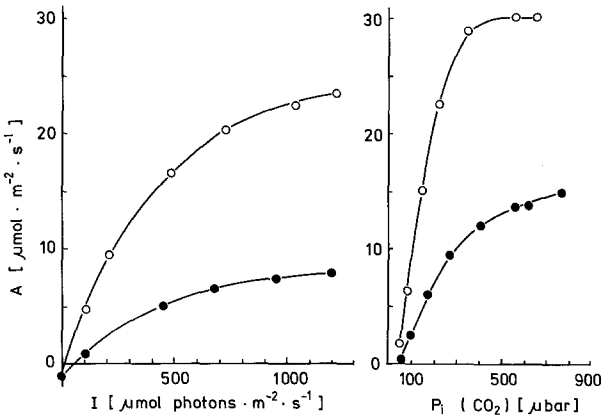

Fig. 3. Net CO_2 assimiliation, *A*, of a spinach leaf as function of irradiance, *I*, and intercellular CO_2 partial pressure, P_i (CO_2); measurements before (○) and after (●) 2 h irradiation with 1200 μmol photons m^{-2} s^{-1} white light in an atmosphere of pure N_2. Other conditions as for Figure 1

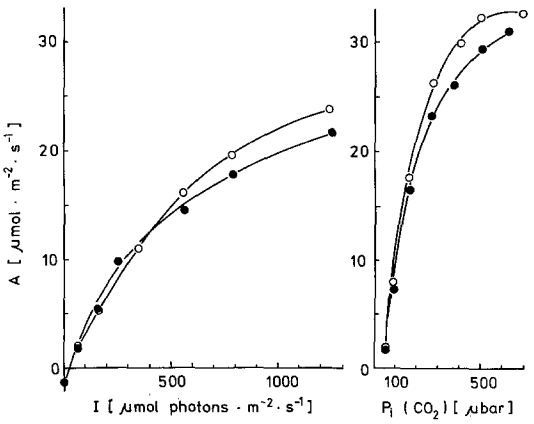

Fig. 4. Net CO_2 assimiliation, *A*, of a spinach leaf as function of irradiance, *I*, and intercellular CO_2 partial pressure, P_i (CO_2); measurements before (○) and after (●) 2 h irradiation with 80 μmol photons m^{-2} s^{-1} white light in an atmosphere of pure N_2. Other conditions as for Figure 1

leaves were irradiated in the specified gas phase for 2 h with 1200 µmol photons m^{-2} s^{-1} white light, corresponding to the maximum irradiance during growth. Photosynthesis of individual leaves was measured as a function of irradiance and of intercellular partial pressure of CO_2 before this treatment. Subsequent to photoinhibitory irradiation, a recovery period of 1–2 h under standard conditions (air with 340 µbar CO_2; 1200 µmol photons m^{-2} s^{-1} white light) was allowed to restore a stationary rate of photosynthesis (see below). Then A–I and A–P_i curves were measured again. Figure 1 shows that irradiation in nitrogen containing 10 mbar (1%) O_2 caused a moderate degree of photoinhibition of CO_2 assimilation, as was expected from earlier experiments of Powles and Osmond (1978) and Powles et al. (1979). The decline of A at high P_i in the inhibited leaf appears to be an effect of supraoptimal CO_2 concentration, as described by Woo and Wong (1983). The experiment of Fig. 2 served as a control to show that the assimilation was not appreciably affected by preillumination in normal air; only a slight decrease in activity was observed. In contrast, strong photoinhibition was seen upon illumination in pure nitrogen (Fig. 3). This inhibition was caused by high irradiance in the absence of O_2 but not by anaerobiosis per se, as demonstrated in Fig. 4. Subsequent to low irradiation in N_2, almost full activity, as in normal air (see Fig. 2), was retained. Leaves kept in N_2 in the dark could not serve as controls in these experiments, because they exhibited a strong effect of wilting of the tissue. Apparently, a minimum partial pressure of O_2 or low irradiation are required to maintain turgor. Although the wilting effect was reversed upon return to standard conditions, strong inhibition of photosynthesis resulted from gassing with N_2 in the dark.

In the experiment shown in Figure 5, A–P_i curves were measured at different irradiances prior to photoinhibitory treatment under aerobic or anaerobic conditions. It can be seen that in both cases the A–P_i curve of the photoinhibited leaf (measured at 1200 µmol photons m^{-2} s^{-1}) closely corresponds to an interpolated A–P_i curve at a lower irradiance. Thus, photoinhibitory treatment has the same effect as lowering the photon fluence rate. This is consistent with the assumption of an inhibition of photosynthetic electron transport (see model by von Caemmerer and Farquhar 1981).

In Table 1, the extent of photoinhibition in various experiments, based on measurements under standard conditions is listed. It shows again that in the presence of O_2 (at partial pressures as low as 1 mbar) significantly less inhibition of net CO_2 assimilation occurred than in pure nitrogen. Anaerobiosis was not inhibitory in low light.

As depicted in Fig. 6, subsequent to photoinhibitory treatment a short-term recovery phase (compare Powles et al. 1979) was observed upon return to standard conditions. It should be noted again that all measurements of the extent of photoinhibition were done after this partial

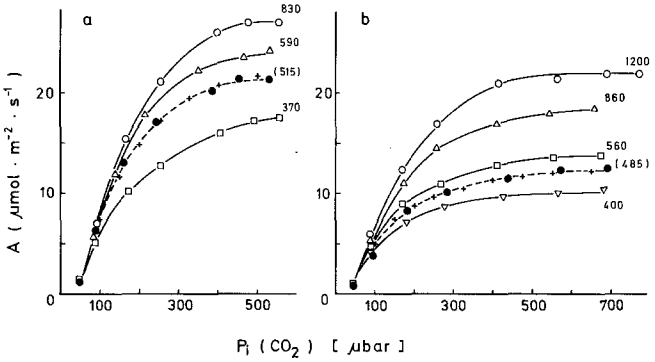

Fig. 5 a, b. Photoinhibition of CO_2 assimiliation, A, of a spinach leave by 2 h photoinhibitory treatment with 1200 µmol photons m^{-2} s^{-1} white light in N_2 containing 20 mbar O_2 (**a**) and in pure N_2 (**b**). Prior to photoinhibitory treatment, A was measured as a function of intercellular CO_2 partial pressure, P_i (CO_2), at different irradiances (*open symbols;* irradiances in µmol photons m^{-2} s^{-1} given in the graph). Subsequent to treatment, A–P_i curves were measured at 1200 µmol photons m^{-2} s^{-1} (●). The O_2 partial pressure for all measurements was 210 mbar. An interpolated A–P_i curve of the state before treatment (+) was constructed for the irradiance given in brackets. Interpolation was based on this "apparent" irradiance corresponding to the value of A at P_i = 250 µbar of the inhibited leaf. The light-saturated A–P_i curve in the inhibited state corresponds to the A–P_i curve in the uninhibited state simulated for lower irradiance

Table 1. Inhibition of net CO_2 assimilation of spinach leaves by 2 h photoinhibitory treatment with 1200 µmol photons m^{-2} s^{-1}, white light (measurements were made in the same light in normal air containing 340 µbar CO_2 and 210 mbar O_2, before and after the 2-h illumination period)

Gas-phase composition during 2 h illumination	Inhibition[a] (%)
Air, 340 µbar CO_2	9±2 (3)
O_2 (20 mbar) in N_2	14±9 (6)
O_2 (10 mbar) in N_2	18±5 (4)
O_2 (1 mbar) in N_2	19±6 (2)
Pure N_2	64±16 (7)
Pure N_2 (low light[b])	11±3 (2)

[a] Mean values and standard deviations are given (number of experiments in brackets). Inhibition is referred to the individual rates of net CO_2 assimilation before the 2-h illumination; the mean of these rates was 20.7±3.2 µmol m^{-2} s^{-1}

[b] Illumination for 2 h with 80 µmol photons m^{-2} s^{-1}, white light

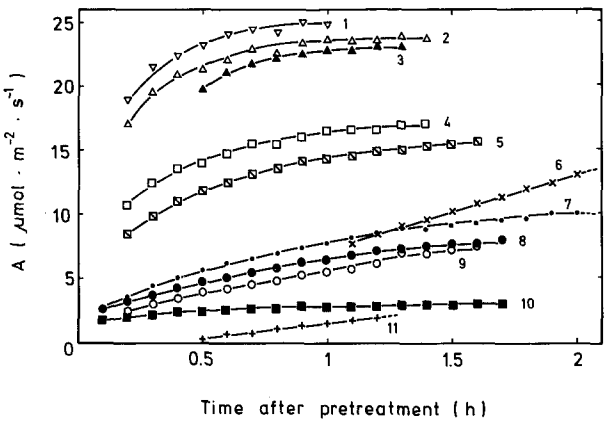

Fig. 6. Short-term recovery of net CO_2 assimilation, *A*, of spinach leaves after various pretreatments as function of time; measurements under standard conditions (air with 340 µbar CO_2; irradiance, 1200 µmol photons m^{-2} s^{-1}). Pretreatments: *Open symbols*, preillumination (1200 µmol photons m^{-2} s^{-1} white light) in the presence of O_2; *curve 1*, 2 h at 20 mbar O_2 in N_2; *curves 2,4*, 2 h at 10 mbar O_2 in N_2; *curves 5,9*, 4 h at 10 mbar O_2 in N_2. *Closed symbols*, treatment in pure N_2; *curve 3*, 2 h low irradiance, 80 µmol photons m^{-2} s^{-1} white light; *curves 8,10*, 2 h high irradiance, 1200 µmol photons m^{-2} s^{-1}; *curves 6,11*, 2 h in the dark; inhibition of *A*, 19% (6 h recovery) and 59% (4 h recovery), respectively

recovery was completed. Long-term recovery within 1–2 d (Powles et al. 1979) was not investigated. In the short term, the recovery was faster in the case of slight inhibition as compared with strong inhibition. Leaves irradiated in pure N_2 exhibited a similar recovery kinetics as those irradiated in the presence of O_2, as seen, e.g., by comparison of curves 8 and 9 in Fig. 6. Notably, the recovery kinetics of photoinhibited leaves was clearly different from that of leaves kept in N_2 in the dark (curves 6 and 11), showing a slow linear recovery for several hours.

Table 2 lists, besides A and P_i, values of leaf conductance, G, from representative experiments. The data indicate increased stomatal opening during photoinhibitory treatment; this is expected as a response to removal of CO_2 from the gas phase. The response is similar in the presence and absence of O_2. Towards the end of the photoinhibitory-treatment period, a decline of leaf conductance was frequently observed. However, after short-term recovery under standard conditions, the inhibited leaves of all experiments were still capable of adjusting conductance to maintain a normal internal CO_2 partial pressure. Stomatal closing was observed in N_2 in the dark followed by partial re-opening upon return to standard conditions. Thus the inhibition of CO_2 assimilation observed in our experiments was not caused by closing of stomates.

Data from thylakoid membranes isolated from single leaves (Table 3) indicate that the electron-transport system was affected by photoinhibitory irradiation under both aerobic and anaerobic conditions. The rates of whole-chain electron transport of thylakoids from individual control leaves varied widely. This is probably the reason why decreased electron-transport rates were only detected in thylakoids from strongly photoinhibited leaves. However, there was a close correlation between the ratio of variable to initial chlorophyll fluorescence, F_v/F_0, of thylakoids and inhibition of CO_2 assimilation in leaves. This indicates damage to PSII reaction centers. Very similar data were found when fluorescence was

Table 2. Magnitudes of CO_2 assimilation, A, intercellular CO_2 partial pressure, P_i (CO_2), and leaf conductance to water vapor transfer, G. For calculations see Wong et al. (1978).

Pretreatment[a]	Condition of measurement	A (µmol m^{-2} s^{-1})	P_i (CO_2) (µbar)	G (mol m^{-2} s^{-1})
–	Standard[b]	19	225	0.38
O_2 (10 mbar) in N_2, 1 h	O_2 (10 mbar)	–	–	0.57
O_2 (10 mbar) in N_2, 4 h	O_2 (10 mbar)	–	–	0.40
O_2 (10 mbar) in N_2, 4 h	Standard	7.5	273	0.40
–	Standard	21	242	0.42
Pure N_2, 1 h	Pure N_2	–	–	0.52
Pure N_2, 2 h	Pure N_2	–	–	0.42
Pure N_2, 2 h	Standard	8.3	286	0.32
Pure N_2 (low light[c]), 2 h	Standard	19	245	0.36
Pure N_2 (dark), 2 h	Pure N_2 (dark)	–	–	0.06
Pure N_2 (dark), 2 h	Standard	6.9	275	0.19

[a] Irradiation in specified CO_2-free atmosphere, 1200 µmol photons m^{-2} s^{-1} (white light), if not indicated otherwise
[b] Standard conditions: air containing 340 µbar CO_2; irradiance, 1200 µmol photons m^{-2} s^{-1}, white light
[c] 80 µmol photons m^{-2} s^{-1}, white light

Table 3. Photoinhibition of net CO_2 assimilation of spinach leaves compared with chlorophyll-fluorescence ratios F_v/F_0 and electron-transport rates of thylakoids isolated after photoinhibitory treatment (1200 μmol photons m^{-2} s^{-1} white light)

Conditions of preillumination	Expt. No.	Inhibition of CO_2 assimilation[a] (%)	Chl fluorescence[b] (F_v/F_0)	Electron transport	
				Rate[c]	Inhibition (%)
Controls[d]	1–6	–	4.0 ± 0.5	109 ± 16	–
O_2 (2 h, 10 mbar) in N_2	7	20	3.1	124	0
	8	10	4.3	131	0
O_2 (4 h, 10 mbar) in N_2	9	62	1.7	83	24
	10	18	2.9	119	0
Pure N_2 (2 h)	11	81	0.9	57	48
	12	61	1.5	75	31
	13	86	0.7	39	64
	14	62	1.7	88	19

[a] Net CO_2 uptake was measured before and after the preillumination period in normal air containing 340 μbar CO_2 and 210 mbar O_2, at 1200 μmol photons m^{-2} s^{-1} white light

[b] Chlorophyll-fluorescence induction (25 °C) of isolated thylakoids recorded at 685 nm upon actinic illumination with 400 μmol photons m^{-2} s^{-1} blue light. F_v = variable fluorescence in the peak of emission; F_0 = initial fluorescence

[c] Uptake of O_2 in the presence of 2.5 mM NH_4Cl, 1 mM NaN_3, and 25 μM methylviologen; actinic blue light, 660 μmol photons m^{-2} s^{-1} (about 70% saturating); rates in μmol mg^{-1} Chl h^{-1}

[d] Controls, leaves without preillumination; rates of net CO_2 assimilation were 22.0 ± 1.7 μmol m^{-2} s^{-1}

Table 4. Chlorophyll-fluorescence emission at 77 K of PSII and PSI (band peaks at 695 and 735 nm) of thylakoids isolated from photoinhibited spinach leaves (photoinhibitory treatment as for Table 3). Values (fluorescence intensity in relative units) are normalized at the 535 nm emission of fluorescein (sodium salt) added in 1.8 μM concentration as an internal standard

Condition of preillumination	Expt.[a] No.	F_{695}		F_{735}	
		Intensity (relative)	Inhibition (%)	Intensity (relative)	Inhibition (%)
Controls[b]	1–3	113 ± 14	–	135 ± 6	–
O_2 (2 h, 10 mbar) in N_2	7	93	18	126	7
Pure N_2 (2 h)	12	76	33	96	29
Pure N_2 (2 h)	13	51	55	77	43

[a] Compare Table 3

[b] No preillumination

measured in the presence of 20 μM DCMU (not shown). Destruction of PSII is also apparent from the lowering of fluorescence bands at 695 (and 735) nm in fluorescence spectra recorded at 77 K (Table 4; compare Powles and Björkman 1982; Barényi and Krause 1984).

Photoinhibition in vitro. Promotion of photoinhibition by anaerobiosis is not confined to the intact leaves, as shown by preillumination of thylakoid membranes in vitro (Fig. 7). Electron transport was inhibited much faster in the absence of oxygen than in air-saturated medium, as first observed by Trebst (1962). The decrease in activity was accompanied by characteristic changes in the signals for chlorophyll-fluorescence induction measured in the presence of DCMU. The maximum variable fluorescence F_v and the F_v/F_0 ratio strongly decreased. A large increase in the F_0 level (see also inset to Fig. 7) was observed upon irradiation under anaerobiosis, in agreement with results by Satoh (1971). In contrast, only a small increase in F_0 was seen when a comparable inhibition of electron transport had been caused by irradiation under aerobic conditions (Barényi and Krause 1985). The enhanced F_0 level caused by anaerobic light treatment was also visible when induction was recorded in the absence of DCMU subsequent to weak far-red illumination. Thus, the high F_0 level is probably not based on partially reduced electron acceptors of PSII, Q_A. Preincubation of thylakoids in O_2-free medium in the dark neither affected electron transport nor fluorescence induction. It should be noted that removal of O_2 by the glucose/glucose oxidase system or by gassing the medium with N_2 gave similar results. At low levels of oxygen corresponding to 1–2% O_2 in the atmosphere, the photoinhibition effect was not different from that in an air-saturated medium.

Discussion

Our results show an enhancement of photoinhibition under anaerobic as compared with aerobic conditions. This refers to photoinhibition caused

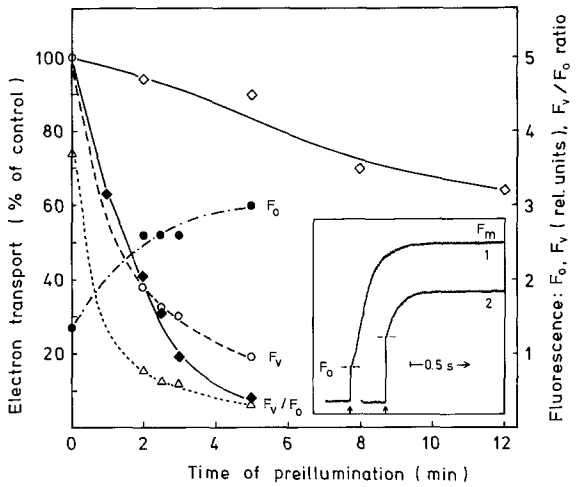

Fig. 7. Effects of preillumination of isolated spinach thylakoids with 3000 µmol photons m^{-2} s^{-1} white light on electron transport and chlorophyll-fluorescence induction at 20 °C. Thylakoids were irradiated in aerobic or anaerobic medium for the times given in the graph. Anaerobiosis was achieved by means of the glucose/glucose oxidase system. Subsequent to this pretreatment the thylakoids were centrifuged and resuspended in air-saturated medium for measurement of electron transport and fluorescence induction (for details see Material and methods). Uncoupled electron transport was measured in broadband red light (1000 µmol photons m^{-2} s^{-1}) in the presence of ferricyanide. ◇ Electron transport rates after light pretreatment in air-saturated medium (as % of dark-incubated controls); control rates of samples incubated in the dark for 2 and 12 min, respectively, were 154 and 132 µmol O_2 mg^{-1} Chl h^{-1}. ◆ Electron-transport rates after light pretreatment in anaerobic medium (as % of dark-incubated controls); control rate after 2.5 min dark-incubation, 207 µmol O_2 mg^{-1} Chl h^{-1}. Fluorescence induction was recorded in the presence of 20 µM DCMU in blue actinic light (5 µmol photons m^{-2} s^{-1}). *Broken lines* denote initial fluorescence, F_0 (●), maximum variable fluorescence, F_v (○), and F_v/F_0 ratios (△), plotted as function of time of light pretreatment in anaerobic medium. *Inset.* Fluorescence-induction signals of thylakoids after 2 min preincubation in anaerobic medium in the dark (*trace 1*) and in 3000 µmol photons m^{-2} s^{-1} white light (*trace 2*). Levels of F_0 and maximal fluorescence, F_m, are indicated. *Arrows* denote onset of actinic illumination

by irradiation both of spinach leaves in vivo (Figs. 1–4, Table 1) and of thylakoid membranes in vitro (Fig. 7). The A–P_i curves of leaves (Fig. 5) and electron-transport rates (Table 3) measured after photoinhibitory treatment in vivo and in vitro indicate a partial inactivation of the electron-transport system. Evidence for strong damage to PSII is provided by chlorophyll-fluorescence data. Activity of PSI was not tested here; Satoh (1970) and Powles and Björkman (1982) have shown that in vitro under anaerobic conditions there is no significant photoinhibition of PSI. Enhancement of photoinhibition in leaves caused by anaerobiosis has not been reported before, although the increased photoinactivation of *Sinapis alba* leaves observed at very low O_2 levels by Cornic (1978) might represent the same phenomenon. The effect seen in pure N_2 is clearly one of light and not of anaerobiosis per se, as the assimilatory activity of leaves remained almost unaffected upon treatment with low irradiation in a nitrogen atmosphere (Fig. 4). Stomatal closure can be excluded as a cause of inhibition of photosynthesis (Table 2). Damage to the leaves occurring in N_2 in the dark appears to be of a different nature, as indicated by its different recovery kinetics (Fig. 6). This latter effect was not investigated further. Notably, isolated thylakoids retain their activity when kept in O_2-free media in the dark (Fig. 7).

Oxygen thus provides appreciable protection from photoinhibition despite of the potential toxicity of reactive oxygen products such as O_2^- and OH radicals, H_2O_2, and singlet oxygen (see Elstner 1982). We confirmed the finding of Satoh (1971) that photoinhibition under anaerobiosis is related to a strong increase in the F_0 level of fluorescence induction. Increased F_0 and decreased F_v levels are also characteristic properties of heat-damaged chloroplasts (see Krause and Weis 1984) and have been interpreted to reflect an inhibition of the photochemical reaction in the PSII centers (Schreiber and Armond 1978). Much less enhancement of F_0, relative to loss of F_v is caused by aerobic photoinhibitory treatment (Satoh 1971, Barényi and Krause 1985). This supports Satoh's suggestion of two distinct mechanisms of photoinactivation.

The mechanisms of photoinhibition under anaerobiosis and of the protection by oxygen are largely unknown. High irradiation of chloroplasts or leaves in the absence of CO_2 and O_2 leads to "over-reduction" of the electron-transport chain, blocking cyclic electron transport around PSI (Köster and Heber 1982; Kobayashi et al. 1982). This would eliminate a possible way to dissipate excess excitation energy by electron cycling. In the presence of O_2, this "over-reduction" is relieved probably by reduction of O_2 (Mehler reaction), which poises cyclic electron transfer (Arnon and Chain 1977; Heber et al. 1978; Ziem-Hanck and Heber 1980). As the affinity of O_2 for the Mehler reaction is high (Heber and French 1968; Heber 1969; Takahashi and Asada 1982), this reaction may be involved in the protecting effect of oxygen which requires only low O_2 concentrations. This assumption needs, however, further experimental proof.

Photoinhibition in the presence of oxygen may, to a considerable extent, be caused by reactive

oxygen species. Scavengers of these toxic reaction products of oxygen limit the damage. In the study by Barényi and Krause (1985) partial but not full protection was achieved, e.g. by simultaneous addition of superoxide dismutase and catalase. Possibly, to some degree, photoinhibition by the "anaerobic" mechanism also occurs in the presence of oxygen.

As a working hypothesis, we assume there are two principal mechanisms leading to photoinhibition. The first is based on "over-reduction" of the electron-transport chain under anaerobiosis and is largely, but perhaps not completely prevented by low levels of O_2. On the other hand, oxygen as a protectant may give rise to photoinhibition by a second mechanism based on toxic effects of reactive oxygen products. Numerous protective systems present in the chloroplasts (see Halliwell 1981) may limit or prevent this effect. Both mechanisms may have taken part in photoinhibition effects reported in the literature; conflicting evidence regarding the effect of O_2 on photoinhibition (see Introduction) might be the result of different contributions of the two mechanisms depending on experimental conditions and plant material studied.

Promotion of photoinhibition of CO_2 assimilation by high O_2 concentration (air level and above) was observed in isolated intact chloroplasts and cells of spinach (Krause et al. 1978), as well as at low temperatures in chilling-sensitive leaves (Rowley and Taylor 1972; van Hasselt and van Berlo 1980; Powles et al. 1983). At first sight, this seems to contradict the above hypothesis. One may speculate however, that in these cases high O_2 levels may cause depletion of intermediates of the carbon-reduction cycle by conversion of a high proportion of ribulose-1,5-bisphosphate to products of the photorespiratory pathway. This may cause an overall decline in utilization of photosynthetic energy. Kirk and Heber (1976) have shown that in intact chloroplasts illuminated in an air-saturated medium at limiting CO_2, virtually all intermediates were converted to glycolate. Carbon dioxide at high concentrations, which opposed this effect, indeed acted protectively in most instances where high O_2 levels promoted photoinhibition.

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Errata

Planta (1985) **163**, 208–213, paper by L.D. Polley and D.D. Doctor: Potassium transport in *Chlamydomonas reinhardtii*: isolation and characterization of transport-deficient mutant strains.

On page 213, right-hand column, after line 2 add:

This research was supported by a grant from Research Corporation.

Planta (1985) **163**, 563–568, paper by V. Sharma and D. Strack: Vacuolar localization of l-sinapoylglucose:L-malate sinapoyltransferase in protoplasts from cotyledons of *Raphanus sativus*.

On page 564, right-hand column, line 10 and 13 should read:

"... in medium C containing 0.5 M mannitol ..."

Planta (1985) **165**, 12–22, paper by B. Gomez-Silva, M.P. Timko and J.A. Schiff: Chloroplast biosynthesis from glutamate or 5-aminolevulinate in intact *Euglena* chloroplasts.

On page 13, right-hand column, paragraph 5, line 5 should read:

1.0 mM $MgCl_2$; 7.0 mM $NaHCO_3$ and 40 mM Tricine-KOH, pH 7.8 ...