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Effects of severe CO_2 starvation on the photosynthetic electron transport chain in tobacco plants

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Abstract

Tobacco plants (*Nicotiana tabacum*) were kept in CO_2 free air for several days to investigate the effect of lack of electron acceptors on the photosynthetic electron transport chain. CO_2 starvation resulted in a dramatic decrease in photosynthetic activity. Measurements of the electron transport activity in thylakoid membranes showed that a loss of Photosystem II activity was mainly responsible for the observed decrease in photosynthetic activity. In the absence of CO_2 the plastoquinone pool and the acceptor side of Photosystem I were highly reduced in the dark as shown by far-red light effects on chlorophyll fluorescence and P700 absorption measurements. Reduction of the oxygen content of the CO_2 free air retarded photoinhibitory loss of photosynthetic activity and pigment degradation. Electron flow to oxygen seemed not to be able to counteract the stress induced by severe CO_2 starvation. The data are discussed in terms of a donation of reducing equivalents from mitochondria to chloroplasts and a reduction of the plastoquinone pool via the NAD(P)H-plastoquinone oxidoreductase during CO_2 starvation.

Abbreviations: DCPIP – 2,6-dichlorophenolindophenol; F_M – maximum fluorescence level; F_o – dark level of fluorescence; 'F_o' – dark level of fluorescence in stressed plants; F_V – maximum variable fluorescence; FR – far-red; P700 – reaction center of Photosystem I; PQ – plastoquinone; PS – photosystem; Q_A – primary electron acceptor of Photosystem II

Introduction

Photosynthesis and plant growth is often limited by the availability of CO_2. Even under optimal growth conditions, the photosynthetic carbon fixation is limited in C3 plants by the CO_2 concentration in the atmosphere. In nature, severe CO_2 deficiency occurs frequently when the water supply to the plant is not sufficient and the stomata are closed due to drought stress. Under such conditions, photosynthetic pigments absorb more light than can be utilized for CO_2 fixation. An excess of absorbed light energy leads to photoinhibition followed by destruction of the photosynthetic apparatus (e.g. Prášil et al. 1992; Aro et al. 1993).

In the course of CO_2 starvation, all endogenous electron acceptors become reduced and oxygen is the main available electron acceptor. Oxygen can serve as electron acceptor in photorespiration (Osmond and Björkman 1972) and in the Mehler reaction (Mehler 1951; Schreiber and Neubauer 1990). The products of the Mehler reaction such as superoxide, hydroxyl radicals and hydrogen peroxide are toxic, and can cause the inhibition of photosynthetic processes. Photosystem (PS) II is the most sensitive target to photooxidat-

ive damage of the photosynthetic electron chain (e.g. Prášil et al. 1992; Aro et al. 1993).

However, there are several reports in the literature that the activity of the photosynthetic electron transport chain, especially of PS II is controlled by the Mehler reaction (Schreiber and Neubauer 1990), cyclic electron flow (Heber and Walker 1992; Heber et al. 1995) and photorespiration (Osmond et al. 1997). These reactions result in formation of a proton gradient, which leads to a down regulation of the activity of PS II and of the turnover rate of the cytochrome b_6f complex, the so called photosynthetic control (e.g. Genty and Harbinson 1996). The down regulation of PS II correlates with the proton gradient dependent formation of zeaxanthin (Demmig 1987). Zeaxanthin is involved in the dissipation of excess energy as heat.

The mechanisms which control the photosynthetic electron transport chain in leaves of water stressed plants were investigated by several groups (Cornic 1994; Loreto et al. 1995). It has been suggested that the Mehler reaction participates in the dissipation of excess energy under water stress (Biehler and Fock 1996), that PS I dependent cyclic electron flow is important in controlling PS II activity (Katona et al. 1992; Heber et al. 1995) and that photorespiration participates in the protection of the photosynthetic electron chain against photoinhibition (Smirnoff 1993). Additionally, it has been suggested that under conditions of CO_2 limitation, photosynthetic control might act to limit the extent of the Mehler reaction and thereby limit the production of reactive oxygen species (Polle 1996; Ott et al. 1999). Despite these protection reactions, photoinhibition is enhanced when the CO_2 concentration in the air is lowered as has been shown in *Phaseolus vulgaris* (Daniel 1997).

Here we describe the effects of CO_2 starvation on the photosynthetic electron transport chain of tobacco plants. Plants were grown for several days in CO_2 free but water saturated air. Tobacco plants, starving for several days in carbon dioxide free air, have served previously as a model system to simulate CO_2 deficiency (Šiffel and Vácha 1998; Šiffel and Braunová 1999; Vácha and Durchan 1995). In this study we investigated the effect of CO_2 starvation on the capacity of CO_2 fixation by measuring gas exchange rates. Chlorophyll fluorescence and absorption measurements at 816 nm were measured to follow the reduction state of the intersystem electron acceptors. Furthermore, we studied the role of oxygen under photoinhibitory conditions. By varying the oxygen content of the CO_2 free air, the importance of photorespiration (Wu et al. 1991; Heber et al. 1996; Kozaki and Takeba 1996) in counteracting ongoing photoinhibition was studied under conditions of CO_2 starvation. In addition, pigment decomposition was analyzed.

Materials and methods

Plant cultivation

Tobacco (*Nicotiana tabacum* L. cv. White Burley) plants were grown on agar medium according to Murashige and Skoog (1962). The micropropagation technique was used to obtain plant material with the same genome. The stalk of one plant was divided in pieces with at least two leaves and rooted on the same medium until new roots appeared. From this source material, particular plants were carefully selected for each experiment. Additional leaves were cut to achieve the same number of leaves for each plant, and afterwards, plants were transferred in the 3-liter glass vessels which could be closed. The plants were grown hydroponically (Heldt and Sauer 1971) for 1–2 weeks in well defined conditions in plant growth chamber (SGC170.PHX.J, Sanyo Gallenkamp, 16/24 °C, 8/16 hours dark/light of 400 μmol quanta m^{-2} s^{-1}).

CO_2 starvation

To remove carbon dioxide from the air, a solid absorber of CO_2 (based on sodium hydroxide, Natrocalcid, Spolana, Velvary, Czech Republic) was added before the vessels were closed. The final concentration of carbon dioxide in the growing vessels was well under the compensation point as checked by a gas exchange system (20-30 μmol (CO_2) mol^{-1}, see Šantrůček et al. 1991). Every day at the beginning of the light period, the vessels were purged with either CO_2 free air or a mixture of CO_2 free air: nitrogen = 1:1 (v/v), both water saturated, for 20 min to adjust 21% or 10.5% (v/v) of oxygen, respectively. The atmosphere in the vessel was water saturated during the stress experiments. Day/night temperatures and light conditions were the same as in the course of plant cultivation.

Exchange of CO_2

Photosynthetic activity was measured as CO_2 exchange rate using a portable photosynthetic system (LI-6200, LI-COR, Lincoln, USA). Plants, starving

for 2 days in CO_2 deficiency, were kept in normal CO_2 laden air (380 μmol (CO_2) mol^{-1}) at moderate light (70 μmol quanta m^{-2} s^{-1}) for 1 hour before the light curves were measured. Light saturation curves were measured under an irradiance of 0-2000 μmol quanta m^{-2} s^{-1}, at a CO_2 concentration of 380 μmol (CO_2) mol^{-1}, a leaf temperature of 25-27 °C, and air humidity of 75-80%.

In vivo *measurements of oxygen evolution*

Oxygen evolution *in vivo* was measured by a leaf disc electrode (Hansatech, Kings Lynn, UK) at the normal, atmospheric concentration of oxygen (21%) in the presence of carbon dioxide (saturated solution of $NaHCO_3$ at 20 °C, i.e. high, saturating concentration of CO_2) in the measuring atmosphere.

Chlorophyll fluorescence in vivo *and P700$^+$ measurements*

Chlorophyll fluorescence *in vivo* was measured with a pulse-amplitude modulation fluorometer (PAM 101-3, Walz, Effeltrich; KL 1500, Schott, Cologne, Germany) as described by Schreiber et al. (1986). The fluorescence ratio, F_V/F_M, was measured prior to the oxygen evolution measurement on the leaf disc kept for 10 min in darkness in the Hansatech leaf disc electrode chamber. The effect of far-red light on chlorophyll fluorescence of intact leaf discs was measured with far-red (725 ± 15 nm) light intensity of 70 μmol quanta m^{-2} s^{-1}. The intensity of the red (650 ± 25 nm) actinic light was 150 μmol quanta m^{-2} s^{-1}. The intensity of the measuring analytic light (standard PAM 101 set) was low enough (integral intensity about 10^{-8} mol quanta m^{-2} s^{-1}, frequency of modulated light: 1.6 kHz) to prevent the reduction of PQ as it was tested by Mini-PAM (Walz, Effeltrich, Germany) in the burst mode (integral intensity about 10^{-9} mol quanta m^{-2} s^{-1}). The duration of the saturation pulses was 700 ms. The intensity of the saturation pulses (KL 1500 lamp, with Calflex X optical filter, Balzers, Liechtenstein) was tested on every leaf disc to be able to take the lowest intensity necessary to evoke the maximum fluorescence response.

The temperature dependence of 'dark' recovery of fluorescence was measured with a special home-made sample holder in which the temperature of the leaf disc was changed in approximately 1–2 seconds. The actinic and measuring light was the same as described above.

P700$^+$ absorbance changes measurements were performed in the transmission mode (Schreiber et al. 1988) with the PAM 101-2 accessories (Walz, Effeltrich; Germany). Far-red light (725 ± 15 nm) with an intensity of 70 μmol quanta m^{-2} s^{-1} was used as it is preferentially absorbed in PS I (light I). As actinic blue light (light II), a halogen lamp and fibre optics (Fibre-Lite A 3200, Dolan-Jenner, Woburn, USA) with appropriate optical filter (DT-Cyan, Balzers, Liechtenstein) were used. Three different blue light intensities (150, 250, and 500 μmol quanta m^{-2} s^{-1}) were applied.

Isolation of thylakoid membranes, PS I and PS II activities

Thylakoid membranes were prepared according to Leegood and Malkin (1986). The grinding medium contained 0.4 M sorbitol, 50 mM HEPES, 5 mM $MgCl_2$, 10 mM KCl, 1 mM $MnCl_2$, 2 mM EDTA, 0.1% (w/v) BSA, 0.2% (w/v) ascorbate, pH 7.6. The centrifuged chloroplasts were osmotically disrupted by suspension in a medium containing 50 mM HEPES, 5 mM $MgCl_2$, 20 mM KCl, pH 7.6. After the second centrifugation, the pellet, which contained washed thylakoid membranes, was resuspended in a small volume of medium containing 0.4 M sorbitol, 50 mM HEPES, 15 mM $MgCl_2$, 10 mM KCl, 1 mM $MnCl_2$, pH 7.6.

Oxygen evolution activity was measured in the suspension medium with a chlorophyll concentration of 30 μg/ml using 1 mM ferricyanide and 0.5 mM phenyl-para-benzoquinone as the electron acceptors and 1 μM nigericin as uncoupler. The PS I activity was measured as the rate of oxygen uptake using 30 μg Chl/ml, 10 μM DCMU, 5 mM ascorbate, 30 μM DCPIP, 0.5 mM methylviologen, 1 mM NaN_3, 1 μM nigericin. The measurements were performed in the absence of superoxide dismutase which might lead to an overestimation of the PS I activity.

Pigment extraction and analysis; high light treatment

Leaves were homogenized in 100% acetone in a glass homogenizer. The homogenate was spun down at 14,000 *g*; the pellet was reextracted with 80% acetone and spun down. Both supernatants were combined and filtered through a 0.2 μm Teflon filter prior to the injection. The pigment composition was analyzed by HPLC on the C-18, 15 × 3 mm, 5 μm (Tessek, Czech Republic) reverse phase column using the isocratic elution of a mixture of acetonitrile, methanol

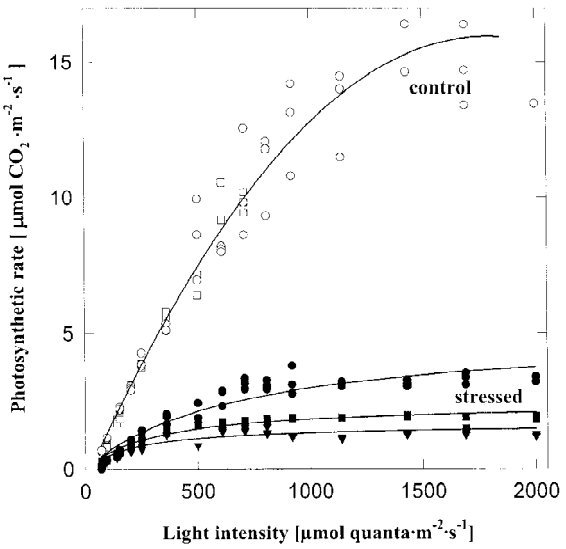

Figure 1. Light saturation curves of photosynthetic electron transport in leaves of tobacco plants. Photosynthetic activity was measured as CO_2 exchange rates. Before measuring the light saturation curves, the plants, starving for 2 days (400 μmol quanta m^{-2} s^{-1}) and nights in CO_2 deficiency (marked as 'stressed'), were relaxing in normal CO_2 laden air (380 μmol (CO_2) mol^{-1}) at a moderate light intensity (70 μmol quanta m^{-2} s^{-1}) for 1 hour. Open symbols: control plants. Closed symbols: stressed plants. Circles: young leaf, squares: mature leaf, triangles: older leaf.

and water = 68:12:6 for the first 5 min followed by a linear gradient to absolute methanol for 2 min and isocratic elution with absolute methanol until the β-carotene was eluted. The flow rate was 2 ml min^{-1}, pigments were detected at 445 nm.

In order to estimate the capacity of zeaxanthin formation, leaves were placed on the water surface in gas chambers under nitrogen atmosphere and subjected to high light for 15 min. A slide projector with heat filter was used as a light source; the light intensity was 1200 μmol quanta m^{-2} s^{-1}.

Results

Figure 1 shows the carbon assimilation of leaves as a function of light intensity in control plants and plants grown under condition of CO_2 starvation (stressed plants). The photosynthetic activity at saturating light intensity was reduced in stressed plants by 80–90% compared to the control. In addition in stressed plants light saturation was reached at much lower light intensities (600 μmol quanta m^{-2} s^{-1} or lower) than in the control (1500 μmol quanta m^{-2} s^{-1}).

Table 1. PS II and PS I activities of isolated thylakoid membranes. Washed thylakoid membranes were isolated from control plants and stressed plants which were kept for 2 days in a CO_2 free atmosphere. PS I activity was measured as O_2-uptake using 0.5 mM methylviologen as electron acceptor; PS II activity was measured as O_2-evolution using 1 mM ferricyanide and 0.5 mM phenyl-para-benzoquinone as electron acceptors. The measurements were performed in the presence of 1 μM nigericin

μmol O_2 / mg Chl · hour	PS II activity	PS I activity
Stressed	114	380
Control	362	397

To test whether this effect is caused by inhibition of the CO_2 fixation or, as a more primary effect, by inhibition of the photosynthetic electron transport chain, we isolated thylakoid membranes from leaves of control and stressed plants and measured PS II and PS I activities in the presence of an uncoupler (Table 1). PS II activity was reduced by 70% in thylakoid membranes obtained from stressed plants while the PS I activity stayed unchanged in comparison with the control. This indicates that inhibition of PS II was mainly responsible for the reduction in carbon assimilation seen in stressed plants (Figure 1).

Figure 2 shows measurements of chlorophyll fluorescence obtained from plants stressed for 2 days in CO_2 free air. As depicted in Figure 2A, the difference between the fluorescence in the dark, monitored with a low intensity pulse-modulated light, and the maximum fluorescence induced by a saturating pulse ($F_V = F_M - F_o$) is very small. This effect can be caused by either high fluorescence in the dark or by a low yield of variable fluorescence (quenching of F_M). In control plants, the ratio F_V/F_M is 0.8 (data not shown). In stressed plants, far-red (FR) illumination (λ = 725 nm) lowered the dark fluorescence by 50%. After switching off the FR light, the high yield of dark fluorescence was restored to its initial value in less than 30 min in complete darkness. This indicates a high reduction state of the PQ pool in the dark in the CO_2 starved plants (Diner 1977; Groom et al. 1993). Interestingly, F'_M was smaller under FR illumination than in the dark-adapted system. The PS II activity seems to be partially down-regulated by far-red light. Down-regulation of PS II might be caused by a proton gradient formed by cyclic electron flow under FR illumination. Measurements of 9-aminoacridine fluorescence quenching in thylakoid membranes of stressed plants showed that, after the addition of purified ox-

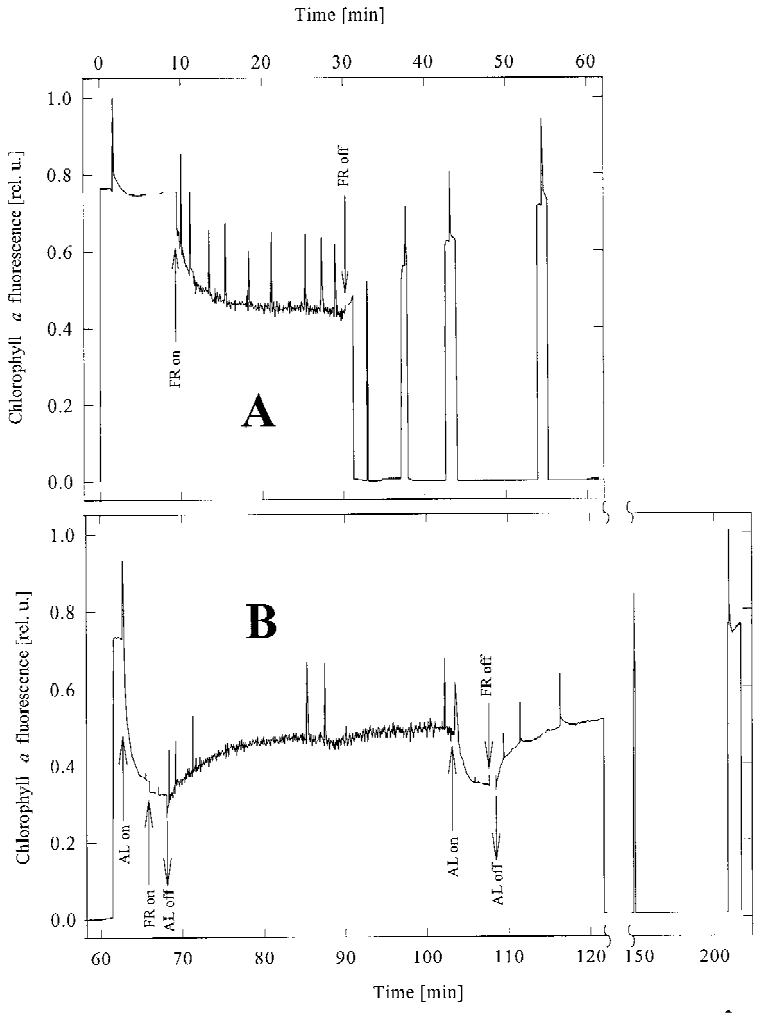

Figure 2. Effect of far-red light on the 'dark' chlorophyll fluorescence of an intact leaf starved for 2 days in a CO_2 deficient atmosphere. (A) Effect of illumination with far-red light. (B) Effect of illumination with actinic and far-red light. The data shown in (B) were obtained with the same leaf disc as in (A) after almost full relaxation of fluorescence to the dark level (see 'Time' axis). Actinic light (AL): red (650 $\pm$ 25 nm) light; 150 μmol quanta m^{-2} s^{-1}. Far-red light (FR): 725 $\pm$ 15 nm; 70 μmol quanta m^{-2} s^{-1}. For measuring light, and saturation pulse parameters, see 'Materials and methods'.

idized ferredoxin, a proton gradient was formed of a similar size compared to thylakoid membranes from unstressed plants (data not shown). Figure 2B shows that a few second illumination with actinic light (150 μmol quanta m^{-2} s^{-1}) decreased the fluorescence yield even further than the FR illumination alone. A combination of FR and actinic light lowered the fluorescence only slightly more. Switching off the actinic light but keeping the FR illumination increased fluorescence, indicating that FR alone at the used light intensity is not sufficient to keep the PQ pool oxidized.

The relaxation of light-induced 'F_o' quenching is temperature dependent as shown in Figure 3. When the stressed leaves were kept at 0 °C after the light-induced quenching, very little recovery to the original 'F_o'-level could be seen. A change of the leaf temperature to 35 °C stimulated the dark recovery drastically which indicates that enzymatic reactions are responsible for this increase in the fluorescence in the dark. In control leaves, the 'F_o'-level did not increase at elevated temperatures.

Figure 4 shows absorption measurements of $P700^+$ in control and stressed leaves. Absorption changes were measured at 816 nm. Illumination of a dark-adapted leaf with far-red light (725 nm, 70 μmol quanta m^{-2} s^{-1}) increased the absorption in-

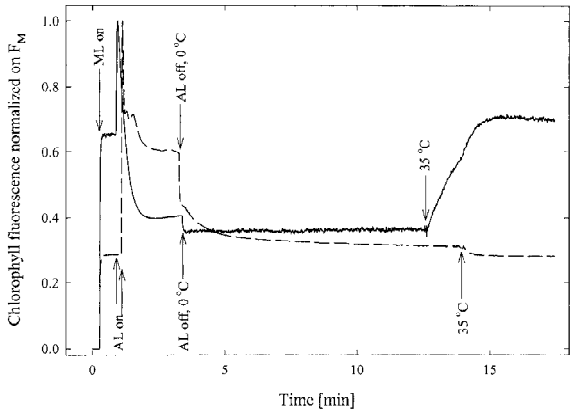

Figure 3. Temperature dependent relaxation of 'F_o' level of intact leaves. The stressed plant (solid line) was kept for 2 days under CO_2 starvation; broken line: leaf of a control plant. Actinic light (AL): red (650 ± 25 nm) light; 150 μmol quanta m^{-2} s^{-1}. For measuring light (ML) parameters, see 'Materials and methods'.

dicating oxidation and accumulation of $P700^+$. After the steady state was reached, blue light was switched on and the $P700^+$ reduction kinetics were measured. In control leaves (Figure 4A), upon illumination with blue light, most of amplitude of the $P700^+$ signal disappeared during a transient period. In the following 10 to 30 s a steady state was reached again which indicated a greater proportion of $P700^+$ than observed in the first seconds after starting the blue light illumination. Depending on the intensity of the blue light, more (500 μmol quanta m^{-2} s^{-1}) or less (150 μmol quanta m^{-2} s^{-1}) $P700^+$ was present in the steady state.

In stressed leaves (Figure 4B), the absorption changes look completely different. Far-red illumination induced only one third of the absorption change seen in the leaf of a control plant, showing that most of the P700 was reduced when plants were grown under CO_2 deficiency. The small amplitude of the $P700^+$ signal formed under far-red illumination indicates that cyclic electron flow takes place. Blue light illumination (500 μmol quanta m^{-2} s^{-1}) gave rise to a much larger absorption change, $P700^+$ accumulates. This effect is very different to the blue light effect seen in control leaves. P700 gets only partially oxidized by far-red light but fully oxidized to $P700^+$ by actinic light. Lower light intensities of the actinic light had much less effect but induce in principle the same effect: oxidation of P700 by actinic light.

In the following we investigated the effect of CO_2 deficiency on photosynthetic activity of plants grown in 21% and 10.5% oxygen (Figure 5). F_V/F_M and oxygen evolution were measured every day to monitor photosynthetic activity. Reduction of the oxygen content in the CO_2 free air from 21% to 10.5% protected the plants against damage (photoinhibition). The rate of photoinhibition depended on the age of the plants, in young plants maximal photoinhibition was reached in three days while mature plants could stand this treatment for ten days.

In addition to the photosynthetic activity the pigment content changed during the starvation of tobacco plants in CO_2 free air. As shown in Figure 6, the chlorophyll (Figure 6A) and the β-carotene content (Figure 6B) decreased by 50% when the plants were kept in 21% oxygen. In 10.5% oxygen the decrease in the pigment content was slowed down. The ability to produce zeaxanthin decreased also drastically (Figure 7). One day after the plants had been transferred to the CO_2 free air, zeaxanthin was 30% of the total carotenoid content. Zeaxanthin, antheraxanthin and violaxanthin together contributed to 37% to the total amount of carotenoids. After 3 days of CO_2 starvation in 21% oxygen the amount of zeaxanthin was reduced to approximately 5% while the sum of the three xanthophylls decreased by only 5% or less.

Discussion

Tobacco plants grown under CO_2 deficiency show a dramatic decrease in photosynthetic activity (Figure 1) which seems to be mainly caused by the inhibition of PS II activity (Table 1). A similar response of the photosynthetic electron transport chain was observed with drought-stressed plants (Stuhlfauth et al. 1990; Brestic et al. 1995). Phosphorus or sulphur starvation of *Chlamydomonas* cells results also in a comparable decrease of the photosynthetic activity (Wykoff et al. 1998). As shown in Figures 2 and 3, not only the photosynthetic electron transport activity is inhibited but, in addition, the reduction state of the plastoquinone pool is high in these chloroplasts as indicated by high dark fluorescence. Far-red illumination, which stimulates cyclic electron flow around PS I, causes a partial oxidation of the plastoquinone pool seen as a decrease of the high 'F_o' fluorescence (Figure 2). Cyclic electron flow leads to the formation of a proton gradient which is sufficiently high to control the PS II activity. However, the decrease of the 'F_o' fluorescence induced by far-red illumination is far from being complete and the extent of $P700^+$ accumulation is rather low compared to unstressed leaves (Figure 4). FR light alone at the given intensity is not able to oxidize

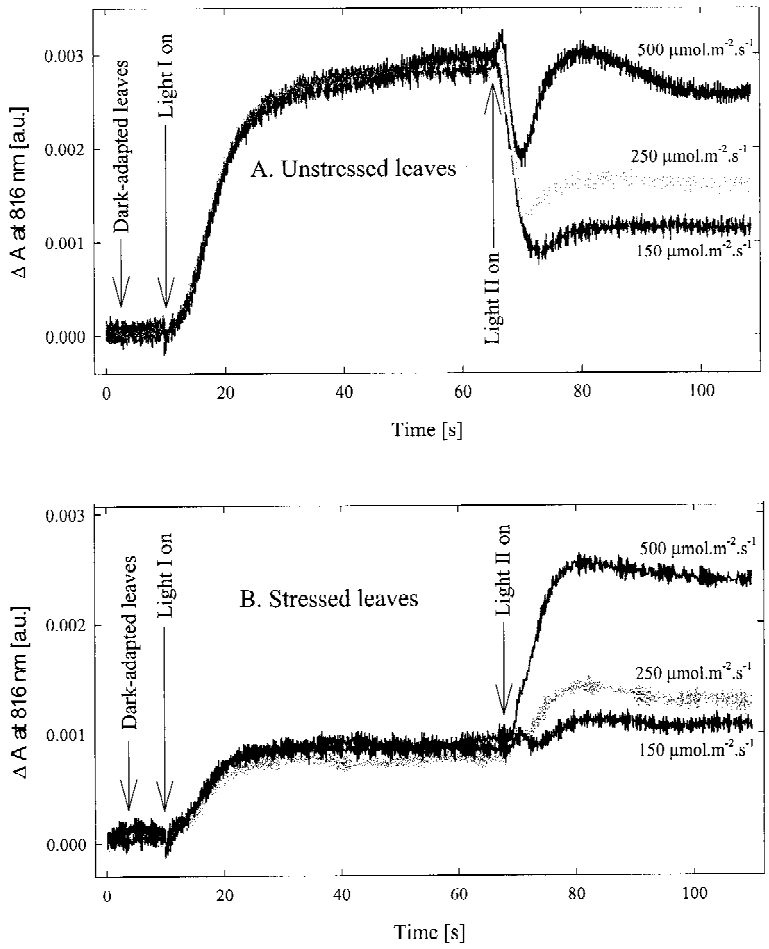

Figure 4. Redox state of Photosystem I. Absorption signals recorded at 816 nm after a dark–light transition in tobacco leaves. (A) Control; (B) stressed leaves starved for 2 days in CO_2 free air. Light I was far-red (725 ± 15 nm; 70 μmol quanta m^{-2} s^{-1}); light II was blue (Balzers DT Cyan filter), intensity as shown in inset.

the intersystem electron transport chain completely. In leaves of CO_2-starved plant, additional illumination with saturating actinic light for both photosystems (blue light or red light at 650 nm) further decreases the 'F_o' fluorescence and leads to an extent of $P700^+$ accumulation which is comparable to the one seen in unstressed leaves (Figures 2 and 4). Under these illumination conditions, cyclic electron transport is favored compared to the reactions which lead to a reduction of the PQ pool and a fluorescence level is reached which is close to the F_o in control plants.

As can be seen in Figures 2 and 3, the reduction of the PQ pool starts immediately after switching off the actinic or far-red light, needs several minutes to reach the steady state and is temperature dependent. From our data, we can only speculate about its biochemical nature. In the literature different mechanisms are discussed which can be responsible for a reduction of the PQ pool in the dark. One possibility is a thermodynamically driven reduction of the acceptor site of PS II by reverse electron flow, which can occur in the presence of ATP (Schreiber and Avron 1977), or DHAP (Mano et al. 1995). Another possibility is the reduction of the PQ pool as a first step in the electron transport chain of chlororespiration (Bennoun 1998). It is known that common metabolites can be exchanged between chloroplasts and mitochondria (see Hoefnagel et al. 1998). A closer interaction between these organelles seems to occur under CO_2 starvation (M. Šimková, personal communication); the mitochondrial electron transport chain may strictly control the redox state of the PQ pool (for

review see Bennoun 1998; Gardeström and Lernmark 1995). Reducing equivalents can be exchanged across the mitochondrial and chloroplastic membrane via the malate/oxaloacetate and to a minor extent via the malate/aspartate shuttle (see Hoefnagel et al. 1998). Via these shuttles reducing equivalents and thereby indirectly NAD(P)H can be transported from the mitochondria to the chloroplast and the PQ pool could be reduced in the dark by a chloroplastic NAD(P)H-plastoquinone-oxidoreductase (ndh). The ndh genes were shown to be transcribed (Matsubayashi et al. 1987) and the polypeptides of some ndh genes have been found in thylakoid membranes of higher plants (e.g. Nixon et al. 1989; Guedeney et al. 1996). The involvement of a NAD(P)H-PQ-oxidoreductase in the increase of fluorescence in the dark was recently shown in thylakoid membranes of higher plants (Corneille et al. 1998).

During illumination with actinic light, electron flow and dark reduction of the PQ pool by ndh (or another enzyme) might occur at the same time and compete with each other. Photosynthetic electron flow is dominating, because the dark reduction of the PQ pool is very slow (see Figures 2 and 3). Dark reduction of the PQ pool might be even inhibited (or down regulated) when effective electron transport takes place.

In addition to the high reduction state of the plastoquinone pool, the photosynthetic activity of stressed plants decreases with the duration of starvation. Reduction of the oxygen content from 21% to 10.5% protects the stressed plants partially against fast inhibition of the photosynthetic electron transport chain as seen by measurements of F_V/F_M and oxygen evolution (Figure 5). It is likely that this fast decrease of activity is caused by photoinhibition produced by toxic reactive oxygen species because lowering the oxygen content of the air slows down the process of inactivation (Figures 5 and 6). The content of chlorophyll, β-carotene (Figure 6) and zeaxanthin also decreases with longer starvation time while the xanthophyll content stays relatively unchanged (Figure 7). Recombination reactions in PS II and also in PS I leading to a high yield of chlorophyll triplet formation can occur under conditions of a completely reduced acceptor side (van Mieghem et al. 1989; Vass et al. 1992). Triplet chlorophyll reacts with oxygen under the formation of singlet oxygen which might be responsible for the observed decrease of activity and damage of pigments (see Prášil et al. 1992; Aro et al. 1993). In addition, active oxygen radicals like superoxide (and subsequently hydrogen peroxide and

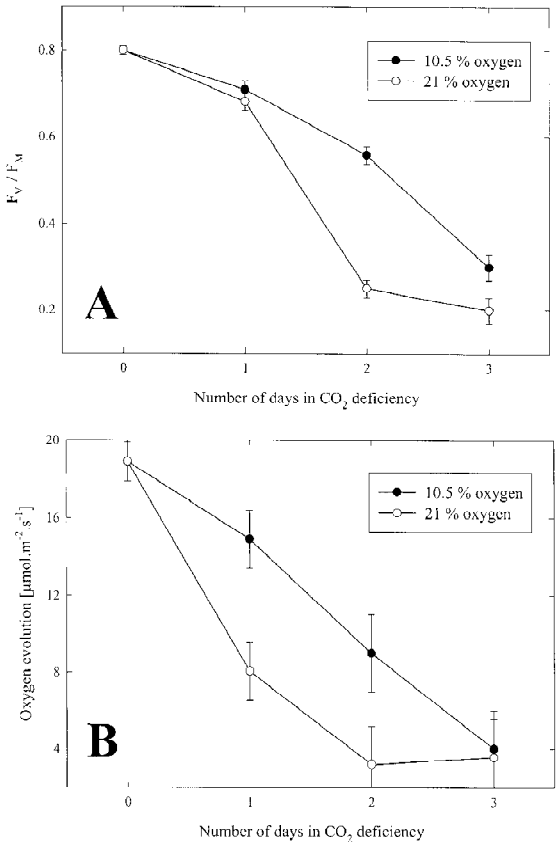

Figure 5. Decrease of photosynthetic activity in the course of CO_2 starvation: Effect of a decreased oxygen concentration. Tobacco plants were transferred into an atmosphere without CO_2 with 21% or 10.5% of oxygen. (A) Photochemical efficiency of PS II assessed by the chlorophyll fluorescence ratio, F_V/F_M, in intact leaves and (B) oxygen evolution of intact leaves measured at saturating CO_2 in a Hansatech leaf-disc electrode.

hydroxyl radicals) will be produced by a direct reduction of oxygen as the only available electron acceptor thereby increasing the photooxidative stress.

The presented data show that, in the absence of CO_2, oxygen cannot compete as an efficient electron acceptor but instead triggers photoinactivation. The electron acceptors stay partially reduced even under far-red illumination (Figures 2 and 4) indicating that neither photorespiration (Kozaki and Takeba 1996; Osmond et al. 1997) nor cyclic electron flow (Katona et al. 1992; Bendall and Manasse 1995) nor Mehler reaction (Schreiber and Neubauer 1990; Biehler and Fock 1996) are sufficient to keep the electron transport chain oxidized under the conditions of CO_2 starvation although it has been shown that the rate of photorespiration is slightly increased at low CO_2 concentrations

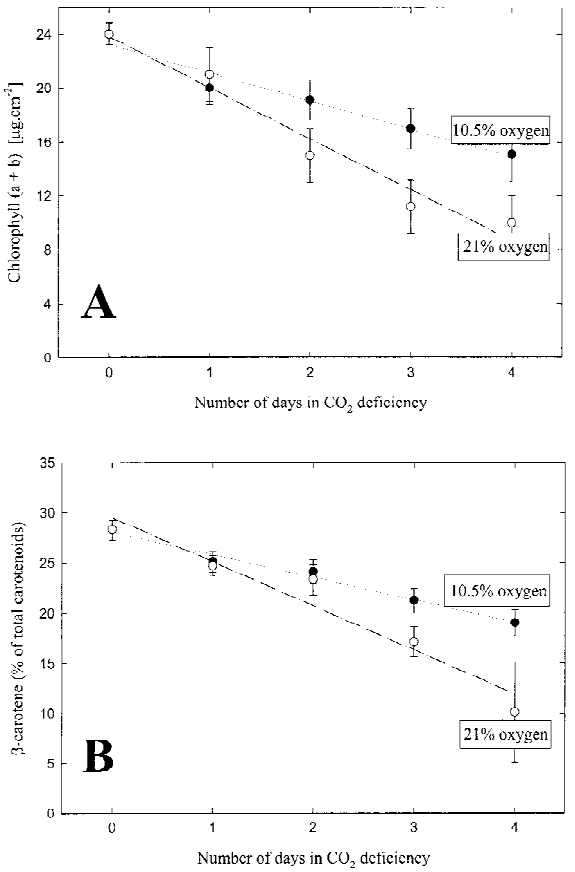

Figure 6. Alterations in pigment composition in the course of CO_2 starvation with respect to the oxygen concentration in the atmosphere. Tobacco plants were transferred into an atmosphere without CO_2 with 21% or 10.5% oxygen. The decrease in chlorophyll (a + b) (A), and β-carotene (B) content was measured by HPLC. Mean values and error bars were calculated from 10 measurements.

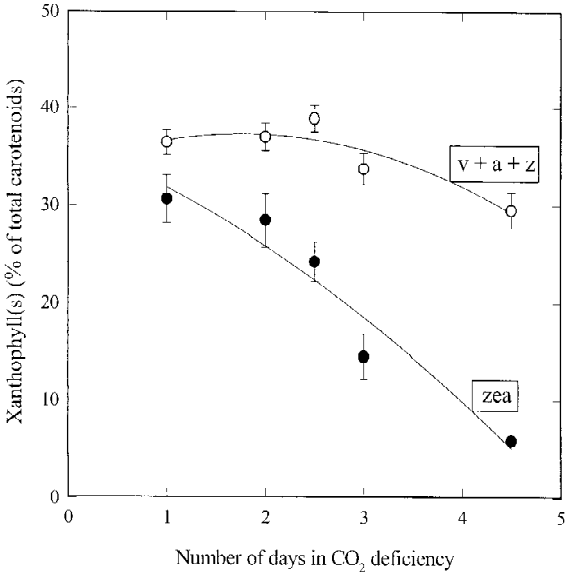

Figure 7. Content of xanthophylls and capacity of zeaxanthin formation during CO_2 starvation in an atmosphere with 21% of O_2. The xanthophyll content was measured by HPLC. Mean values and error bars were calculated from 10 measurements. Zea, z – zeaxanthin, v – violaxanthin, a – antheraxanthin. Leaves were placed on a water surface in gas chambers under nitrogen atmosphere and subjected to high light (1200 μmol quanta m^{-2} s^{-1}) for 15 min prior to measurements of the xanthophyll content.

(Dietz and Heber 1986). Photorespiration can not serve as an effective pathway for photoprotection under the conditions of severe CO_2 starvation used here because carbon hydrate reserves have been already consumed during the first days of CO_2 starvation. Zeaxanthin formation decreases with the duration of the stress showing that also protection mechanisms of energy dissipation in PS II cannot counteract the general process of death. In addition, under prolonged CO_2 starvation, the morphological structure changes, grana stacks are dissolved (M. Šimková, personal communication) and, during prolonged starvation, the plants die.

CO_2 starvation inducing alterations of the redox state of the photosynthetic electron transport chain, of the pigment composition and morphological modifications can be used as a model system to study the capacity of available storage compounds and the metabolic flexibility of the plant to respond to stress. In general this kind of stress may be of interest as a model system to study senescence phenomena.

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References

Aro E-M, Virgin I and Anderson B (1993) Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. Biochim Biophys Acta 1143: 113–134

Bendall DS and Manasse RS (1995) Cyclic photophosphorylation and electron transport. Biochim Biophys Acta 1229: 23–38

Bennoun P (1998) Chlororespiration, sixteen years later. In: Rochaix J-D Goldschmidt-Clermont M and Merchant S (eds) Advances in Photosynthesis, Vol 7, The Molecular Biology of

Chloroplasts and Mitochondria in *Chlamydomonas*, pp 675–683. Kluwer Academic Publishers, Dordrecht, The Netherlands

Biehler K and Fock H (1996) Evidence for the contribution of the Mehler-peroxidase reaction in dissipating excess electrons in drought-stressed wheat. Plant Physiol 112: 265–272

Brestic M, Cornic G, Fryer MJ and Baker NR (1995) Does photorespiration protect the photosynthetic apparatus in French bean leaves from photoinhibition during drought stress? Planta 196: 450–457

Corneille S, Cournac L, Guedeney G, Havaux M and Peltier G (1998) Reduction of the plastoquinone pool by exogenous NADH and NADPH in higher plant chloroplasts. Characterization of a NAD(P)H-plastoquinone oxidoreductase activity. Biochim Biophys Acta 1363: 59–69

Cornic G (1994) Drought stress and high light effects on leaf photosynthesis. In: Baker NR and Bowyer JR (eds) Photoinhibition of Photosynthesis, pp 297–313. Bios Scientific Publishers, Oxford

Daniel E (1997) The temperature dependence of photoinhibition in leaves of *Phaseolus vulgaris* (L.). Influence of CO_2 and O_2 concentrations. Plant Sci 124: 1–8

Demmig B, Winter K, Krüger A and Czygan FC (1987) Photoinhibition and zeaxanthin formation in leaves. Plant Physiol 84: 218–224

Dietz K-J and Heber U (1986) Light and CO_2 limitation of photosynthesis and states of the reactions regenerating ribulose 1,5-bisphosphate or reducing 3-phosphoglycerate. Biochim Biophys Acta 848: 392–401

Diner BA (1977) Dependence of the deactivation reactions of Photosystem II on the redox state of plastoquinone pool A varied under anaerobic conditions. Equilibria on the acceptor side of Photosystem II. Biochim Biophys Acta 460: 247–258

Genty B and Harbinson J (1996) Regulation of light utilization for photosynthetic electron transport. In: Baker NR (ed) Photosynthesis and the Environment, pp 69–99. Kluwer Academic Publishers, Dordrecht, The Netherlands

Gardeström P and Lernmark U (1995) The contribution of mitochondria to energetic metabolism in photosynthetic cells. J Bioenerg Biomembrane 27: 415–421

Groom QJ, Kramer DM, Crofts AR and Ort DR (1993) The non-photochemical reduction of plastoquinone in leaves. Photosynth Res 36: 205–215

Guedeney G, Corneille S, Cuiné S and Peltier G (1996) Evidence for an association of Ndh-B, Ndh-J gene products and ferredoxin-NADP-reductase as components of a chloroplastic NAD(P)H dehydrogenase complex. FEBS Lett 378: 277–280

Heber U and Walker D (1992) Concerning a dual function of coupled cyclic electron transport in leaves. Plant Physiol 100: 1621–1626

Heber U, Gerst U, Krieger A, Neimanis S and Kobayashi Y (1995) Coupled cyclic electron transport in intact chloroplasts and leaves of C3 plants: Does it exist? If so, what is its function? Photosynth Res 46: 269–275

Heber U, Bligny R, Streb P and Douce R (1996) Photorespiration is essential for the protection of the photosynthetic apparatus of C3 plants against photoinactivation under sunlight. Bot Acta 109: 307–315

Hoefnagel HN, Atkin OK and Wiskich JT (1998) Interdependence between chloroplasts and mitochondria in the light and in the dark. Biochim. Biophys. Acta 1399: 235–255

Heldt HW and Sauer F (1971) The inner membrane of the chloroplast envelope as the site of specific metabolite transport. Biochim Biophys Acta 234: 83–91

Katona E, Neimanis S, Schönknecht G and Heber U (1992) Photosystem I-dependent cyclic electron transport is important in controlling Photosystem II activity in leaves under conditions of water stress. Photosynth Res 34: 449–464

Kozaki A and Takeba G (1996) Photorespiration protects C3 plants from photoinhibition. Nature 384: 557–560

Leegood RC and Malkin R (1986) Isolation of sub-cellular photosynthetic systems. In: Hipkins MF and Baker NR (eds) Photosynthesis Energy Transduction. A Practical Approach, pp 9–26. IRL Press, Oxford

Loreto F, Tricoli D and Di Marco G (1995) On the relationship between electron transport rate and photosynthesis in leaves of the C_4 plant *Sorghum bicolor* exposed to water stress, temperature changes and carbon metabolism inhibition. Aust J Plant Physiol 22: 885–892

Mano J, Miyake C, Schreiber U and Asada K (1995) Photoactivation of the electron flow from NADPH to plastoquinone in spinach chloroplasts. Plant Cell Physiol 36, 1589–1598

Matsubayashi T, Wakasugi T, Shinozaki K, Yamaguchi-Shinozaki K, Zaita N, Hidaka T, Meng BY, Ohto C, Tanaka M, Kato A, Maruyama T and Sugiura M (1987) Six chloroplast genes (ndhA-F) homologous to human mitochondrial genes encoding components of the respiratory chain NADH dehydrogenase are actively expressed; determination of the splice sites in ndhA and ndhB pre-mRNAs. Mol Gen Genet 210: 385–393

Mehler AH (1951) Studies on the reaction of illuminated chloroplasts. II. Stimulation and inhibition of the reaction with molecular oxygen. Arch Biochem Biophys 34: 339–351

Murashige T and Skoog F (1962) A revised medium for rapid growth and bio-assay with tobacco tissue cultures. Physiol Plant 15: 473–497

Nixon PJ, Gounaris K, Coomber SA, Hunter CN, Dyer TA and Barber J (1989) PsbG is not a Photosystem two gene but may be a ndh gene. J Biol Chem 264: 14129–14135

Osmond CB, Björkman Ö (1972) Simultaneous measurements of O_2 effects on net photosynthesis and glycolate metabolism in C_3 and C_4 species of *Atriplex*. Carnegie Inst Washington Year Book 71: 141–148

Osmond CB, Badger M, Maxwell K, Björkman Ö and Leegood R (1997) Too many photons: Photorespiration, photoinhibition and photooxidation. Trends Plant Sci 2: 119–121

Ott T, Clarke J, Birks K and Johnson GN (1999) Regulation of the photosynthetic electron transport chain. Planta 209: 250–258

Polle A (1996) Mehler reaction: Friend or foe in photosynthesis? Bot Acta 109: 84–89

Prášil O, Adir N and Ohad I (1992) Dynamics of Photosystem II. Mechanism of photoinhibition and recovery processes. In: Barber J (ed) The Photosystems: Structure, Function and Molecular Biology, pp 295–348. Elsevier, Amsterdam

Šantrůček J, Šiffel P, Polišenská H and Pešková H (1991) Species-specific response of the photosynthetic apparatus of tobacco and potato to low CO_2 concentrations. Photosynthetica 25: 203–209

Schreiber U and Avron M (1977) ATP-induced chlorophyll luminescence in isolated spinach chloroplasts. FEBS Lett 82: 159–162

Schreiber U and Neubauer C (1990) O_2-dependent electron flow, membrane energization and the mechanism of non-photochemical quenching of chlorophyll fluorescence. Photosynth Res 25: 279–293

Schreiber U, Schliwa U and Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10: 51–62

Schreiber U, Klughammer C and Neubauer C (1988) Measuring P700 absorbance changes around 830 nm with a new type of pulse modulation system. Z Naturforsch 43: 686–698

Šiffel P and Braunová Z (1999) Release and aggregation of the light-harvesting complex in intact leaves subjected to strong CO_2 deficit. Photosynth Res 61: 217–226

Šiffel P and Vácha F (1998) Aggregation of the light-harvesting complex in intact leaves of tobacco plants stressed by CO_2 deficit. Photochem Photobiol 67: 304–311

Smirnoff N (1993) Tansley Review No. 52. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125: 27–58

Stuhlfauth T, Scheuermann R and Fock HP (1990) Light energy dissipation under water stress conditions. Plant Physiol 92: 1053–1061

Vácha F and Durchan M (1995) The O_2 dependent inhibition of photosynthesis in tobacco plants. In: Mathis P (ed) Photosynthesis: From Light to Biosphere, Vol IV, pp 223–226. Kluwer Academic Publishers, Dordrecht, The Netherlands

van Mieghem FJE, Nitschke W, Mathis P and Rutherford AW (1989) The influence of the quinone-iron electron acceptor complex on the reaction centre photochemistry in Photosystem II. Biochim Biophys Acta 977: 207–214

Vass I, Styring S, Hundall T, Koivuniemi A, Aro AM and Andersson B (1992) Reversible and irreversible intermediates during photoinhibition of Photosystem II; stable reduced Q_A species promote chlorophyll triplet formation. Proc Natl Acad Sci USA 89: 1408–1412

Wu J, Neimanis S and Heber U (1991) Photorespiration is more effective than the Mehler reaction in protecting the photosynthetic apparatus against photoinhibition. Bot Acta 104: 283–291

Wykoff DD, Davies JP, Melis A and Grossman AR (1998) The regulation of photosynthetic electron transport during nutrient deprivation in *Chlamydomonas reinhardtii*. Plant Physiol 117: 129–139