Photochemistry and Photobiology, 1998, 67(3): 304–311

Aggregation of the Light-Harvesting Complex in Intact Leaves of Tobacco Plants Stressed by CO_2 Deficit

Pavel Šiffel* and František Vácha

Institute of Plant Molecular Biology, Academy of Sciences of the Czech Republic, Branišovská, Czech Republic

Received 28 August 1997; accepted 17 December 1997

ABSTRACT

Pronounced aggregation of the photosystem II light-harvesting complex (LHC II) was observed in low-light-grown tobacco plants stressed with a strong CO_2 deficit for 2–3 days. The LHC II aggregates showed a typical band at 697–700 nm (F_{699}) in low-temperature emission spectra. Its excitation spectrum corresponded to that of detergent-solubilized LHC II. Formation of F_{699} in stressed plants was not reversed in the dark and leaves did not contain any zeaxanthin showing that neither a light-induced transthylakoid pH gradient nor zeaxanthin was required for LHC II aggregation. The CO_2-stressed plants showed clear signs of photodamage: depression of the potential yield of photosystem II photochemistry (F_V/F_M) by 50–70% and a decline in chlorophyll content by 10–15%. Therefore, we propose that the photodamage to the photosynthetic apparatus is the cause of the LHC II aggregation in plants. The F_{699} exhibited a reversible decrease of its intensity upon irradiation of leaves with intensive light. There was no or only slight decrease around 700 nm in unstressed plants. The nonphotochemical quenching of chlorophyll fluorescence showed the opposite relation, being higher before than after the strong CO_2 deficit. This discrepancy was likely related to the different LHC II aggregation state in control and stressed plants.

INTRODUCTION

About half of the chlorophyll (Chl)† of higher plants and green algae is bound to a Chl *a* and *b*-containing protein, the light-harvesting complex II (LHC II). Besides its main function, *i.e.* collection of light energy, it plays an important role in the stacking of thylakoid membrane (1) and in the regulation of the energy distribution between the two photosystems (PS) (2,3). Recently, Horton *et al.* (4,5) proposed that the LHC II is involved in the regulation of the dissipation of excitation energy to heat in the pigment antenna. It was hypothesized that the mechanism underlying this regulation is the reversible aggregation of the LHC II controlled by a proton gradient across the thylakoid membrane.

The ability of the LHC II to aggregate was demonstrated *in vitro*. Pronounced LHC II aggregation was stimulated by lowering of the pH from 7 to 4.5–5.5 (4,6,7). Similarly, the xanthophyll cycle, which is generally accepted to regulate heat dissipation in the pigment antenna, seems to control the organization of the light-harvesting complex. Violaxanthin has been shown to inhibit strongly the LHC II aggregation, whereas zeaxanthin had no or a stimulatory effect on it (4,7–9). The LHC II, isolated from thylakoids by mild detergent treatment, has been shown to aggregate upon removal of detergent, on addition of cations (1,4,6,7) and upon an increase of the LHC II/lipid ratio in proteoliposomes (6,10). The LHC II aggregation was also done within the thylakoid membrane by mild treatment with nonionic detergent (11). Also specific changes in lipid composition of the thylakoid membrane may be the cause of LHC II aggregation. Siegel *et al.* (12) found that addition of phospholipids to thylakoids caused extensive aggregation of the LHC II into semicrystalline arrays. This aggregation can be eliminated by use of the uncharged digalactosyldiglyceride instead of phospholipids (13).

Despite great progress in the characterization of isolated LHC II aggregates, little is known about their occurrence in intact tissues. The mechanism of the formation of LHC II aggregates *in vivo* remains unknown. There are several reports of LHC II aggregates *in vivo*. In these studies, LHC II aggregates were monitored by their characteristic emission band with a maximum at about 700 nm. A shoulder at 699 nm was observed in a mutant of *Chlamydomonas reinhardtii* lacking both PS (14). Relative intensity of this shoulder increased with increasing Chl concentration in the algae culture, which seems to be in line with the LHC II aggregation observed in proteoliposomes upon an increase in the LHCII/lipid ratio (6,10). A similar shoulder at 701 nm has been found in the emission spectrum of the LHC I of yellow lime leaves in which both PS, but not LHC II, were completely degraded (15). A reversible quenching at about 700 nm was monitored upon formation of high-energy quenching in thylakoids isolated from high light-stressed spinach leaves (16)

*To whom correspondence should be addressed at: Institute of Plant Molecular Biology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic. Fax: +42 38 41475; e-mail: siffel@umbr.cas.cz

†*Abbreviations:* Chl, chlorophyll; F_0, dark level of Chl fluorescence yield; F_V, variable Chl fluorescence yield; F_M, maximum Chl fluorescence yield; F_{699}, fluorescence emission band peaking at 699 nm; LHC II, light-harvesting complex of photosystem II; PAR, photosynthetically active radiation; PS, photosystem; q_{NP}, nonphotochemical quenching of Chl fluorescence; q_{NP}', rapidly reversible part of nonphotochemical quenching.

© 1998 American Society for Photobiology 0031-8655/98 $5.00+0.00

and in intact whole leaves of the tropical plant *Guzmania monostachia* (7,17,18). It can be noted that fitting of emission spectra of both the constant and the variable fluorescence by Gaussian components required a component near 703 nm (19,20).

In this paper, formation of the emission band at about 700 nm is reported in tobacco plants stressed by CO_2 deficit. Spectral analysis showed that this band originates from LHC II aggregates and exhibits reversible light-induced quenching. A mechanism for the formation of LHC II aggregates in intact leaves is discussed.

MATERIALS AND METHODS

Plant cultivation and treatment. Plantlets of tobacco (*Nicotiana tabacum* L. cv. White Burley) were cultivated in sterile conditions on Murashige and Skoog agar medium (21) in glass vessels (volume about 85 cm^3) covered with aluminum foil (so-called *in vitro* culture). Vessels with plantlets were placed in a controlled chamber under 20°C, 12 h photoperiod and irradiance of 50 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR). For details of cultivation and photosynthetic characteristics of the *in vitro* cultures see Lees (22) and Pospíšilová *et al.* (23), respectively. Then the aluminum covers were replaced with hermetically sealed glass stoppers with a solid CO_2 absorber on the base of sodium hydrate (Natrocalcid, Chemopetrol Spolana Neratovice, Czech Republic) fixed on the inner surface using Ramsay grease. The CO_2 concentration in the glass vessels with the CO_2 absorber was well below the CO_2 compensation point (25–30 ppm) as determined by infrared gas analysis described in Šantrůček *et al.* (24). Ten hermetically closed plants with CO_2 absorber and 10 control plants (covered with aluminum foil) were used for analysis each day after closing the vessels with glass stoppers. The plants were taken from cultivation room 3 h after the beginning of the photoperiod.

In vivo Chl fluorescence. The capacity of PS II photochemistry and fluorescence quenching were assayed with a PAM fluorometer (Walz, Germany; see Schreiber *et al.* (25)). Leaf discs (0.5 cm^2) taken from the central parts of tobacco leaves were placed on a drop of distilled water and predarkened for 15 min in a black metal holder. Then the following sequence of measurements was conducted: measurement of a dark fluorescence yield, F_0, determination of a maximum fluorescence yield, F_M, using a saturation pulse (3200 μmol m^{-2} s^{-1} for 1 s), 3 min dark, measurement of the slow fluorescence decline induced by red actinic light (300 μmol m^{-2} s^{-1}) for 5 min, application of the saturation pulse to determine a maximum fluorescence yield upon actinic irradiation, F_M', relaxation for 7 min and application of the saturation pulse to determine a relaxed maximum fluorescence yield, F_M''. The capacity of PS II photochemistry was calculated as the ratio of variable ($F_V = F_M - F_0$) and maximum fluorescence. High energy fluorescence quenching was accessed as a part of nonpotochemical quenching relaxing within 7 min of darkening. This part, further referred to as q_{NP}', was calculated as the $(F_M'' - F_M')/F_M'$ ratio (for description of symbols see the legend to Fig. 1). It was preferred to Shreiber's ratio (25) because of its proportionality to the concentration of a quencher in the Stern–Volmer equation. An example of fluorescence measurement is given in Fig. 1.

Fluorescence spectra. Fluorescence emission and excitation spectra were measured on leaf disks and diluted leaf homogenates. Homogenates were prepared as follows: Three leaf disks of total area of 0.8 cm^2 were homogenized in 0.3 mL of medium (0.4 *M* sorbitol, 50 m*M* Tricine buffer, pH 7.6, 10 m*M* KCl, 2.5 m*M* $MgCl_2$, 10 m*M* sodium ascorbate) using a glass homogenizer and filtered through cotton wool. The homogenate was soaked into a glass microfiber filter (Whatmann) and immediately frozen in liquid nitrogen. The Chl concentration in samples was about 0.5 μg Chl($a+b$) cm^{-2}. The time from the start of the homogenization to freezing of the sample was less than 1 min. The disks of the total area of 1–1.3 cm^2 taken from the same leaf were used for determination of the pigment content. Fluorescence emission and excitation spectra were measured with a Fluorolog spectrofluorometer (SPEX, USA) using the spectral bandwidth of emission or excitation monochromator of 2 nm. The slit of the second monochromator was adjusted to give a bandwidth of 4 nm. The excitation wavelength was 490 nm. All spectra were measured at 77°K.

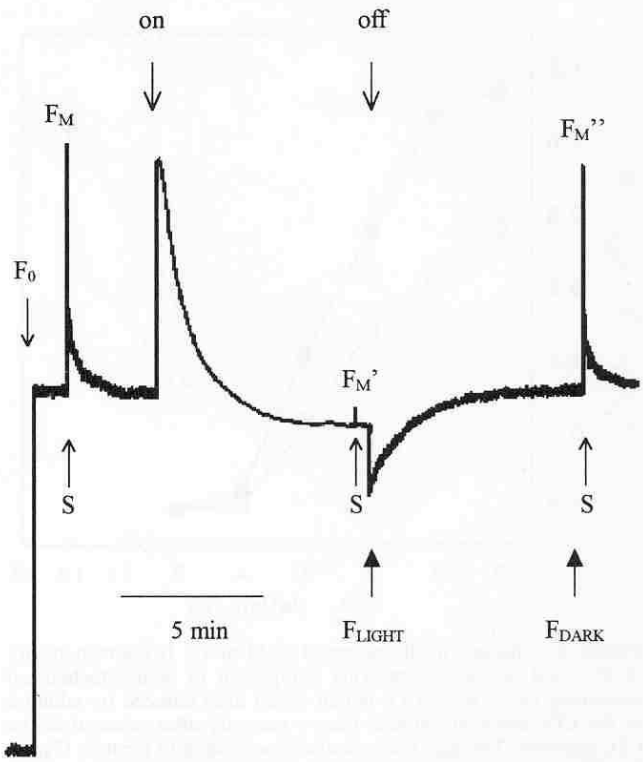

Figure 1. An example of measurement of Chl fluorescence yield from a tobacco leaf. The tobacco plant was stressed for 2 days by CO_2 deficit. The term F_0 refers to the dark level of chlorophyll fluorescence; F_M, F_M' and F_M'' refer to a maximum fluorescence yield in the dark, under irradiation with actinic light and after dark relaxation, respectively. F_M, F_M' and F_M'' were determined using a 1 s saturation pulse (3200 μmol PAR m^{-2} s^{-1}) of white light (indicated by arrows with "s"). "On" and "off" refers to the start and the end of irradiation with red actinic light (300 μmol PAR m^{-2} s^{-1}). Full arrows indicate the freezing of samples for measurement of ratios of fluorescence emission spectra presented in Fig. 8.

Absorption spectra. Absorption spectra of tobacco were measured with whole leaves frozen on a Plexiglas holder in liquid nitrogen. Spectra were recorded with a PU 88OO spectrophotometer (Philips-Pye Unicam, UK) using a bandwidth of 2 nm.

Isolation of LHC II. The LHC II was isolated using sodium dodecylsulfate polyacrylamide gel electrophoresis according to Anderson (26) as described in Šiffel *et al.* (27).

Zeaxanthin content. 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 injection. The pigment composition was analyzed using the modified HPLC method of Gilmore and Yamamoto (28) on a C-18 15 × 3 mm, 5 μm (Tessek, Czech Republic) reverse-phase column using isocratic elution of a mixture of acetonitrile, methanol 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 for 5 min until 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 a gas chamber under nitrogen atmosphere and subjected to high intensive light (slide projector with a heat filter, 1000 μmol m^{-2} s^{-1}) for 20 min.

Chlorophyll content. Amounts of Chl *a* and *b* in leaf homogenates were determined in 80% acetone extracts with a PU 88OO spectrophotometer (Philips-Pye Unicam, UK) according to Lichtenthaler (29).

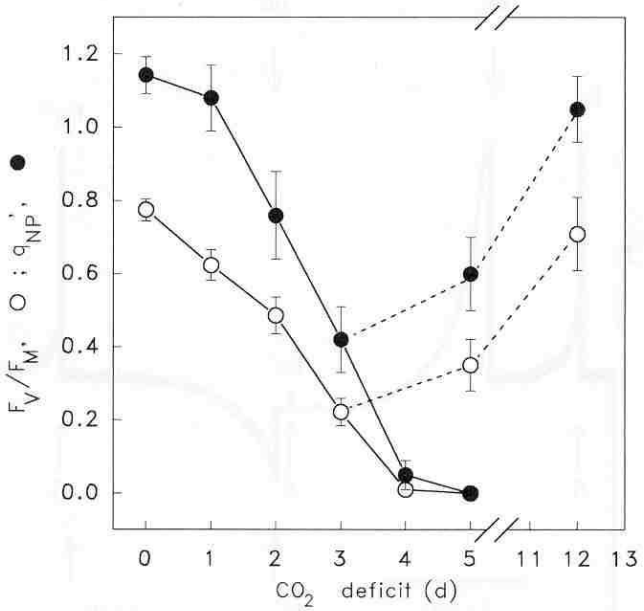

Figure 2. Changes in the potential yield of PS II photochemistry (F_V/F_M) and the rapidly relaxing component of nonphotochemical quenching (q_{NP}') upon CO_2 deficit (solid line) induced by addition of the CO_2 absorber. Broken line = recovery after removal of the CO_2 absorber. The q_{NP}' was calculated according to formula $(F_M'' - F_M')/F_M'$. Both parameters were measured with a PAM fluorometer. Intensities of saturation pulse and red actinic light were 3200 and 300 μmol m^{-2} s^{-1}, respectively.

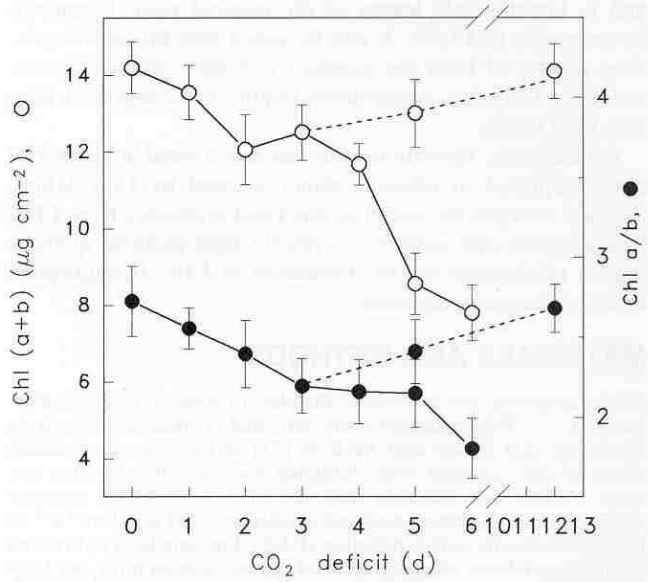

Figure 3. Changes in Chl content and Chl *a*/*b* ratio upon CO_2 deficit (solid line) induced by addition of the CO_2 absorber. Broken line = recovery after removal of the CO_2 absorber.

RESULTS AND DISCUSSION

A carbon dioxide deficit has been proven to increase considerably the sensitivity of the photosynthetic apparatus to photoinhibitory damage. Under CO_2 deficit, even very low light intensities are able to cause depression of the activity of PS II (30). The changes in PS II function upon CO_2 depletion were tested using the ratio of variable to maximal fluorescence, F_V/F_M (Figs. 1 and 2), characterizing the yield of PS II photochemistry. In our experimental conditions (50 μmol PAR m^{-2} s^{-1}, 20°C, 25–30 ppm of CO_2), the F_V/F_M ratio declined to zero within 4–5 days. Depression of the PS II yield was reversible in atmospheric CO_2 concentration until the third day of the stress. The recovery of the PS II photochemistry lasts about 9 days. Later, the recovery of the PS II yield was much slower or was not observed at all. Similar time dependence was found also for nonphotochemical quenching (q_{NP}, results not shown) and q_{NP}', the rapidly relaxing component of q_{NP} (Fig. 2). Thus, the tobacco plants stressed by CO_2 deficit for 2 or 3 days were used for further analyses. These plants did not exhibit any visible signs of damage. The Chl content in their leaves was lowered only by 10–15% relative to unstressed plants (Fig. 3).

Spectral characterization of LHC II aggregates

A qualitative difference between the plants stressed for 2 or 3 days and unstressed ones was found in fluorescence emission spectra. The emission spectrum of unstressed plants consists of three main bands with maxima at 685, 695 and at about 735 nm. Two former bands originate from PS II and the third one from PS I. In addition, there is a small shoulder at the short-wavelength edge of the spectrum (at 681 nm) that represents the LHC II emission (31,32). The shoulder can be clearly detected in intact tissues using a second derivative analysis (see, *e.g.* Šiffel *et al.* (15)). In the stressed plants a new shoulder at 699 nm appeared (Fig. 4A). This shoulder was clearly detectable in whole leaves but not in diluted leaf homogenates. The reason was the reabsorption. A strong reabsorption of fluorescence in the leaf depressed the short-wavelength emission including the main band at 685 nm and, thus, the minor bands positioned at longer wavelengths (*i.e.* at 695 and around 699 nm) became more pronounced. For comparison of emission spectra of the leaf disk and the diluted homogenate prepared from the same leaf see Fig. 4B. It is possible that the observed changes can be explained by reabsorption. Reabsorption is sensitive to changes in pigment concentration and changes in the form of the absorption spectrum. Chlorophyll concentration declined by 10–15% during 2 or 3 days of stress. At this time, the absorption spectra of the stressed leaves showed only small changes in the region 680–720 nm. Even at later stages of stress, when absorbance changes were higher (see Fig. 7), these changes did not exceed about 10%. Such small changes are not sufficient to explain dramatic changes in the emission spectrum. In the emission spectrum, at later stages of the stress, a similar band was found in highly diluted samples where reabsorption can be excluded (see below).

The emission band peaking at about 700 nm is usually ascribed to aggregates of the LHC II (1,4). We performed more detailed spectral analysis of F_{699} on diluted leaf homogenates to confirm this statement in intact plants. In plants stressed for 2 or 3 days, only a small shoulder at 699 nm was formed. Its spectral analysis was complicated because of high overlapping with PS II emission. Nevertheless, under prolonged CO_2 deficit, photodamage caused a marked decline of PS II emission (Fig. 5). Thus, in order to take excitation spectra of F_{699}, several plants were kept in CO_2 deficit for 5–6 days. In these samples, emission bands of

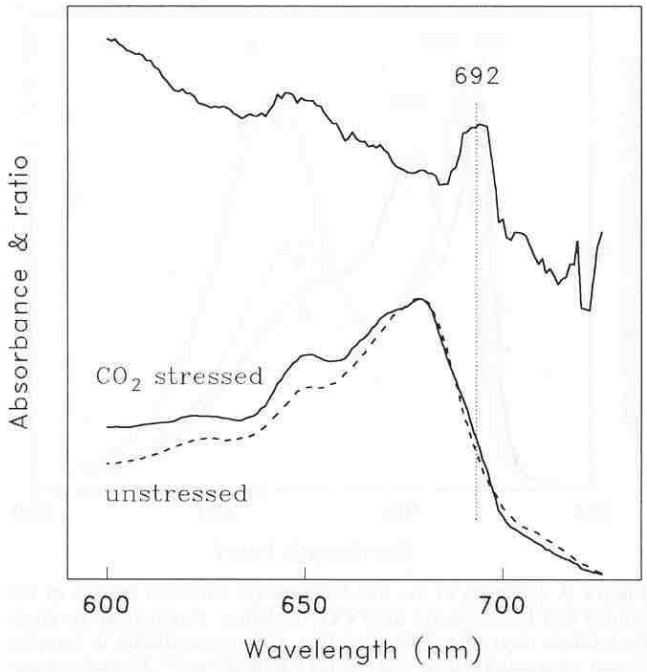

Figure 7. Comparison of 77°K absorption spectra for tobacco leaves before (broken line) and 6 days after CO_2 depletion (solid line). The ratio of the spectra (CO_2 depleted/control), depicted on the top of the figure, clearly shows the band near 692 nm arising on CO_2 depletion.

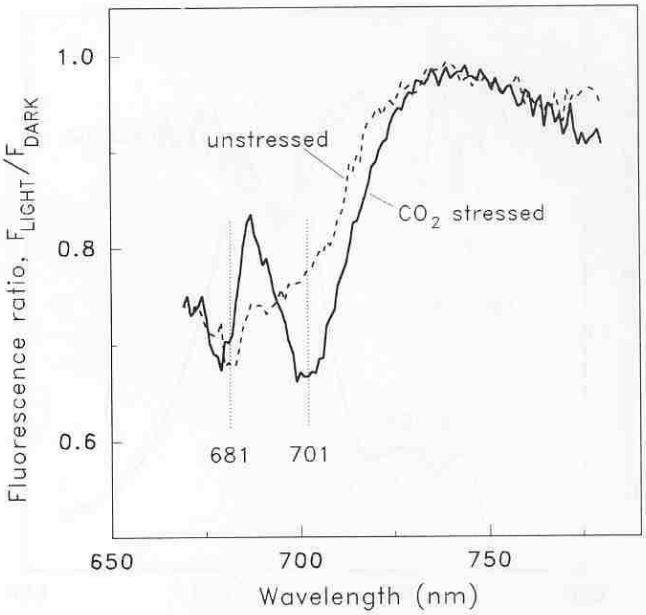

Figure 8. Comparison of reversible light-induced changes in the fluorescence emission spectra for the plant stressed by CO_2 deficit for 2 days (solid line) and the unstressed plant (broken line). The figure depicts the ratios of spectra of irradiated and dark relaxed samples. The samples were frozen (in the dark) 7 min after irradiation with actinic light (F_{LIGHT}) or after dark relaxation for 7 min (F_{DARK}) as indicated by arrows in Fig. 1.

ference around 690 nm. In this spectral region, a shoulder with the maximum at 691–693 nm was formed upon CO_2 deficit of tobacco plants (see also the ratio of the spectra at the top of Fig. 7). The shoulder likely represents a Chl form produced upon LHC II aggregation. A similar band could be found in difference spectra of light-scattering samples. Absorption bands became flatter and broader upon scattering of light, exhibiting relative depression of absorbance at the maximum. The difference between the spectra of scattering and transparent samples gives two maxima at both sides of the peak. This is true for difference spectra but not for their ratio, which was therefore used in our paper. The ratio of ''scattering/transparent'' should give a negative peak positioned at the absorption maximum.

Other differences (in the region of Chl *b* absorption and above 700 nm) are related to the different abundance of LHC II and complexes of PS in control and stressed plants and reflect the degradation of both PS under CO_2 deficiency.

Thus, the spectral analysis shows that the long-wavelength Chl species with absorption and emission maxima at 691–693 and 699 nm, respectively, formed upon CO_2 deficit of tobacco plants, originates from aggregates of LHC II.

Light-induced decrease of fluorescence intensity of LHC II aggregates

Ruban *et al.* (16,17) demonstrated a reversible light-induced decrease of Chl fluorescence near 700 nm in isolated thylakoids and in intact leaves of tropical plant *G. monostachia.* This decrease was interpreted to reflect a change in the aggregation state of the LHC II *in vivo,* and has been taken as evidence supporting the involvement of LHC II aggregation in high-energy quenching of Chl fluorescence. Therefore, we tested whether the emission band formed upon CO_2 deficit in tobacco plants undergoes a similar light-induced decline. We compared the light-induced decrease of F_{699} with the rapidly relaxing component of q_{NP} for both stressed and control tobacco plants. This component is usually taken to reflect the high-energy quenching (5,18). Segments cut from one leaf of stressed tobacco were irradiated with actinic light for 7 min to induce q_{NP} and, then, relaxed in the darkness for 7 min (Fig. 1). Samples for measurement of emission spectra were frozen in liquid nitrogen before and after dark relaxation as indicated by full arrows in Fig. 1. In order to emphasize the light-induced changes, ratios of the spectra taken before and after dark relaxation (F_{LIGHT}/F_{DARK}) were calculated (Fig. 8). The ratio F_{LIGHT}/F_{DARK} gives similar information as the ratio $(F_{DARK} - F_{LIGHT})/F_{DARK} = 1 - F_{LIGHT}/F_{DARK}$ used by Ruban *et al.* (16). The form of the spectral dependence of the ratio F_{LIGHT}/F_{DARK} does not depend on relative intensities of individual spectra and on fluorescence reabsorption in the sample and, thus, it is easier to use it when measuring the spectra without normalization on an internal fluorescence standard (*e.g.* rhodamine). The F_{LIGHT}/F_{DARK} ratios show that both the stressed and control plants exhibited a reversible light-induced decrease of fluorescence intensity of the PS II. In addition, the CO_2-stressed plants exhibited a reversible light-induced decline around 700 nm and at 680 nm similar to that described by Ruban *et al.* (16,17). In unstressed plants, however, no or only a slight shoulder around 700 nm was found in the ratio. This shows that the LHC II aggregates formed during CO_2 deficit undergo reversible light-induced changes which parallel the high-energy quenching.

On the other hand, there was an apparent discrepancy between the extent of q_{NP}' and light-induced changes at 700 nm in CO_2-stressed and control plants. The CO_2-stressed plants showed a lower q_{NP}' than the control ones and a highly pronounced depression around 700 nm. On the contrary, the control plants exhibited a higher q_{NP}' without a pronounced decrease around 700 nm. Therefore, the reversible light-induced depression of emission intensity around 700 nm does not depend only on q_{NP}'. The CO_2-stressed plants differed from control plants in the aggregated state of the LHC II as indicated by the presence of the F_{699} in emission spectra of stressed material (Fig. 4A) and, thus, the light-induced depression of fluorescence around 700 nm seems to depend, in addition to the extend of q_{NP}', on the aggregation state of the LHC II present in thylakoids before induction of q_{NP}' by actinic light. Similarly, a light-induced decrease around 700 nm observed even in unstressed plant material (16) can depend on preexistent LHC II aggregation, the presence of which in unstressed plants has been indicated by several experiments (33–35).

What can be the cause of LHC II aggregation in intact plants?

By analogy with the aggregation induced in isolated LHC II and isolated thylakoids, one can consider that LHC II aggregation in intact tissues was stimulated by low intrathylakoid pH and/or by zeaxanthin (4,5). A pH gradient is formed upon irradiation of the photosynthetic apparatus and is dissipated within several minutes of darkness. Our samples prepared for measurement of fluorescence spectra were frozen under dim light, which is not able to form a marked pH gradient. In order to exclude the effect of light at all, several samples were predarkened for 15 min before freezing. No effect of predarkening on the fluorescence intensity of F_{699} was observed. The F_{699} was observed even at the end of the dark period of cultivation, *i.e.* after 12 h of darkness. Moreover, the delay of several days between the CO_2 depletion and appearance of the LHC II aggregates can hardly be explained by means of lumen protonation, which occurs within minutes after irradiation. Hence, low pH does not seem to be responsible for the observed LHC II aggregation. To test the role of the xanthophyll cycle, we determined the content of zeaxanthin in tobacco leaves in both CO_2-stressed and control plants. Surprisingly, no zeaxanthin was found in either group of plants (Fig. 9). Neither was anteraxanthin, an intermediate in deepoxidation of violaxanthin to the zeaxanthin, present. In order to verify the ability of our plants to form zeaxanthin, some of them were irradiated by intensive light (1000 μmol m^{-2} s^{-1}) for 20 min in a nitrogen atmosphere and the capacity of the xanthophyll cycle (amount of zeaxanthin formed during high-light irradiation relative to the total amount of xanthopylls involved in the xanthophyll cycle) was determined. This showed values around 10% for unstressed plants. In stressed plants, similar values were found during the first 3 days of the stress and then the capacity of the xanthophyll cycle declined (Fig. 9). Thus, surprisingly, a CO_2 deficit did not induce formation of zeaxanthin or a marked increase in the capacity of the xanthophyll cycle. Although we did not study the reasons for the lack of the response of the zeaxanthin protective

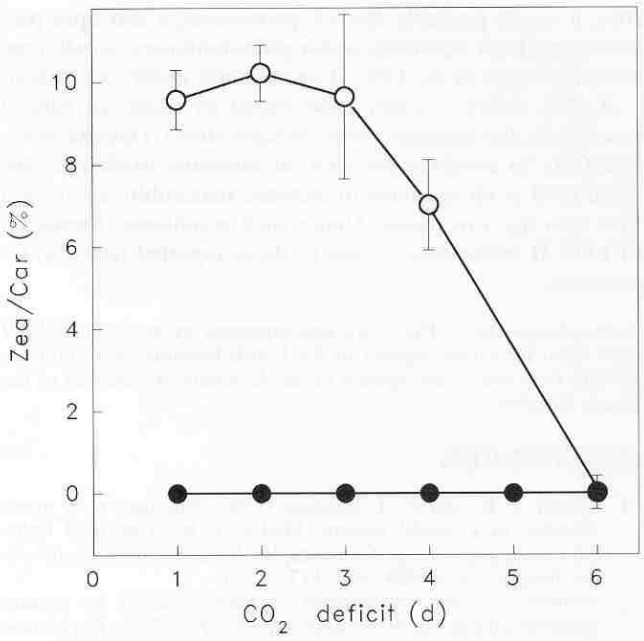

Figure 9. Changes in zeaxanthin content (closed symbols) and the capacity of the zeaxanthin cycle (open symbols) during CO_2 deficit. The capacity was determined as the amount of zeaxanthin (relative to total xanthophyll cycle pigments) formed in leaves under irradiation with 1000 μmol PAR m^{-2} s^{-1} in nitrogen atmosphere for 20 min.

mechanism to CO_2 deficit in our plants, it seems reasonable to mention here at least one possible cause of such unusual plant response—cultivation under very low irradiance (50 μmol m^{-2} s^{-1}).

A CO_2 deficit is generally considered to be strong photoinhibitory treatment, which, when applied for a long time, causes pronounced photodamage to the photosynthetic apparatus even at very low irradiance. Because of different sensitivity of individual constituents of thylakoid membranes to photodamage, the composition of thylakoids can be changed by long-term CO_2 deficit. In our experiments, damage to Chl proteins was indicated by changes in the spectra of CO_2-stressed plants (see Fig. 5). They showed that both PS were degraded faster than LHC II. Most of the LHC II is usually bound to PS II. Upon photodamage to PS, LHC II was, as indicated by relative increase in its emission intensity, released from PS II, which could facilitate the LHC II aggregation. A further indicator of photodamage was the Chl content. It decreased slowly during the first 4 days and then, following the full PS II photoinhibition, a rapid decline in Chl content occurred. It coincided with an increase in the emission band at 699 nm relative to the LHC II emission at 681 nm.

Several papers, noted already in the introduction, referred to the LHC II aggregation induced by changes in the lipid/protein ratio (6,10,14) or changes in lipid composition (12,13) in artificial (liposomes) or native membranes. Besides damage to Chl proteins, photooxidation of lipid is known to occur under photoinhibitory conditions (36–38). This can lead to a decrease in the lipid-to-protein ratio and/or to a change in the composition of lipids in the thylakoid membrane and, thus, to cause LHC II aggregation. There-

fore, it seems probable that PS photodamage and lipid peroxidation, both occurring under photoinhibitory conditions, were the cause of the LHC II aggregation under CO_2 deficit.

A CO_2 deficit is often experienced by plants in natural conditions, for instance under drought stress. Deficits of O_2 and CO_2, in combination with an intensive irradiance, are often used as an approach to increase zeaxanthin levels (and thus also q_{NP}') in plants. Under such conditions, formation of LHC II aggregates similar to those reported here can be expected.

Acknowledgements—This work was supported by grant no. 204/94/0261 from the Grant Agency of the Czech Republic and grant no. 651403 from the Grant Agency of the Academy of Sciences of the Czech Republic.

REFERENCES

1. Mullet, J. E. and C. J. Arntzen (1980) Simulation of grana stacking in a model system. Mediation by a purified light-harvesting pigment–protein complex from chloroplasts. *Biochim. Biophys. Acta* **589**, 100–117.
2. Bennett, J. (1984) Regulation of photosynthesis by protein phosphorylation. In *Molecular Aspects of Cellular Regulation* (Edited by P. Cohen), pp. 227–246. Elsevier, Amsterdam.
3. Anderson, J. M. (1986) Photoregulation of the composition, function and structure of thylakoid membrane. *Annu. Rev. Plant Physiol.* **37**, 93–186.
4. Horton, P., A. V. Ruban, D. Rees, A. A. Pascal, G. Noctor and A. J. Young (1991) Control of the light-harvesting function of chloroplast membranes by aggregation of the LHC II chlorophyll–protein complex. *FEBS Lett.* **242**, 1–4.
5. Horton, P., A. V. Ruban and R. G. Walters (1996) Regulation of light harvesting in green plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 665–684.
6. Ryrie, I. J., J. M. Anderson and D. J. Goodchild (1980) The role of the light-harvesting chlorophyll a/b–protein complex in chloroplast membrane stacking. Cation induced aggregation of reconstituted proteoliposomes. *Eur. J. Biochem.* **107**, 345–354.
7. Ruban, A. V. and P. Horton (1994) Spectroscopy of non-photochemical and photochemical quenching of chlorophyll fluorescence in leaves; evidence for a role of the light harvesting complex of photosystem II in regulation of energy dissipation. *Photosynth. Res.* **40**, 181–190.
8. Ruban, A. V., A. J. Young and P. Horton (1996) Dynamic properties of the minor chlorophyll a/b binding proteins of photosystem II, an *in vitro* model for photoprotective energy dissipation in the photosynthetic membrane of green plants. *Biochemistry* **35**, 674–678.
9. Phillip, D., A. V. Ruban, P. Horton, A. I. Asato and A. J. Young (1996) Quenching of chlorophyll fluorescence in the major light-harvesting complex of photosystem II: a systematic study of the effect of carotenoid structure. *Proc. Natl. Acad. Sci. USA* **93**, 1492–1497.
10. Larkum, A. W. D. and J. M. Anderson (1982) The reconstitution of a photosystem II protein complex, P-700–chlorophyll *a*–protein complex and light-harvesting chlorophyll *a/b*–protein. *Biochim. Biophys. Acta* **679**, 410–421.
11. Lyon, M. K. and K. R. Miller (1985) Crystallization of the chlorophyll a/b light-harvesting complex within thylakoid membrane. *J. Cell Biol.* **100**, 1139–1147.
12. Siegel, C. O., A. E. Jordan and K. R. Miller (1981) Addition of lipid to photosynthetic membrane: effects on membrane structure and energy transfer. *J. Cell Biol.* **91**, 113–125.
13. Sprague, S. G., E. L. Camm, B. R. Green and L. A. Staehelin (1985) Reconstitution of light-harvesting complexes and photosystem II core into galactolipid and phospholipid liposomes. *J. Cell Biol.* **100**, 552–557.
14. Lebedev, N. N., R. A. Khatyrov, V. A. Ladygin and A. A. Krasnovskii (1988) Fluorescence excitation spectra and decay kinetics of light-harvesting complex in *Chlamydomonas reinhardtii* mutants. *Photosynthetica* **22**, 364–370.
15. Šiffel, P., M. Kutík and N. N. Lebedev (1991) Spectroscopically analyzed degradation of chlorophyll–protein complexes and chloroplasts ultrastructure during yellowing of leaves. *Photosynthetica* **25**, 395–407.
16. Ruban, A. V., D. Rees, G. D. Noctor, A. Young and P. Horton (1991) Long-wavelength chlorophyll species are associated with amplification of high-energy-state excitation quenching in higher plants. *Biochim. Biophys. Acta* **1059**, 355–360.
17. Ruban, A. V., A. J. Young and P. Horton (1993) Induction of nonphotochemical energy dissipation and absorbance changes in leaves. *Plant Physiol.* **102**, 741–750.
18. Ruban, A. V. and P. Horton (1995) Regulation of non-photochemical quenching of chlorophyll fluorescence in plants. *Aust. J. Plant Physiol.* **22**, 221–230.
19. Shubin, V. V., V. A. Sineshchekov and F. F. Litvin (1976) Variable fluorescence of native forms of chlorophyll at −196°C. *Biofizika* **21**, 760–762.
20. Zucchelli, G., R. C. Jennings and F. M. Garlaschi (1992) Independent fluorescence emission of the chlorophyll spectral forms in higher plants photosystem II. *Biochim. Biophys. Acta* **1099**, 163–169.
21. Murashige, T. and F. Skoog (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**, 473–497.
22. Lees, R. P. (1994) Effect of light environment on photosynthesis and growth *in vitro*. In *Physiology, Growth and Development of Plants in Culture* (Edited by P. J. Lumsden, J. R. Nicholas and W. J. Davies), pp. 31–46. Kluwer Academic Publishers, Dordrecht.
23. Pospíšilová, J., J. Solárová and J. Čatský (1992) Photosynthetic responses to stress during *in vitro* cultivation. *Photosynthetica* **26**, 3–18.
24. Šantrůček, J., P. Šiffel, M. Polišenská and M. Pešková (1991) Species-specific response of the photosynthetic apparatus of tobacco and potato to low CO_2 concentration. *Photosynthetica* **25**, 203–209.
25. Schreiber, U., U. Schliwa and W. Bilger (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res.* **10**, 51–62.
26. Anderson, J. M. (1980) P-700 content and polypeptide profile of chlorophyll–protein complexes of spinach and barley thylakoids. *Biochim. Biophys. Acta* **591**, 113–126.
27. Šiffel, P., E. Šindelková, M. Durchan and M. Zajícová (1992) Photosynthetic characteristics of *Solanum tuberosum* L. plants transformed by *Agrobacterium* strains. 1. Pigment apparatus. *Photosynthetica* **27**, 441–447.
28. Gilmore, A. M. and H. Y. Yamamoto (1991) Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C18 high performance liquid chromatographic column. *J. Chromatogr.* **543**, 137–145.
29. Lichtenthaler, H. K. (1987) Chlorophylls and carotenoids: pigments of photosynthetic membranes. In *Methods in Enzymology* (Edited by L. Packer and R. Douce), pp. 350–382. Academic Press, New York.
30. Osmond, C. B. (1981) Photorespiration and photoinhibition. Some implications for the energetics of photosynthesis. *Biochim. Biophys. Acta* **639**, 77–89.
31. Satoh, K. (1980) F-695 emission from the purified photosystem II chlorophyll a–protein complex. *FEBS Lett.* **110**, 53–56.
32. Hemelrijk, P. W., S. L. S. Kwa, R. van Grondelle and J. P. Dekker (1992) Spectroscopic properties of LHC-II, the main light-harvesting chlorophyll a/b protein complex from chloroplast membranes. *Biochim. Biophys. Acta* **1089**, 159–166.
33. Kolubayev, T., N. E. Geacintov, G. Paillotin and J. Breton (1986) Domain size in chloroplasts and chlorophyll–protein complexes probed by fluorescence yield quenching induced by singlet–triplet exciton anihilation. *Biochim. Biophys. Acta* **376**, 105–115.
34. Garab, G., J. Kieleczava, J. C. Sutherland, C. Bustamante and C. Hind (1991) Organization of pigment–protein complexes into macrodomains in the thylakoid membranes of wild-type and chlorophyll *b*-less mutant of burley as revealed by circular dichroism. *Photochem. Photobiol.* **54**, 273–281.

35. Bassi, R. and P. Dianese (1992) Supramolecular light-harvesting complex from chloroplast photosystem II membranes. *Eur. J. Biochem.* **204**, 317–326.
36. Girotti, A. W. (1990) Photodynamic lipid peroxidation in biological systems. *Photochem. Photobiol.* **51**, 497–509.
37. Hodgson, R. A. J. and J. K. Raison (1991) Lipid peroxidation and superoxide dismutase activity in relation to photoinhibition induced by chilling in moderate light. *Planta* **185**, 215–219.
38. Chauhan, N. P., T. Fatma and R. K. Mishra (1992) Protection of wheat chloroplasts from lipid peroxidation and loss of photosynthetic pigments by quercetin under strong illumination. *J. Plant Physiol.* **140**, 409–413.