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Photosynthetic light-harvesting function of carotenoids in higher-plant leaves exposed to high light irradiances

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Abstract. Exposure of barley (*Hordeum vulgare* L.) leaves to strong white light (1500 μmol photons · m^{-2} · s^{-1}) decreased the quantum yield of photosynthetic oxygen evolution in green light preferentially absorbed by carotenoids (Φ-510) but not in red light exclusively absorbed by chlorophylls (Φ-650). This phenomenon was observed to be (i) rapidly induced (within a few minutes), (ii) slowly reversible in darkness (within about 1 h), (iii) insensitive to dithiothreitol and (iv) maximally induced by photon flux densities higher than about 1000 μmol · m^{-2} · s^{-1}. Determination of the carotenoid composition of the major light-harvesting complex of PSII (LHCII) and analysis of the thylakoid membrane lipid fluidity before and after strong illumination of barley leaves in the presence or the absence of dithiothreitol showed that the light-induced decrease in the Φ-510/Φ-650 ratio did not require the physical detachment of carotenoids from the pigment antennae. Compared to barley plants grown under moderate light and temperature conditions, plants grown in sustained high irradiance at elevated temperature exhibited (i) a lower Φ-510/Φ-650 ratio, (ii) a reduced size of the functional PSII pigment antenna in green light (but not in red light) and (iii) a marked increase in the amount of free carotenoids found in non-denaturing Deriphat-containing electrophoretic gels of thylakoid membranes. Similarly, the Φ-510/Φ-650 ratio of the LHCII-deficient *chlorina-f2* barley mutant was very low compared to the wild type. Separation and quantification of the *cis*/*trans* carotenoid isomers of barley leaves revealed that strong illumination did not induce pronounced *cis-trans* isomerization of xanthophylls. Taken together, the data suggest that the efficiency of energy transfer from carotenoids to chlorophylls varies with the light environment both in the short term and in the long term, with excess light energy noticeably inhibiting the photosynthetic light-harvesting function of carotenoids. The photoprotective significance of this carotenoid decoupling from the chlorophyll antennae is discussed.

Key words: Carotenoid – *Hordeum* (carotenoid, photoprotection) – Light harvesting – Light stress – Photosynthetic oxygen evolution – Xanthophyll isomers

Introduction

The carotenoids serve at least two important functions in photosynthesis, namely light harvesting and photoprotection (Mathis and Schenck 1982; Lichtenthaler 1987; Siefermann-Harms 1990; Koyama 1991; Frank and Cogdell 1996). Carotenoids are vital as photoprotective agents which prevent the chlorophyll-photosensitized formation of highly destructive singlet oxygen by intercepting the chlorophyll triplet states and by scavenging any additional singlet oxygen present. Carotenoids also act as light-harvesting pigments by absorbing sunlight and transferring the excitation energy to (bacterio)chlorophyll, which eventually reaches the reaction center. By this mechanism, photosynthetic organisms can utilize sunlight more efficiently, because carotenoids absorb green light which is abundant in sunlight but is weakly absorbed by chlorophyll. The efficiency of energy transfer from carotenoids to (bacterio)chlorophylls has been mainly studied in the reaction centers and light-harvesting complexes (LHCs) of photosynthetic bacteria. Particularly, it has been shown that the energy-transfer efficiency varies considerably, depending on various factors including the carotenoid species, the distance and geometrical arrangement between carotenoids and bacteriochlorophylls, and the

Abbreviations and symbols: Ant, A = antheraxanthin; DCMU = 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LHCII = light-harvesting complex of PSII; PFD = photon flux density; SASL = spin-labeled stearic acid; $t_{1/2}$ = half time of the induced chlorophyll fluorescence rise; Vio, V = violaxanthin; Zea, Z = zeaxanthin; τ_C = apparent rotational correlation time; Φ = quantum yield of oxygen evolution

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stereochemical structures of the polyene chain of carotenoids (Nogushi et al. 1990; Cogdell et al. 1992; Frank and Cogdell 1996). The existence of carotenoids which do not transfer the absorbed light energy to bacteriochlorophyll has also been reported in photosynthetic bacteria (Nogushi et al. 1992).

In the major LHC of PSII (LHCII) of green plants, lutein has been reported to transfer singlet-state energy to chlorophyll *a* with approx. 100% efficiency (Siefermann-Harms and Ninnemann 1982). This high efficiency is believed to result from the dense packing of pigments in the LHCII with carotenoids being in close contact with chlorophylls. Consequently, slight perturbations of the pigment organization of the LHCs are likely to impede the photosynthetic light-harvesting activity of carotenoids. Accordingly, various treatments such as incubation in low concentrations of detergent and urea or exposure to elevated temperatures were shown to selectively interrupt the carotenoid-to-chlorophyll singlet-singlet energy transfer in isolated LHCII (Siefermann-Harms and Ninneman 1982; Siefermann-Harms 1990). A similar phenomenon was suggested to occur in vivo in intact leaves submitted to mild heating (Havaux and Tardy 1996) and to strong light stress (Havaux et al. 1991). More precisely, exposure of pea leaves to intense white light was observed to reduce the relative contribution of carotenoids in the chlorophyll fluorescence excitation spectrum. This effect was interpreted as the manifestation of a lowered energy flow from accessory carotenoid pigments to antenna chlorophylls. In the present study, we investigate this phenomenon more extensively, taking advantage of the photoacoustic method. Indeed, as recently pointed out by Kramer and Crofts (1996), one of the most useful applications of the photoacoustic technique is the in-vivo determination of the spectral dependence of the quantum yield of photosynthesis. The quantum yield of oxygen evolution (Φ) was measured in red light (directly absorbed by chlorophylls) and in green light (preferentially absorbed by carotenoids) in barley leaves exposed to short-term and long-term light treatments. This photoacoustic study was complemented by fluorimetric measurements of the functional PSII antenna size, by biochemical analyses of the photosynthetic pigments and by electron-resonance spectroscopic measurements of the thylakoid membrane lipid fluidity.

Materials and methods

Plant material and treatments. Two barley genotypes (*Hordeum vulgare* L.) were used: the Plaisant cultivar (wild type) and the chlorophyll-*b*-less *chlorina-f2* mutant. Plaisant seeds were procured from GAE-Recherche (Maisse, France) and *chlorina-f2* seeds were kindly provided by Dr. B. Genty (University of Orsay, Paris, France). Plants were grown in a phytotron under controlled conditions of temperature (23 °C/18 °C), light (350–400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 12 h/d) and air humidity (70%) or in a greenhouse, as previously described (Havaux and Tardy 1997). Leaves were exposed to a strong white light of photon flux density (PFD) 1000 or 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at constant temperature (23 °C), as described elsewhere (Havaux and Tardy 1997). Leaves were infiltrated with 5 mM DTT for 4 h through the transpiration stream. Wild-type barley plants aged three weeks were transferred to a 700-l growth chamber: light and temperature were progressively increased to final values of 1400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 36.5 °C. Plants were grown for 10–12 d under those extreme conditions. Control plants were kept under the same conditions as those prevailing in the phototron. Photon flux densities were measured with a Li-Cor quantum-meter (Li 185B/Li 190SB; Li-Cor, Lincoln, Neb., USA).

Photoacoustic and chlorophyll-fluorescence measurements. Chlorophyll fluorescence was measured in modulated light with a PAM-type fluorometer (Walz, Effeltrich, Germany). The initial fluorescence level (Fo) was excited by a weak red light (650 nm) modulated at 16 kHz. The fluorescence signals were recorded with a computer using the DA-100 data acquisition system (Walz). The maximal quantum yield of PSII photochemistry was calculated as $Fv/Fm = (Fm-Fo)/Fm$ where Fm is the maximal yield of chlorophyll fluorescence induced by a 800-ms pulse of intense white light. After 30 min infiltration with 50 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) in darkness, chlorophyll fluorescence was induced by a red light (655 nm, 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or a blue light (470 nm, 12.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$) produced by a light-emitting diode (PAM-102-L or ED-101-PL, respectively; Walz). The half-time ($t_{1/2}$) of the fluorescence rise was taken as a measure of the functional antenna size of PSII (Malkin et al. 1981).

Photosynthetic oxygen evolution was measured in green light or in red light by the photoacoustic method, as previously described (Havaux and Tardy 1996, 1997). The measuring light was modulated at 19 Hz and was filtered through a red or green interference filter (Oriel 57610 or 57550) peaking at 650 nm or 510 nm, respectively. The PFDs of the red and green light beams were 55 and 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Photosynthesis was saturated with a strong white light of PFD 4000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The quantum yield of oxygen evolution (Φ) was estimated from the ratio of the photobaric signal amplitude to the photothermal signal amplitude as described by Malkin and Canaani (1994).

Preparation of LHCII. Thylakoid membranes were prepared according to Robinson and Yocum (1980) except that 5 mM DTT was added to the isolation medium and that Tricine was replaced by Hepes in the last extraction step. The major LHC of PSII (LHCII) was purified using the Mg^{2+} precipitation method of Krupa et al. (1987). Purified LHCII was stored at −80 °C in 20 mM Tricine-NaOH (pH8), 2 mM EDTA, and 200 mM sorbitol before further analysis.

Pigment determinations. Pigments were extracted from leaf discs or LHCII in methanol. After centrifugation and filtration, pigments were separated and quantified by HPLC as described by Havaux and Tardy (1996).

The *cis*/*trans* carotenoid isomers were separated using the HPLC method of Schoefs et al. (1995). This method allows the xanthophylls to be well resolved in their *cis* and *trans* isomers whereas carotene isomers are poorly separated. The chromatograph was equipped with a photodiode-array detector in order to determine the electronic absorption spectrum of each peak during the HPLC analysis. In order to prevent triplet-sensitized carotenoid photo-isomerization, all the procedures of extraction and HPLC analysis were done in darkness or in dim green light at about 4 °C.

Deriphat-polyacrylamide gel electrophoresis of thylakoid membranes. Thylakoid membranes were prepared according to Robinson and Yocum (1980) except that 5 mM DTT was added to the isolation medium and that Tricine was replaced by Hepes in the last extraction step. Thylakoids were subjected to gentle solubilization with a mild detergent mixture and the solubilized photosynthetic complexes were subsequently separated at 4 °C by non-denaturing Deriphat-PAGE as previously described (Tardy and Havaux 1996). The thylakoids were fractionated into five well-separated bands: PSI, core complex of PSII (CCII), trimeric LHCIIb, monomeric LHCIIs, and free pigments. The unstained gels were scanned

(EASY-Store system; Herolab, Wiesloch, Germany) and the abundance (intensity × area of the green bands) of the pigmented complexes was determined using the EASY analysis software. The free pigment band was excised from the gels and pigments were extracted in *sec*-butanol using the method described by Martinson and Plumley (1995). Deriphat-160 was a generous gift from Sidobre-Sinova (Saint-Fargeau-Ponthierry, France).

Electron spin resonance (ESR) spectroscopy. Thylakoid membranes were prepared according to Robinson and Yocum (1980) except that 5 mM DTT was added to the isolation medium and that Tricine was replaced by Hepes in the last extraction step. Thylakoid membranes were washed twice, first with 100 mM sorbitol, 5 mM EDTA, 20 mM Tricine at pH 8, then with 100 mM sorbitol. The resulting unstacked membranes were finally resuspended in 0.1 M Tris (pH 8.5) at a chlorophyll concentration of 1 mg · ml^{-1}. A 100-µl sample of this preparation was vigorously mixed with 2 µl of a methanolic solution of the spin label 12-doxylstearic acid (12-SASL; 2 mg · ml^{-1}). After centrifugation, free spin label could not be detected in the supernatant. The labeled membrane suspension was rapidly introduced into a 50-µl glass disposable pipette (Corning, Giesen, Germany) sealed at one end. Paramagnetic resonance signals of the samples were recorded with a Bruker ESP 300 (9.5 GHz) spectrometer equipped with a temperature-control unit cooled with liquid nitrogen (Bruker Analytische Messtechnik, Karlsruhe, Germany). The ESR measurements were done at 13 °C. Typical ESR parameter settings were: magnetic-field strength, 3390 G; microwave power, 20 mW; modulation frequency, 100 kHz; scan range, 100 G; modulation amplitude, 1.0 G; usual scan time, 6–8 min (4–5 scans); gain, 2×10^5. The apparent rotational correlation time (τ_c) was calculated from: $\tau_C = 6.5 \times 10^{-10} \times W_0 \times [(ho/h_{-1})^{1/2}-1]$ where W_0 is the width of the mid-field line and h_0 and h_{-1} are the heights of the mid- and highfield lines, respectively (Keith et al. 1970; Cannon et al. 1975).

Results

The quantum yield of photosynthetic oxygen evolution (Φ) was measured in green light centered at 510 nm and in red light centered at 650 nm using the photoacoustic technique (Malkin and Canaani 1994). Green light is absorbed by both chlorophyll and carotenoid pigments whereas red light is absorbed by chlorophylls only. The PFDs of the green and red light beams were adjusted so that the absorption of red light by the leaf samples, as estimated by the amplitude of the light-saturated photothermal signal (Malkin and Canaani 1994), was comparable to that of green light. So, the Φ measurements were performed with approximately the same number of quanta absorbed in the green and red spectral regions. Figure 1 shows that illuminating leaves with a strong white light of PFD 1500 µmol · m^{-2} · s^{-1} caused a marked decrease in the oxygen yield measured in green light (Φ-510) relative to the yield measured in red light (Φ-650). The ratio Φ-510/F-650 fell from around 0.75 to around 0.6 within 20 min. It is important to note that Φ-650 remained virtually unchanged during the light treatment. Similarly, the quantum yield of PSII photochemistry determined by chlorophyll fluorometry (Fv/Fm, determined after 20-min dark-adaptation) was little affected, slightly decreasing from 0.79 to 0.75 after a 20-min exposure to 1500 µmol · m^{-2} · s^{-1}. The selective decrease in the efficiency of green light for photochemistry suggests that strong light brought about the excitonic uncoupling of some carotenoids from the

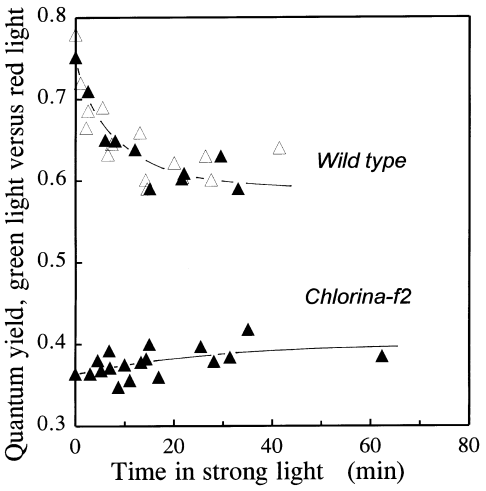

Fig. 1. Ratio of the quantum yield of oxygen evolution measured in green light (Φ-510) to that measured in red light (Φ-650) in barley leaves (wild type and *chlorina-f2* mutant) suddenly exposed to strong white light (1500 µmol · m^{-2} · s^{-1}). *Closed triangles*, control leaves; *open triangles*, leaves pre-infiltrated with 5 mM DTT

chlorophyll antennae. We also measured Φ in red light beams preferentially absorbed by chlorophyll *a* or chlorophyll b (680 nm or 640 nm, respectively). No differential effect of the strong light treatment was observed between Φ-680 and Φ-640 (data not shown). This indicates that the observed changes in Φ-510/ Φ-650 did not involve uncoupling between the chlorophyll-*a*-containing core complex of the photosystems and the chlorophyll-*a*/*b*-protein complexes which are associated with the light-state transitions. In reality, we can consider that the Φ measurements (before or after the strong light treatment) were done in state 2 since the leaf samples were illuminated for several minutes with the red or green light beams in the photoacoustic cell. This was checked by measurements of the far-red-light-induced Emerson enhancement (E) of oxygen evolution in red light (640 nm): E was 10% before strong illumination and 13% after 30 min in strong light, indicating no appreciable changes in the red light distribution between the two photosystems. These low E values are typical of state 2 (Canaani and Malkin 1984).

The decrease in Φ-510/Φ-650 at 1500 µmol · m^{-2} · s^{-1} was not affected by pre-infiltrating leaves with 5 mM DTT, a potent inhibitor of the violaxanthin (Vio) de-epoxidase. One can then conclude that the selective reduction of Φ-510 did not require the synthesis of antheraxanthin (Ant) and zeaxanthin (Zea). As compared to wild-type barley leaves, leaves of the *chlorina-f2* barley mutant had a much lower Φ-510/Φ-650 value of ca. 0.35. In contrast to the wild type, the Φ-510/Φ-650 ratio of *chlorina-f2* leaves was virtually insensitive to strong illumination. Seemingly, a large fraction of the carotenoids in LHCII-deficient *chlorina-f2* chloroplasts are not coupled to chlorophylls. The results obtained with *chlorina-f2* indicate that the Φ-510/Φ-650 changes measured in illuminated wild-type leaves are indicative of photoinduced changes in LHCII. Constitutive

Table 1. The Φ510/Φ650 photoacoustic ratio, $t_{1/2}$ of the chlorophyll fluorescence induction rise in the presence of DCMU in blue light and in red light, and carotenoids/chlorophylls ratio (Car/Chl) in barley leaves grown at 23 °C/400 μmol · m^{-2} · s^{-1} or 36.5 °C/1400 μmol · m^{-2} · s^{-1}. $n = 4 \pm$ SD

	23 °C/ 400 μmol · m^{-2} · s^{-1}	36.5 °C/ 1400 μmol · m^{-2} · s^{-1}
$t_{1/2}$(ms)		
In red light	19.1 ± 2.2	20.4 ± 2.1
In blue light	45.0 ± 3.3	59.2 ± 2.4
Φ510/Φ650	0.69 ± 0.03	0.60 ± 0.02
Car/Chl	0.19	0.25

uncoupling of carotenoids from chlorophylls was also observed in barley leaves grown under extreme conditions of temperature and light (Table 1). Indeed, the Φ-510/Φ-650 ratio was 0.7 in leaves grown under 'normal' conditions (23 °C/400 μmol photons · m^{-2} · s^{-1}) and noticeably decreased to 0.6 in leaves grown at 36.5 °C and 1500 μmol photons · m^{-2} · s^{-1}.

The size of the functional PSII-pigment antennae was also estimated from chlorophyll fluorescence induction measurements in the presence of DCMU (Malkin et al. 1981). When chlorophyll fluorescence was induced by red light (655 nm), the half time ($t_{1/2}$) of the chlorophyll-fluorescence induction rise, which is inversely proportional to the functional antenna size of PSII, was close to 20 ms for both growth conditions (Table 1). When fluorescence was induced by blue light (470 nm), $t_{1/2}$ noticeably increased in plants grown in high-light/elevated-temperature conditions compared to control plants, indicating a decrease in the functional PSII antenna size in the former plants. As the carotenoid/chlorophyll ratio increased in the high-light-grown leaves (Table 1), the selective increase in $t_{1/2}$ in blue light confirms the conclusion derived from the Φ measurements that a significant part of the carotenoid pool was uncoupled from the chlorophyll antennae of PSII. The half-time was also measured in control barley leaves before and after exposure to 1000 μmol photons · m^{-2} · s^{-1} for 30 min (data not shown). Again, $t_{1/2}$ in red light was little affected (+5%) by the light treatment, whereas $t_{1/2}$ in green light increased by more than 35%.

Figure 2 shows that the photoinduced pigment uncoupling as monitored by the Φ-510/Φ-650 changes was fully reversible in darkness. The time course of the Φ-510/Φ-650 reversal appeared to be slower than the time course of the light-induced decrease in Φ-510/Φ-650 (compare Fig. 1 and Fig. 2). In addition, the dark recovery of Φ-510/Φ-650 was much slower than the state 2-state 1 transitions which are known to occur in the minute time range. As shown in Fig. 3, the phenomenon was saturated at a PFD of ca. 1000 μmol · m^{-2} · s^{-1}.

When the pigmented complexes of barley thylakoids were separated by non-denaturing Deriphat-PAGE (Fig. 4), little change in the pigment distribution within or between the photosystems was observed after growth in strong light at high temperature. However, the abundance of free pigments was observed to markedly

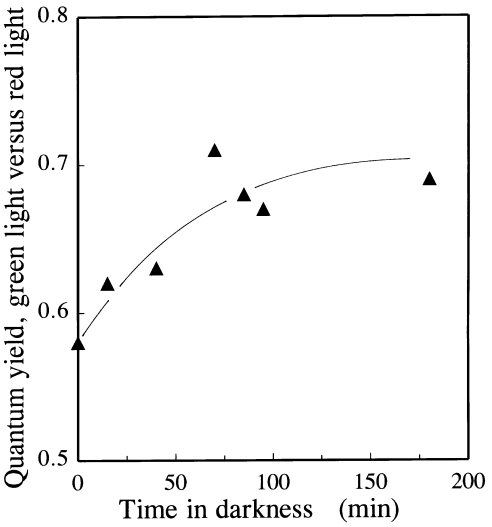

Fig. 2. Dark recovery of the Φ-510/Φ-650 ratio in wild-type barley leaves pre-exposed for 10 min to a strong white light of PFD 1500 μmol · m^{-2} · s^{-1}

increase. The free-pigment bands were excised from the gels and the pigments were extracted and analyzed by HPLC (Table 2). The free chlorophylls generated during electrophoresis of control thylakoids (isolated from plants grown at 23 °C and 350 μmol photons · m^{-2} · s^{-1}) represented approx. 0.2% of the chlorophylls loaded on the gels. This value is compatible with the amount of free chlorophyll estimated in vivo by chlorophyll-fluorescence lifetime measurements (Marder and Raskin 1993). For thylakoids prepared from plants grown under extreme light and temperature conditions, 0.6% of the loaded chlorophylls were found in the free pigments band. The free-pigment band was observed to be strongly enriched in carotenoids, Vio and lutein in particular. The carotenoid/chlorophyll (Chl) ratio of the free pigments was 0.56 for control thylakoids and 1.25 for thylakoids prepared from plants grown in high light/high temperature. These values are much higher than

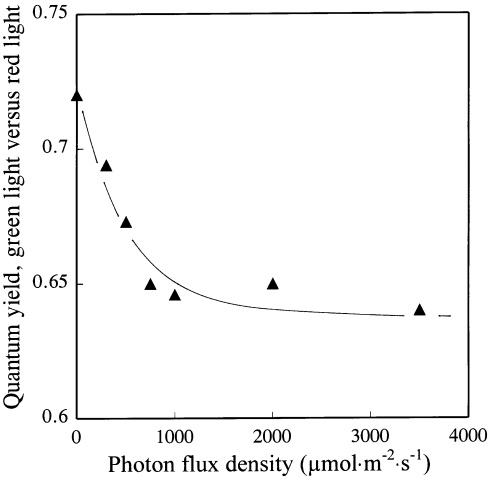

Fig. 3. The Φ-510/Φ-650 ratio in barley leaves exposed for 20 min to different PFDs of white light

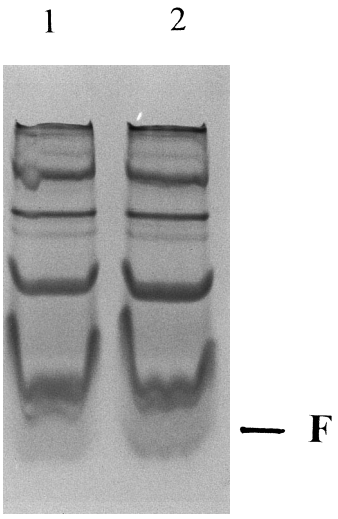

Fig. 4. Non-denaturing Deriphat-PAGE of thylakoid pigmented complexes. *Lane 1*, control barley leaves; *lane 2*, barley leaves grown at 36.5 °C and 1500 μmol photons · m^2 · s^{-1}. *F*, free pigments

those measured in leaves (see Table 1). We estimated that approx. 0.6% and 3.5% of the carotenoids were found in the free-pigment zone for control and light-acclimated thylakoids, respectively.

Table 3 shows the pigment content of the major LHC of PSII (LHCII) prepared from barley leaves before and after strong illumination in the presence or absence of DTT. As expected, strong light (1000 μmol · m^{-2} · s^{-1} for 15 min) induced a marked decrease (more than 50%) in the Vio content from 39 to 18 μg · (mg Chl)$^{-1}$. Concomitantly, the level of Ant and Zea found in LHCII increased but, remarkably, this increase did not fully compensate for the loss of Vio. Indeed, the total xanthophyll-cycle pigments content was 42 μg · (mg Chl)$^{-1}$ in dark-adapted LHCII and only 35 μg · (mg Chl)$^{-1}$ in LHCII isolated from the illuminated leaves. Then, a substantial fraction of Vio converted to Zea was lost from LHCII. This finding indicates that Ant and Zea were released from LHCII during illumination or that they were loosely bound to LHCII, causing their partial loss during the LHCII preparation. When the same experiment was performed with leaves pretreated with the Vio de-epoxidase inhibitor DTT, Zea did not accumulate in the LHCII, as expected, and no loss of Vio was observed during illumination. Clearly, Vio and Zea behave differently regarding their interaction with LHCII.

Table 2. Composition of the free-pigment bands in the Deriphat-PAGE gels of thylakoid membranes (see Fig. 4, band F) prepared from barley leaves grown at 23 °C/400 μmol · m^{-2} · s^{-1} and 36.5 °C/1400 μmol · m^{-2} · s^{-1}. Data are expressed as μg pigment per mg chlorophyll loaded on the gels. Car/Chl is the ratio of total carotenoids to total chlorophylls. $n = 4 \pm$ SD

	23 °C/ 400 μmol · m^{-2} · s^{-1}	36.5 °C/ 1400 μmol · m^{-2} · s^{-1}
Neoxanthin	0.11 ± 0.09	0.30 ± 0.16
Vio	0.35 ± 0.14	2.30 ± 1.10
Ant	0.03 ± 0.01	0.40 ± 0.40
Lutein	0.46 ± 0.06	2.55 ± 1.05
Zea	0.05 ± 0.01	0.22 ± 0.15
β-Carotene	0.19 ± 0.03	1.45 ± 0.65
Chlorophyll *b*	0.46 ± 0.16	1.16 ± 0.44
Chlorophyll *a*	1.60 ± 0.41	4.58 ± 1.76
Car/Chl	0.56	1.25

In Table 4, we examined the effects of a strong light treatment (10 min at 1000 μmol · m^{-2} · s^{-1}) on the relative proportions of the xanthophyll *cis* and *trans* isomers. Neoxanthin was present mainly in the *cis* isomeric form; about 3.5% of neoxanthin was found in the *trans* configuration in dark-adapted barley leaves. In contrast, the other xanthophylls were almost exclusively found in the *trans* configuration. In particular, in dark-adapted leaves, the *cis*/*trans* Vio and *cis*/*trans* lutein ratios were lower than 2%. Purified LHCII was not enriched in *cis*-Vio. Light stress had little effect on the *cis*/*trans* xanthophylls ratio. However, *cis*-*trans* isomerization of neoxanthin was occasionally detected in some high-light-treated leaves.

The lipid phase of barley thylakoid membranes was analyzed before and after strong light treatment by measuring its fluidity using electron spin resonance and the spin label 12-SASL (Table 5). The rationale of these experiments is that diffusive displacement of carotenoids from the LHCs to the membrane (if any) and subsequent interaction of the released carotenoids with the thylakoid membrane lipid phase should modify the fluidity properties of the thylakoid membrane, as demonstrated in carotenoid-containing artificial lipid membrane (Milon et al. 1986; Subczynski et al. 1992; Gabrielska and Gruszecki 1996). In other words, decreased membrane fluidity can be used as an indirect indicator of the interaction between carotenoids and thylakoid membrane

Table 3. Carotenoid content [in μg · (mg total chlorophyll)$^{-1}$] of LHCII purified from dark-adapted and light-treated barley leaves infiltrated with 0 and 5 mM DTT. V + A + Z is the sum Vio + Ant + Zea. The light treatment was 1000 μmol · m^{-2} · s^{-1} for 10 min. $n = 3 \pm$ SD

Pigments	Light		Light and DTT	
	Before	After	Before	After
Neoxanthin	38.8 ± 2.0	41.8 ± 2.5	38.9 ± 3.8	41.6 ± 3.2
Vio	39.0 ± 2.5	17.5 ± 2.1	43.1 ± 3.7	38.9 ± 5.9
Ant	1.8 ± 1.3	6.7 ± 1.9	1.7 ± 0.6	2.2 ± 1.5
Lutein	126.5 ± 7.6	134.4 ± 4.5	124.1 ± 8.5	131.9 ± 9.5
Zea	0.9 ± 0.6	10.5 ± 1.2	0.3 ± 0.5	1.3 ± 0.8
β-carotene	14.5 ± 5.7	11.0 ± 5.7	13.8 ± 4.8	12.1 ± 5.3
V + A + Z	41.7	34.7	45.1	42.4

Table 4. *Cis* and *trans* isomers of xanthophylls (in ng · mm^{-2}) in barley leaves before and after illumination (1000 μmol · $m^{-2} \cdot s^{-1}$ for 10 min) in the presence or the absence of 5 mM DTT. The isomer content of LHCII prepared from dark-adapted barley leaves is also shown [in ng · (μg total chlorophyll)$^{-1}$]. n.d., not detected. $n = 3 \pm$ SD

Xanthophyll isomers	Leaves			LHCII
	Dark	Light	Light + DTT	
trans-Neoxanthin	0.2 ± 0.1	1.4 ± 1.4	0.2 ± 0.1	1.7 ± 0.5
cis-Neoxanthin	5.9 ± 0.6	5.1 ± 0.1	5.3 ± 0.1	39.6 ± 6.7
trans-Vio	12.2 ± 1.4	4.3 ± 0.5	10.5 ± 1.8	28.3 ± 3.3
cis-Vio	0.15 ± 0.1	0.05 ± 0.07	0.2 ± 0.1	1.1 ± 0.4
trans-Ant	0.6 ± 0.1	1.4 ± 0.4	0.4 ± 0.1	0.6 ± 0.4
trans-Lutein	14.8 ± 1.7	14.3 ± 0.9	13.4 ± 0.1	86.8 ± 4.3
cis-Lutein	0.17 ± 0.17	n.d.*	0.5 ± 0.1	2.8 ± 2.0
trans-Zea	n.d.	7.7 ± 0.4	n.d.	n.d.

*Could have been masked by the Zea peak

Table 5. The (A + Z)/(A + Z + V) ratio and lipid fluidity (measured by the τ_C value of 12-SASL) of thylakoid membranes prepared from dark-adapted and light-treated barley leaves (wild type and *chlorina f2*) in the presence or the absence of 5 mM DTT. The light treatment was 10 min at 1000 μmol · $m^{-2} \cdot s^{-1}$. n.d., not determined. $n = 4 \pm$ SD

	Wild type		*Chlorina f2*	
	τ_C (ns)	(A + Z)/(A + Z + V)	τ_C (ns)	(A + Z)/(A + Z + V)
Dark	4.1 ± 0.2	0.1	4.4 ± 0.4	0.1
Light	5.1 ± 0.1	0.6	4.2 ± 0.1	0.85
Dark + DTT	4.4 ± 0.2	0.1	n.d.	n.d.
Light + DTT	4.5 ± 0.2	0.1	n.d.	n.d.

lipids. The apparent rotational correlation time (τ_C) of 12-SASL incorporated in thylakoid membranes (wild type) significantly increased after a short illumination of the leaves, indicating a decrease in thylakoid membrane fluidity. When the Vio cycle was blocked by DTT, the light-induced change in membrane fluidity was not observed. Those findings confirm previous observations by Gruszecki and Strzalka (1991). No change in τ_C was noticed after illumination of *chlorina-f2* leaves although Vio was massively converted to Zea.

Discussion

This study has shown that bright light significantly reduces the quantum yield of oxygen evolution (Φ) monitored in green light, which preferentially excites carotenoid pigments, relative to Φ measured in red light, which is exclusively absorbed by the chlorophylls. This suggests that a substantial fraction of the carotenoid pool bound to the light-harvesting pigment complexes of the photosystems ceased to work as efficient accessory pigments in leaves exposed to strong light. A similar phenomenon was previously observed by fluorescence spectroscopy in pea leaves exposed to strong white light: chlorophyll fluorescence emission excited by orange light (550–600 nm) was enhanced relative to the fluorescence emission excited at lower wavelengths in the blue spectral region (400–500 nm; Havaux et al. 1991), indicating loss of excitation energy transfer from carotenoids to chlorophylls. Moreover, the finding that carotenoid fluorescence in the 500–600 nm spectral range significantly increased in photoinhibited leaves (Gruszecki et al. 1991) supports the idea of a photophysical separation of carotenoids and chlorophylls during strong illumination. Indeed, decreased depopulation of the excited singlet states of carotenoids by neighbouring chlorophylls should obviously increase the probability of carotenoid singlet deactivation by other processes, including fluorescence. However, in those previous works, extreme light stresses were imposed on leaves, leading to marked photooxidative damage of the photochemical apparatus of photosynthesis. This was not the case in the present study where Φ-650, the quantum yield of PSII photochemistry (Fv/Fm) and the pigment content of the leaf samples were very little affected by the light treatments. Additionally, for kinetic and spectroscopic reasons (mentioned in the *Results*), one can exclude the involvement of state 1-state 2 transitions in the light-induced Φ-510/Φ-650 changes.

Singlet-singlet energy transfer between carotenoid and chlorophyll molecules presumably requires a very close contiguity of the pigments (Mathis and Schenck 1982; Siefermann-Harms 1990; Cogdell et al. 1992; Frank and Cogdell 1996). For instance, in synthetic carotenoporphyrins consisting of a carotenoid part covalently linked to a synthetic tetraarylporphyrin, the transfer yield has been shown to be dependent on the geometry of the system. Efficient energy transfer was measured when the chromophores closely approached, with the carotenoid chromophore close to the porphyrin π-electron system (Bensasson et al. 1981; Moore et al. 1982). In natural systems, such intimate and specific pigment-pigment interactions are doubtless mediated by the higher-order structure of the pigment-protein complexes (Siefermann-Harms and Ninnemann 1982; Siefermann-Harms 1990). In photosynthetic bacteria, the efficiency of energy transfer from bound carotenoids to bacteriochlorophylls was demonstrated to depend on the apoprotein which determines not only the distance

and orientation between carotenoids and bacteriochlorophylls but also the molecular structure of bound carotenoids (Nogushi et al. 1990). Strong light is believed to provoke pigment aggregation phenomena in the minor and major LHCIIs linked to protonation of LHCII polypeptides (Horton et al. 1996). Consequently, it is conceivable that the conformational/structural changes induced by light in the light-harvesting pigment-protein complexes modify the mutual orientation and intermolecular distance between antenna pigments as well as the molecular structure of bound carotenoids, impeding the short-distance singlet-singlet energy transfers from carotenoids to chlorophylls.

In view of its insensitivity to DTT (Fig. 1), the observed carotenoid-chlorophyll excitonic uncoupling was not directly dependent on the conversion of Vio to Zea. We did not examine, however, whether the Vio cycle modulates the kinetics of the LHC rearrangements associated with the carotenoid, as is the case for the non-photochemical energy dissipation (Ruban et al. 1993a; Horton et al. 1996). Our data also indicate that the pigment decoupling does not involve the physical detachment of carotenoids from the LHCs: neither the pigment content of LHCII (Table 3) nor the fluidity of the thylakoid membrane lipid phase in which the LHCs are embedded (Table 5) was modified by the strong light treatment in the presence of DTT. From in-vitro studies of carotenoids incorporated in artificial lipid membranes (Milon et al. 1986; Subczynski et al. 1992; Gabrielska and Gruszecki 1996), one would expect that if xanthophylls were released from the LHCs to the surrounding lipid domain then membrane lipid rigidification would have taken place. Therefore, our data do not support the suggestion previously made by ourselves (Havaux et al. 1991; Havaux and Tardy 1995) and by others (Gruszecki 1995; Gruszecki et al. 1997) that the fluorimetrically measured pigment disconnection is the spectral manifestation of the process of making Vio available to the de-epoxidase, understood to be the physical detachment of Vio from the chlorophyll antennae. Most probably, the Φ-510/Φ-650 changes (Fig. 1), as well as the observed decrease in fluorescence yield excited by blue light (Havaux et al. 1991), reflect molecular rearrangement of the antennae complexes. From the data of Table 4, one can exclude the possibility that this molecular rearrangement involved major changes in the geometrical configuration of the LHC-bound xanthophylls. Recently, in an in-vitro study of isolated LHCII incorporated in liposomes, Gruszecki et al. (1997) have reported light-induced spectroscopic changes which could be interpreted as the manifestation of *cis-trans* isomerization of carotenoids. These authors have hypothesized that these *cis-trans* rearrangements involve mainly Vio and are combined with the detachment of *trans* Vio from the LHCs and migration to the membrane lipid phase where *trans*-Vio is enzymatically converted to *trans*-Zea. These hypotheses seem to be highly improbable in native thylakoid membranes. Indeed, the present study has shown that *cis*-Vio represents a very small fraction ($< 2\%$) of the total Vio pool. Consequently, Zea is synthesized from the pre-existing *trans*-Vio pool. In addition, as already mentioned above, no detachment of Vio from LHCII was observed in strong light (in DTT-infiltrated samples). As far as the carotenoids of the reaction centers are concerned, the HPLC method used here does not allow a good separation of the β-carotene isomers. In an early study of the pigment distribution in PSII-enriched membrane fractions using an HPLC system equipped with a calcium hydroxide column, both *cis* and *trans* β-carotenes were detected in PSII (Lemoine et al. 1992). More recently, β-carotene was found in the configuration 15,15′-*cis* in the reaction center of a higher plant (Bialek-Bylka et al. 1995), in agreement with the Koyama's proposal that carotenoids selectively adopt a *cis*-configuration in the reaction centers of the photosystems (Koyama 1991).

Actually, a light-induced decrease in membrane fluidity did occur in thylakoid membranes when Vio was allowed to convert to Zea (Table 5). We also observed that LHCII prepared from illuminated leaves was impoverished in xanthophyll-cycle pigments compared to LHCII purified from dark-adapted leaves (Table 3). The concomitant membrane rigidification and decrease in the Zea content of LHCII is compatible with a diffusive displacement of Zea from the pigment-protein complexes to the surrounding lipid domain, as previously suggested (Gruszecki 1995; Havaux and Tardy 1995; Gruszecki et al. 1997). The differential behavior of Zea and Vio with respect to their interaction with the LHCs probably results from their chemical features. Zeaxanthin differs from Vio in its polarity, its number of carbon double bonds and its spatial conformation (Ruban et al. 1993b). The observation that the amount of Zea in LHCII prepared from light-treated leaves is smaller than the amount of Vio in LHCII from dark-adapted leaves was previously made by Lee and Thornber (1995). However, this was not mentioned in other reports (Ruban et al. 1994; Philipp and Young 1995). As the latter reports dealt with extreme light stresses in CO_2-free nitrogen/low oxygen mixtures, these discrepancies could be explained by the type of light treatments used in the different works. Indeed, it is conceivable that, if release of Zea takes place during the operation of the xanthophyll cycle, this should result in a complex and dynamic equilibrium between bound and unbound xanthophylls depending on the illumination conditions.

The reversibility of the light-induced Φ-510/Φ-650 (Fig. 2) could suggest a regulatory function of the carotenoid disconnection. Strong-light-induced loss of energy transfer from carotenoids to chlorophylls can be interpreted as a protective mechanism since part of the light absorbed by accessory pigments is diverted from the sensitive reaction centers. The light dependence of the phenomenon (Fig. 3) is compatible with an adaptive function to high light irradiances close to the light saturation of photosynthesis. The photoprotective nature of the pigment-uncoupling phenomenon reported here is also supported by the observation that growth of plants under stressful light and temperature conditions simultaneously increased the amount of carotenoids that

are weakly or not bound to the LHCs (Fig. 4, Table 2) and decreased the functional antenna size of PSII in green light (Table 1). Thus, in plants adapted to high light intensities, some of the carotenoids permanently lost their usual function of accessory pigments. This may reflect a special organization of the pigment antennae in plants adapted to extreme light and temperature environments. It is tempting to relate this long-term rearrangement of the pigment antennae with accumulation of the strong-light-induced ELIPs, a protein family with homology to the LHC polypeptides (Green and Kühlbrandt 1995). It has been suggested that early-light inducible proteins (ELIPs) are pigmented proteins that function as substitutes for the LHC proteins when plants are grown under potentially harmful light conditions (Adamska and Kloppstech 1994). Possibly, the photophysics of xanthophyll bound to ELIPs differs from that of LHC-bound xanthophylls.

The chlorophyll-*b*-less *chlorina f2* barley mutant seems to be an extreme example of functional disconnection of carotenoids from chlorophyll antennae. The quantum yield of oxygen evolution in green light was dramatically reduced compared to Φ in red light, suggesting that carotenoids do not play a major role as light-harvesting pigments. A similar phenomenon was previously reported in a pale-green aurea mutant (*Su*/*su*) of tobacco by Canaani et al. (1985) who hypothesized that carotenoids may reside outside the thylakoid membrane in this mutant. Similarly, Schindler and Lichtenthaler (1994) suggested the possibility of an incomplete transfer of excitation energy from carotenoids to chlorophyll *a* in the aurea tobacco mutant. *Chlorina-f2* is completely deficient in LHCII and lacks most of the minor LHCs of PSII (Krol et al. 1995; Preiss and Thornber 1995). In addition, the carotenoid/chlorophyll ratio of *chlorina f2* is higher than that of the wild type (0.32 ± 0.04 vs. 0.23 ± 0.01). Unfortunately, the localization of the excess carotenoids in *chlorina f2* is unknown but, according to Jahns and Krause (1994), they are not necessarily bound to distinct proteins. The observation that light stress did not modify the fluidity properties of the thylakoid membrane lipids in *chlorina-f2* is consistent with this idea. Indeed, no change in the interaction between xanthophyll-cycle pigments and the membrane lipid phase is expected in strong light if Vio/Zea are already free in the thylakoid membrane before the light treatment. Alternatively, carotenoids of *chlorina-f2* are bound to pigment-protein complexes but they do not meet the criterion of close proximity required for efficient energy transfer. In this context, it is worth mentioning that, in LHCII trimers isolated from wild-type *Arabidopsis thaliana*, energy is transferred to chlorophyll *a* mainly via chlorophyll *b* and not directly from carotenoids (Connelly et al. 1997). Therefore, absence of chlorophyll *b* in *chlorina-f2* photosystems may perturb carotenoid-to-chlorophyll energy transfers.

To summarize, the efficiency of energy transfer from carotenoids to chlorophylls in barley leaves seems to vary with the light environment both in the short term and in the long term, with excess light energy reducing the photosynthetic light-harvesting function of carotenoids. We do not know the carotenoid species involved in this phenomenon but, considering the extent of the Φ-510/Φ-650 changes (about −20%, Fig. 1), it is possible that an appreciable fraction of the LHC-bound carotenoids is concerned. As the free pigment band in the Deriphat-PAGE gels is indicative of the pigments that are the less firmly bound to proteins, its enrichment in Vio and lutein identifies these carotenoids as candidates for the photoinduced pigment decoupling. Experiments with DTT show that the carotenoid disconnection does not require the physical detachment of carotenoids from the LHCs. The physiological significance of the reversible pigment uncoupling reported here deserves to be studied more extensively in the future.

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References

Adamska I, Kloppstech K (1994) Considerations to the role of ELIPs during light stress. In: Baker NR, Bowyer JR (eds) Photoinhibition of photosynthesis – from molecular mechanisms to the field. Bios Scientific, Oxford, pp 209–224

Bensasson RV, Land EJ, Moore AL, Crouch RL, Dirks G, Moore TA, Gust D (1981) Mimicry of antenna and photoprotective carotenoid functions by a synthetic carotenoporphyrin. Nature 290: 329–332

Bialek-Bylka GE, Tomo T, Satoh K, Koyama Y (1995) 15-*cis*-β-Carotene found in the reaction center of spinach photosystem II. FEBS Lett 363: 137–140

Canaani O, Malkin S (1984) Distribution of light excitation in an intact leaf between the two photosystems of photosynthesis – changes in absorption cross-sections following state 1-state 2 transitions. Biochim Biophys Acta 766: 513–524

Canaani O, Motzan Z, Malkin S (1985) Comparison of photosynthetic parameters of an aurea mutant (*Su*/*su*) of tobacco and the wild-type by the photoacoustic method. Planta 164: 480–486

Cannon B, Polnaszek CF, Butler KW, Eriksson LEG, Smith ICP (1975) The fluidity and organization of mitochondrial membrane lipids of the brown adipose tissue of cold-adapted rats and hamsters as determined by nitroxide spin probes. Arch Biochem Biophys 167: 505–518

Cogdell RJ, Andersson PO, Gillbro T (1992) Carotenoid singlet states and their involvement in photosynthetic light-harvesting pigments. J Photochem Photobiol B: Biol 15: 105–112

Connelly JP, Müller MG, Bassi R, Croce R, Holzwarth AR (1997) Femtosecond transient absorption study of carotenoid to chlorophyll energy transfer in the light-harvesting complex II of photosystem II. Biochemistry 36: 281–287

Frank HA, Cogdell RJ (1996) Carotenoids in photosynthesis. Photochem Photobiol 63: 257–264

Gabrielska J, Gruszecki WI (1996) Zeaxanthin (dihydroxy-β-carotene) but not β-carotene rigidifies lipid membranes: a ^{1}H-NMR study of carotenoid-egg phosphatidylcholine liposomes. Biochim Biophys Acta 1285: 167–174

Green BR, Kühlbrandt W (1995) Sequence conservation of light-harvesting and stress-response proteins in relation to the three-dimensional molecular structure of LHCII. Photosynth Res 44: 139–148

Gruszecki WI (1995) Different aspects of protective activity of the xanthophyll cycle under stress conditions. Acta Physiol Plant 17: 145–152

Gruszecki WI, Strzalka K (1991) Does the xanthophyll cycle take part in the regulation of fluidity of the thylakoid membrane? Biochim Biophys Acta 1060: 310–314

Gruszecki WI, Veeranjaneyulu K, Leblanc RM (1991) Qualitative changes in the fluorescence spectra of intact pea leaves after photoinhibition. Biochem Cell Biol 69: 399–403

Gruszecki WI, Matula M, Ko-chi N, Koyama Y, Krupa Z (1997) *Cis-trans*-isomerization of violaxanthin in LHCII: violaxanthin isomerization cycle within the violaxanthin cycle. Biochim Biophys Acta 1319: 267–274

Havaux M, Tardy F (1995) Short-term adaptive responses of photosynthesis to elevated temperatures and strong light. Possible role and mode of action of the xanthophyll-cycle pigments. In: Mathis P (ed) Photosynthesis: from light to biosphere, vol IV. Kluwer, Dordrecht, pp 777–782

Havaux M, Tardy F (1996) Temperature-dependent adjustment of the thermal stability of photosystem II in vivo: possible involvement of xanthophyll-cycle pigments. Planta 198: 324–333

Havaux M, Tardy F (1997) Thermostability and photostability of photosystem II in leaves of the *chlorina-f2* barley mutant deficient in light-harvesting chlorophyll *a*/*b* protein complexes. Plant Physiol 113: 913–923

Havaux M, Gruszecki WI, Dupont I, Leblanc RM (1991) Increased heat emission and its relationship to the xanthophyll cycle in pea leaves exposed to strong light stress. J Photochem Photobiol B: Biol 8: 361–370

Horton P, Ruban AV, Walters RG (1996) Regulation of light harvesting in green plants. Annu Rev Plant Physiol Plant Mol Biol 47: 655–684

Jahns P, Krause GH (1994) Xanthophyll cycle and energy-dependent fluorescence quenching in leaves from pea plants grown under intermittent light. Planta 192: 176–182

Keith A, Bulfield G, Snipes W (1970) Spin-labeled *Neurospora* mitochondria. Biophys J 10: 618–629

Koyama Y (1991) Structures and functions of carotenoids in photosynthetic systems. J Photochem Photobiol B: Biol 9: 265–280

Kramer DM, Crofts AR (1996) Control and measurement of photosynthetic electron transport in vivo. In: NR Baker (ed) Photosynthesis and the environment. Kluwer Academic Publishers, Dordrecht, pp 25–66

Krol M, Spangfort MD, Huner NPA, Öquist G, Gustafsson P, Jansson S (1995) Chlorophyll *a*/*b*-binding proteins, pigment conversions, and early light-induced proteins in a chlorophyll *b*-less barley mutant. Plant Physiol 107: 873–883

Krupa Z, Huner NPA, Williams JP, Maissan E, James DR (1987) Development at cold-hardening temperatures. The structure and composition of purified rye light harvesting complex II. Plant Physiol 84: 19–24

Lee A I-C, Thornber JP (1995) Analysis of the pigment stoichiometry of pigment-protein complexes from barley (*Hordeum vulgare*). Plant Physiol 107: 565–574

Lemoine Y, Zabulon G, Brody SS (1992) Pigment distribution in photosystem II. In: Murata N (ed) Research in photosynthesis, vol 1. Kluwer, Dordrecht, pp 331–334

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148: 349–382

Malkin S, Canaani O (1994) The use and characteristics of the photoacoustic method in the study of photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 45: 493–526

Malkin S, Armond PA, Mooney HA, Fork DC (1981) Photosystem II photosynthetic unit sizes from fluorescence induction in leaves. Plant Physiol 67: 570–579

Marder JB, Raskin VI (1993) The assembly of chlorophyll into pigment-protein complexes. Photosynthetica 28: 243–248

Martinson TA, Plumley FG (1995) One-step extraction and concentration of pigments and acyl lipids by *sec*-butanol from in vitro and in vivo samples. Anal Biochem 228: 123–130

Mathis P, Schenck CC (1982) The functions of carotenoids in photosynthesis. In: Britton G, Goodwin TW (eds) Carotenoid chemistry and biochemistry. Pergamon, Oxford, pp 339–352

Milon A, Wolff G, Ourisson G, Nakatani Y (1986) Organization of carotenoid-phospholipid bilayer systems. Helv Chim Acta 69: 12–24

Moore AL, Joy A, Tom R, Gust D, Moore TA, Bensasson RV, Land EJ (1982) Photoprotection by carotenoids during photosynthesis: motional dependence of intramolecular energy transfer. Science 216: 982–984

Nogushi T, Hayashi H, Tasumi M (1990) Factors controlling the efficiency of energy transfer from carotenoids to bacteriochlorophyll in purple photosynthetic bacteria. Biochim Biophys Acta 1017: 280–290

Nogushi T, Hayashi H, Shimada K, Takaichi S, Tasumi M (1992) In vivo states and functions in an aerobic photosynthetic bacterium, *Erythrobacter longus*. Photosynth Res 31: 21–30

Phillip D, Young AJ (1995) Occurrence of the carotenoid lactucaxanthin in higher plant LHCII. Photosynth Res 43: 273–282

Preiss S, Thornber JP (1995) Stability of the apoproteins of light-harvesting complex I and II during biogenesis of thylakoids in the chlorophyll *b*-less barley mutant *chlorina f2*. Plant Physiol 107: 709–717

Robinson HH, Yocum CF (1980) Cyclic photophosphorylation reactions catalyzed by ferredoxin, methyl viologen and anthraquinone sulfonate. Use of photochemical reactions to optimize redox poising. Biochim Biophys Acta 590: 97–106

Ruban AV, Young AJ, Horton P (1993a) Induction of nonphotochemical energy dissipation and absorbance changes in leaves. Plant Physiol 102: 741–750

Ruban AV, Horton P, Young AJ (1993b) Aggregation of higher plant xanthophylls: differences in absorption spectra and in the dependency on solvent polarity. J Photochem Photobiol B: Biol 21: 229–234

Ruban AV, Young AJ, Pascal AA, Horton P (1994) The effects of illumination on the xanthophyll composition of the photosystem II light-harvesting complexes of spinach thylakoid membranes. Plant Physiol 104: 227–234

Schindler C, Lichtenthaler HK (1994) Is there a correlation between light-induced zeaxanthin accumulation and quenching of variable chlorophyll *a* fluorescence? Plant Physiol Biochem 32: 813–823

Schoefs B, Bertrand M, Lemoine Y (1995) Separation of photosynthetic pigments and their precursors by reversed-phase high-performance liquid chromatography using a photodiode-array detector. J Chromatogr 692: 239–245

Siefermann-Harms D (1990) Protective function of the apoprotein of the light-harvesting chlorophyll *a*/*b* protein complex in pigment photo-oxidation. J Photochem Photobiol B: Biol 4: 283–295

Siefermann-Harms D, Ninnemann H (1982) Pigment organization in the light-harvesting chlorophyll *a*/*b* protein complex of lettuce chloroplasts. Evidence obtained from protection of the chlorophylls against proton attack and from excitation energy transfer. Photochem Photobiol 35: 719–731

Subczynski WK, Markowska E, Gruszecki WI, Sielewiesiuk J (1992) Effects of polar carotenoids on dimyristoylphosphatidylcholine membranes: a spin-label study. Biochim Biophys Acta 1105: 97–108

Tardy F, Havaux M (1996) Photosynthesis, chlorophyll fluorescence, light-harvesting system and photoinhibition of a zeaxanthin-accumulating mutant of *Arabidopsis thaliana*. J Photochem Photobiol B: Biol 34: 87–94