Plant Physiol. (1995) 107: 873–883

Chlorophyll *a/b*-Binding Proteins, Pigment Conversions, and Early Light-Induced Proteins in a Chlorophyll *b*-less Barley Mutant[1]

Mariana Król, Michael D. Spangfort, Norman P. A. Huner, Gunnar Öquist, Petter Gustafsson, and Stefan Jansson*

Department of Plant Physiology, University of Umeå, S-901 87 Umeå, Sweden (G.O., P.G., S.J.); Department of Plant Sciences, University of Western Ontario, N6A 5B7 London, Ontario, Canada (M.K., N.P.A.H.); and Department of Biochemistry, Chemical Centre University of Lund, S-221 00 Lund, Sweden (M.D.S.)

Monospecific polyclonal antibodies have been raised against synthetic peptides derived from the primary sequences from different plant light-harvesting Chl *a/b*-binding (LHC) proteins. Together with other monospecific antibodies, these were used to quantify the levels of the 10 different LHC proteins in wild-type and *chlorina f2* barley (*Hordeum vulgare* L.), grown under normal and intermittent light (ImL). *Chlorina f2*, grown under normal light, lacked Lhcb1 (type I LHC II) and Lhcb6 (CP24) and had reduced amounts of Lhcb2, Lhcb3 (types II and III LHC II), and Lhcb4 (CP 29). *Chlorina f2* grown under ImL lacked all LHC proteins, whereas wild-type ImL plants contained Lhcb5 (CP 26) and a small amount of Lhcb2. The *chlorina f2* ImL thylakoids were organized in large parallel arrays, but wild-type ImL thylakoids had appressed regions, indicating a possible role for Lhcb5 in grana stacking. *Chlorina f2* grown under ImL contained considerable amounts of violaxanthin (2–3/reaction center), representing a pool of phototransformable xanthophyll cycle pigments not associated with LHC proteins. *Chlorina f2* and the plants grown under ImL also contained early light-induced proteins (ELIPs) as monitored by western blotting. The levels of both ELIPs and xanthophyll cycle pigments increased during a 1 h of high light treatment, without accumulation of LHC proteins. These data are consistent with the hypothesis that ELIPs are pigment-binding proteins, and we suggest that ELIPs bind photoconvertible xanthophylls and replace "normal" LHC proteins under conditions of light stress.

The vast majority of the photosynthetic pigments in plants do not take part in the actual charge separation of the photosynthetic light reaction. Instead they function as antenna molecules that absorb photons and transfer, via other pigment molecules in the antenna, the absorbed energy to the RCs. The total energy initially harvested by the antenna molecules is, however, not trapped by the RCs. Excess light will force the pigment bed to decrease its efficiency of energy transfer; a larger proportion of the captured energy will be dissipated as heat to prevent overexcitation and potential photodamage to the photosynthetic apparatus. In higher plants, the carotenoids β-carotene and zeaxanthin seem to have a special role in photoprotection (Britton, 1993). Probably this is also true for antheraxanthin (Gilmore and Yamamoto, 1993). How carotenoids protect against photodamage is not known, but the low energy of their triplet state might enable them to quench triplet Chl, which otherwise would have the capacity to produce a highly reactive species, singlet oxygen. A more indirect role of zeaxanthin has also been proposed (Horton et al., 1991).

The photosynthetic pigments (Chls and carotenoids) are bound to specialized pigment-binding proteins with different functions. The higher plant light-harvesting antenna can be separated into three functional layers (Jansson, 1994). The innermost (core) antenna consists of Chl *a*- and β-carotene-binding proteins. The approximately 100 Chl *a* and 10 to 15 β-carotene molecules of the PSI core antenna (Britton, 1993) are bound to the two RC polypeptides, the products of the *PsaA* and *PsaB* genes. The core antenna of PSII consists of two proteins, named CP 43 and CP 47, binding in total 50 Chl *a*, 5 β-carotene, and perhaps a few lutein molecules (Bassi et al., 1993). Most photosynthetic antenna pigments are, however, bound to the different LHC proteins associated with either PSI or PSII (Jansson, 1994). The LHC proteins do not bind only Chl *a* and *b* but also xanthophylls: lutein, neoxanthin, violaxanthin, antheraxanthin, and zeaxanthin. The latter three xanthophylls may be interconverted in the xanthophyll cycle where violaxanthin, under a high trans-thylakoidal pH gradient in the light, can be metabolized to zeaxanthin via antheraxanthin by a de-epoxidasing enzyme (Demmig-Adams, 1990). Violaxanthin can subsequently be regenerated by epoxidation of zeaxanthin. The formation of zeaxanthin is

[1] This work was supported by grants from the Swedish Council for Forestry and Agricultural Research, the Swedish Natural Research Council, and the Natural Science and Engineering Council of Canada.

* Corresponding author; e-mail stefan.jansson@plantphys.umu.se; fax 46–90–16–66–76.

Abbreviations: ELIP, early light-induced protein; EPS, epoxidation state of the xanthophyll pool; HL, high light; ImL, intermittent light; LHC, light-harvesting Chl *a/b* binding; RC, reaction center; SBTI, soybean trypsin inhibitor.

closely correlated to the nonradiative energy dissipation in PSII through increasing q_e, the energy-dependent nonphotochemical quenching component of Chl fluorescence (Demmig-Adams, 1990).

Of the 10 known LHC proteins, four (Lhca1, 2, 3, and 4, nomenclature according to Jansson et al. [1992]) are exclusively associated with PSI and four (Lhcb3, 4, 5, and 6 or type III LHC II, CP 29, CP 26, and CP 24, respectively) are exclusively associated with PSII, whereas the most abundant ones, Lhcb1 and Lhcb2, often referred to as LHC II, can serve as antennae for both photosystems. The architecture of the light-harvesting antenna is not known, but considerable evidence indicates that the PSI- and PSII-specific LHC proteins form inner antennae, onto which trimers of Lhcb1 and Lhcb2 attach (Jansson, 1994). Because the xanthophyll cycle is known to regulate PSII activity, the investigation of its action has almost entirely focused on PSII. Although violaxanthin is found in all PSII LHC proteins (Thornber et al., 1993), several authors have suggested that Lhcb4, Lhcb5, and Lhcb6 (CP 29, CP 26, and CP 24) regulate the energy flow from the outer antenna to the RC through the action of the xanthophyll cycle (Bassi et al., 1993; Jansson, 1994; Ruban et al., 1994). The xanthophyll cycle functions also in PSI (Thayer and Björkman, 1992), but it is not known whether it has any regulatory effect there.

Plants grown under ImL and mutants deficient in Chl *b* have often been used to investigate the functional properties of LHC proteins. Typically, these plants lack the major LHC proteins, Lhcb1 and Lhcb2 (Simpson et al., 1985; White and Green, 1987; Allen et al., 1988) but still contain various amounts of minor LHC proteins (White and Green, 1988; Peter and Thornber, 1991; Marquardt and Bassi, 1993; Jahns and Krause, 1994). The barley (*Hordeum vulgare* L.) *chlorina f2* mutant (Simpson et al., 1985) is the most well-characterized Chl *b*-deficient mutant. Several studies have addressed its LHC polypeptide composition (White and Green, 1987, 1988; Morrissey et al., 1989; Harrison and Melis, 1992; Harrison et al., 1993), but it is still not fully disclosed. A major obstacle for correct identification and quantification lies in the immunological cross-reactivity generally observed between different LHC proteins. In this work, we have circumvented this problem by using monospecific polyclonal antibodies raised against synthetic peptides. Moreover, we have immunologically assayed the level of ELIPs, a protein family with homology to the LHC polypeptides (Green et al., 1991). ELIPs also have a considerable sequence homology to a β-carotene-binding protein of green algae (Lers et al., 1991) and are induced during desiccation in the resurrection plant *Craterostigma plantagineum* (Bartels et al., 1992). Their function is unknown, but involvement in pigment synthesis or in the PSII repair after photoinhibition has been suggested (Adamska et al., 1992). Genes encoding two types of ELIPs, one about 17 kD and one about 14 kD, have been cloned from barley (Grimm et al., 1987).

Our studies lead us to the suggestion that ELIPs are xanthophyll-binding proteins and sites for the xanthophyll cycle.

MATERIALS AND METHODS

Plant Material

Wild-type and *chlorina f2* barley (*Hordeum vulgare* L.) were grown for 14 d at 25°C at a photon flux density of 200 μmol m^{-2} s^{1} and a photoperiod of 16 h. Etiolated seedlings of wild type and *chlorina f2* were grown at 25°C in the dark for 7 d, after which they were exposed to ImL conditions for 36 cycles (2 min of light at 40 μmol m^{-2} s^{-1} and 118 min of dark). Samples were withdrawn for analysis after the 30 cycles prior to or after their exposure to HL.

The HL (1000 μmol m^{-2} s^{1}) treatment was performed on detached leaves laid on wet filter paper using tungsten halogen lamps. The plants were protected from IR radiation by a circulating water system.

Thylakoid Preparation and Western Blotting

Barley thylakoid membranes were prepared according to the method of Harrison and Melis (1992). In the case of the *chlorina f2* mutant and ImL-grown plants, protease inhibitors (2 mM benzamidine and 2 mM ϵ-aminocaproic acid) were added to the homogenization buffer. Spinach thylakoids were isolated as previously described by Spangfort and Andersson (1989).

SDS-PAGE was run according to the procedure of Laemmli (1970). Western blotting was performed by electrophoretically transferring the proteins from SDS-PAGE to an Immobilon membrane, followed by the incubation of the membrane in antibody solution. Antigen-antibody complexes were visualized by alkaline phosphatase reaction.

Antibodies

For this study, four new monospecific LHC antibodies were raised against synthetic oligopeptides derived from the consensus sequences of the different LHC proteins. The selected peptide sequences were SADWMPGQPRP (Lhca1), RQLWFASKQSL (Lhca3), KGEWLPGLASP (Lhca4), and KAPPKKAKAPPT (Lhcb4). A C-terminal Cys residue was added to facilitate subsequent coupling to SBTI. Production of the Lhcb6 antibody (CP 24-m4), raised against a synthetic peptide with the sequence ESKRWVN-FFN, will be described elsewhere. Peptides were synthesized at the Department of Immunology, University of Uppsala, Sweden. The peptide structures were verified by MS and the purity by reverse-phase chromatography as a control of quality. For antibody production, the C-terminal Cys residue of the peptides was coupled to SBTI using a linker according to the method of Gordon et al. (1987), mixed with Freund's complete adjuvant, and injected into rabbits. The peptide-SBTI complexes in incomplete adjuvant were subsequently injected into the rabbits a month later and then at 2-week intervals.

The different antibodies used in this study are listed (with references) in Table I.

Table I. *Antibodies used in this work*

Antigen/ Gene Product	Name of Antibody	Reference
PsaD	PsaD	Andersen et al. (1992)
PsaL	PsaL	Andersen et al. (1992)
Lhca1	Anti-Lhca1	This work
Lhca2	Anti-LHCI	Sigrist and Staehelin (1994)
Lhca3	Anti-Lhca3	This work
Lhca4	Anti-Lhca4	This work
PsbA	PsbA	Mullet et al. (1990)
PsbS	PsbS	Ljungberg et al. (1986)
Lhcb1	T1-A1	Sigrist and Staehelin (1992)
Lhcb2	T2-A1	Sigrist and Staehelin (1992)
Lhcb3	T3-A1	Sigrist and Staehelin (1994)
Lhcb4	Anti-Lhcb4	This work
Lhcb5	Anti-CP26	Falbel and Staehelin (1992)
Lhcb6	CP24-m4	This work
ELIP	ELIP	Pötter and Kloppstech (1993)

Pigment Determination

Photosynthetic pigments were quantified with a modification of the method of Gilmore and Yamamoto (1991). Pigments were extracted at room temperature under dim light. To ensure that only thylakoid pigments were measured, the thylakoid pellets were washed three times with 50 mM Tricine, 330 mM sorbitol to remove plastoglobuli. The pellets were suspended in 1 mL of 100% acetone and centrifuged, and the supernatant was collected. The acetone-dried pellets were re-extracted as above. The combined supernatants were centrifuged and the final supernatant was stored at −20°C under nitrogen. No pigment degradation was observed during this storage period. The acetone extracts were subsequently passed through a 0.2-μm filter (13JP020AN, Micro Filtration Systems, Dublin, CA) and analyzed by HPLC. An end-capped ODS (20) reversed-phase column (Ultracarb 3, 10 μm, 150 × 4.6 mm; Phenomenex, Torrance, CA) was used, and the solvent program was identical with program A (solutions A and B) of Gilmore and Yamamoto (1991). Pigments were detected by measuring A_{440}. A typical chromatogram is shown in Figure 1. In contrast to that used by Gilmore and Yamamoto, we used an end-capped column to separate the photosynthetic pigments. We believe that this represents an improvement, since non-end-capped columns may be more variable than end-capped columns. One of three columns tested by Gilmore and Yamamoto performed rather poorly, but the three different columns we have used so far have produced virtually identical results.

The retention times and response factors for Chl *a*, Chl *b*, lutein, α-carotene, and β-carotene were identified using authentic compounds (Sigma). The retention time and response factor (peak area to mmol) for Pchlide was determined using Pchlide prepared from etiolated wheat. Chlide co-eluted with Pchlide and is in the quantifications included in the Pchlide figure. The retention time and response factor for pheophytine *a* was determined after conversion of Chl *a* to pheophytine *a* with acid. Neoxanthin, violaxanthin, antheraxanthin, and zeaxanthin were identified by their retention times and absorption spectra. The response factors for neoxanthin, violaxanthin, antheraxanthin, and zeaxanthin were assumed to have the same relation to that of lutein as determined by Gilmore and Yamamoto (1991). The validity of this assumption was demonstrated by treating different parts of a detached leaf with different light intensities for 10 min and by checking that the measured amount of violaxanthin plus antheraxanthin plus zeaxanthin did not change during photoconversion. A shoulder on the leading edge of neoxanthin, probably neoxanthin A (Thayer and Björkman, 1990), as well as a trailing peak of violaxanthin was detected as reported before (Young and Britton, 1989; Thayer and Björkman, 1990; Gilmore and Yamamoto, 1991). For quan-

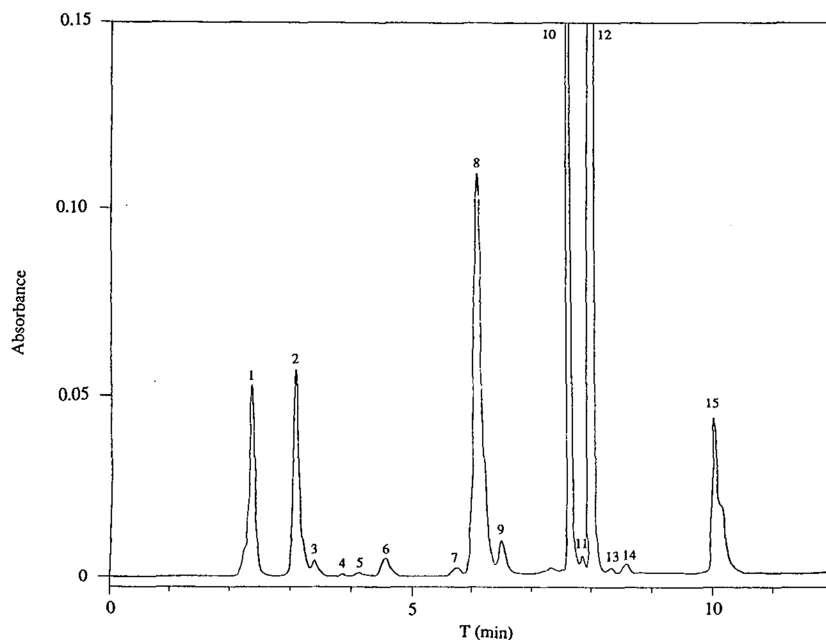

Figure 1. HPLC of thylakoid pigments. Identity of peaks: 1, neoxanthin; 2, violaxanthin; 3, violaxanthin, see text; 4 and 5, lutein epoxides (?); 6, antheraxanthin; 7, carotenoid X, see text; 8, lutein; 9, zeaxanthin; 10, Chl *b*; 11, unknown; 12, Chl *a*; 13, unknown; 14, pheophytine *a*; 15, β-carotene. Pchlide (not present in this sample) eluted after 1.4 min.

Table II. *Peptides used for production of specific antibodies*

Consensus sequences for the various LHC polypeptides are derived from Jansson (1994). Uppercase letter indicates conserved amino acid residue; lowercase letter indicates most abundant amino acid. The sequences used for production of antibodies are underlined.

Lhca1	sAdWMPGqPRPsyLDGS
Lhca2	AadpdRPLWFPGsTPPpWLDGS
Lhca3	astpPVKQGanRqLWFASkQSLSYLDGS
Lhca4	kKGeWLPGLaSPdYLdGS
Lhcb1	...ssgSPWyGpDRVkYLGPfsGEsPSYLTGE
Lhcb2	...vPqSIWYGeDRPKyLGPFSEQTPSYLTGE
Lhcb3	gNDLWYGPDRVKYLGPFSAQTPSYLnGE
Lhcb4	...KApPKKakappTTDRPLWyPGAisPdwLDGs
Lhcb5	...ELAKWYGPDRRIfLPeGLLDRseiPEYLNGE
Lhcb6	AAAvAPKKSWIPAVKsGGNlvDPEWLDGS

tification, these were included in the neoxanthin and violaxanthin peaks. Three minor (apparently carotenoid) compounds were also detected. The first two (4 and 5 in Fig. 1), perhaps lutein epoxides (Siefermann-Harms, 1988; Young and Britton, 1989), were assumed to have the same response factor as antheraxanthin. The third peak (7 in Fig. 1), denoted carotenoid X, was assumed to have the same response factor as lutein. The response factors were: Pchlide/Chlide = 0.65, neoxanthin = 1.31, violaxanthin = 1.31, lutein epoxide 1 and 2 = 1.31, antheraxanthin = 1.31, carotenoid X = 1.71, lutein = 1.71, zeaxanthin = 1.70, Chl b = 4.58, Chl a = 2.51, pheophytine a = 22.1, α-carotene = 1.60, β-carotene = 1.60. One additional unknown compound eluted between Chl b and Chl a (11 in Fig. 1), and one (13 in Fig. 1) eluted after Chl a. We were not able to measure the absorbance spectra of these compounds for identification. Our method was not fully reproducible for carotenes, similar to that reported by Thayer and Björkman (1990), so quantification of these pigments was not possible. The EPS was calculated as (violaxanthin + antheraxanthin/2)/(violaxanthin + antheraxanthin + zeaxanthin) (Demmig-Adams and Adams, 1990). For the presentation of pigment quantifications, total pigment was calculated as the sum of all pigments, excluding the carotenes and the unidentified peaks 11 and 13.

EM

Cross-sections of primary leaves (2 cm from the top) were prepared for EM by fixing in glutaraldehyde and staining with 5% uranyl acetate. Samples were embedded in Spurr's resin and sections were stained with lead citrate according to the method of Król et al. (1988).

RESULTS

Characterization of Specific Antibodies

The sequences of the synthetic peptides used for production of antibodies were derived from consensus sequences of the hydrophilic N-terminal part of individual LHC proteins (Table II). Regions showing high conservation in the primary sequence within each type, but low conservation between the different types, of LHC protein were chosen to minimize immunological cross-reaction between different LHC proteins but still allow detection of the same protein in as many plant species as possible. The antibodies were tested on thylakoid protein samples from spinach and barley, as well as on an LHC I-containing PSI preparation (completely devoid of PSII and LHC II) and a PSII-enriched fraction of barley thylakoids. The anti-Lhca1 antibody reacted against a barley protein with the molecular mass of 21 kD (Fig. 2), but a weaker reaction against proteins of apparent molecular weights much higher than any known LHC polypeptide was also found. The anti-Lhca3 antibody specifically detected a barley protein with the apparent molecular mass of 24 kD and the anti-Lhca4 antibody recognized a 21-kD barley protein, in addition to a very weak signal against an unidentified 50-kD protein. The proteins reacting with these antibodies were highly abundant in the PSI preparation and their gel mobilities were consistent with the ones found by Knötzel et al. (1992). All anti-Lhca antibodies also recognized spinach proteins of the correct (Ikeuchi et al., 1991) apparent mol wt (data not shown). The antibody reactions against the spinach proteins were stronger than against their barley counterparts. This was expected, since the peptides synthesized were designed primarily after dicotyledonous LHC protein sequences and the corresponding barley proteins are slightly divergent. This means that our new anti-Lhca antibodies all were useful for measuring the amounts of the different Lhca polypeptides in both barley and spinach.

In contrast, the anti-Lhcb4 antibody recognized two barley thylakoid proteins with the apparent molecular masses of about 30 and 23 kD (Fig. 2) but did not show specific reaction against any spinach protein. The absence of reaction against spinach Lhcb4 is not surprising, since the Lhcb4 peptides used for raising antibodies were identical with the barley Lhcb4 sequence, which turned out to be quite divergent from the recently determined *Arabidopsis thaliana* Lhcb4 sequence (Green and Pichersky, 1993). This sequence was not available when we synthesized the peptides. We believe, for two reasons, that only the 30-kD

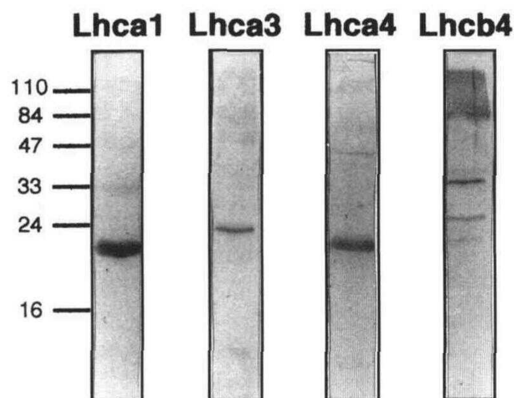

Figure 2. Characterization of the specific antibodies against LHC polypeptides. Barley thylakoid proteins were separated on SDS gels, blotted, and incubated with the indicated antibodies. Mobility of molecular mass markers are to the left.

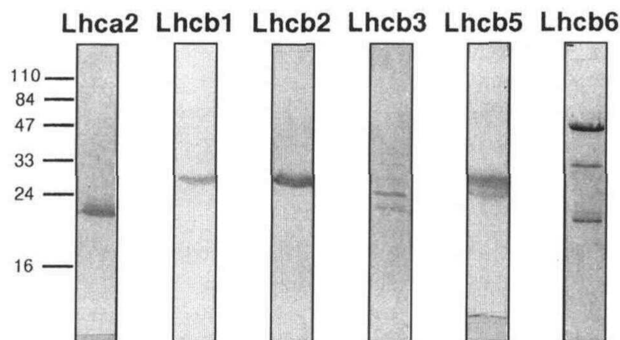

Figure 3. Specificity of the antibodies against LHC proteins. Barley thylakoid proteins were separated on SDS gels, blotted, and incubated with the indicated antibodies. Mobility of molecular mass markers are to the left.

protein represents Lhcb4. First, its size is consistent with earlier reports (White and Green, 1987, 1988; Peter and Thornber, 1991; Harrison and Melis, 1992), and second, a monoclonal antibody (CMpLHCI:1) specific for barley Lhca2 and Lhcb4 proteins (Høyer-Hansen et al., 1988) recognized only the 30-kD protein. Several lines of evidence indicate that the lower molecular mass band corresponds to the 22-kD PsbS protein. The size is correct, and our Lhcb4 peptide shares homology to the PsbS sequence. A PsbS antibody (Ljungberg et al., 1986) also recognized a protein that had the same pattern of appearance (equally abundant in wild-type and *chlorina f2* thylakoids grown under normal light or ImL) as the 23-kD band recognized by our Lhcb4 antibody. Since cross-reactivity of antibodies against PsbS and CP 29 (Lhcb4 and/or Lhcb5) has also been found before (Camm and Green, 1989), we believe that the lower molecular mass band recognized in barley thylakoids probably represents PsbS.

When we tested the other LHC antibodies on barley thylakoid proteins (Fig. 3), we noticed one difference in binding characteristic compared to what has been previously reported. Whereas the Lhca2, Lhcb1, and Lhcb2 antibodies gave a signal from a single band and Lhcb5 from a doublet as expected (Falbel and Staehelin, 1992; Sigrist and Staehelin, 1992, 1994), the Lhcb3 antibody decorated three proteins. This was in contrast to a previous study (Sigrist and Staehelin, 1994), in which only one protein was detected. The middle band corresponds in size to Lhcb3 and the other two bands (30 and 22 kD) might be cross-reacting proteins or posttranslational modification products. This is without relevance for our conclusions, since they are not present in *chlorina f2* or in ImL plants. Anti-Lhcb6 reacted with two polypeptides (approximately 31 and 50 kD) in addition to the 20-kD Lhcb6 protein. Since two other Lhcb6 antibodies (one raised against the purified protein and one against a different synthetic peptide) recognized only the 20-kD protein (data not shown), only the 20-kD protein represents Lhcb6.

Taken together, this shows that our antibody collection allowed us to identify and quantify each specific LHC protein in barley.

The LHC Protein Composition of Wild-Type and *Chlorina f2* Barley, Grown under Normal Light and ImL

The thylakoid protein pattern of the different samples (wild-type and *chlorina f2* barley, both grown under normal light and ImL) showed, as expected, significant differences (Fig. 4), especially in the 20- to 30-kD region where the LHC proteins are found. To quantify the differences in the LHC protein composition, we used western blotting. Equal amounts of thylakoid proteins were separated by SDS-PAGE, transferred to poly(vinylidenedifluoride) membranes, and blotted with our set of antibodies (Table I). Antibodies against RC polypeptides were included to allow us to relate the levels of the individual LHC proteins to the number of PSI and PSII centers. The result is shown in Figure 5.

Chlorina f2 contained, on a protein basis, slightly less PSI but somewhat more PSII then the wild type, consistent with the findings of Harrison et al. (1993). In ImL plants, the PsaD and PsaL antibodies gave conflicting results. The PsaL polypeptide was largely depleted, compared to PsaD. To establish which antibody truly reflects the number of PSI centers, we also analyzed the samples with a PsaE antibody. We found that the distribution of PsaE resembled PsaD, not PsaL, and concluded that the amount of PsaD protein should be used to standardize the data to the number of PSI centers. PsaD and PsaE are also extrinsic, hydrophilic PSI proteins (Bryant, 1992) and should not be associated with thylakoid membranes in the absence of a RC. This means that, even if they should be present in excess in ImL plants, the superfluous proteins should not be found in our membrane preparations. We have chosen to present the level of each LHC protein in a semiquantitative way (Table III). We have estimated the staining intensity in each lane with the relative number of RCs, measured by the intensity in the reaction with PsaD and PsbA antibodies with the different samples. Therefore, data are given on a relative scale: +++, wild-type level; ++, reduced level compared to wild type level; +, trace amounts; −, absence. For example, Lhca1 is slightly less

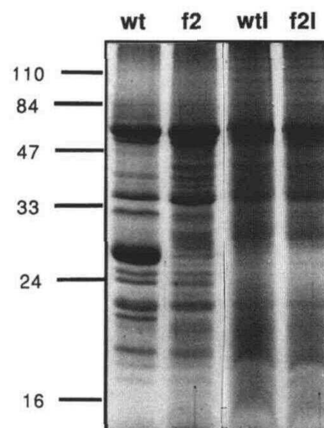

Figure 4. SDS-PAGE, stained with Coomassie blue, of thylakoid membrane proteins of wild-type and *chlorina f2* barley grown under normal light and ImL. wt, Wild-type barley; f2, *chlorina f2*; wtl, wild type grown under ImL; f2l, *chlorina f2* grown under ImL.

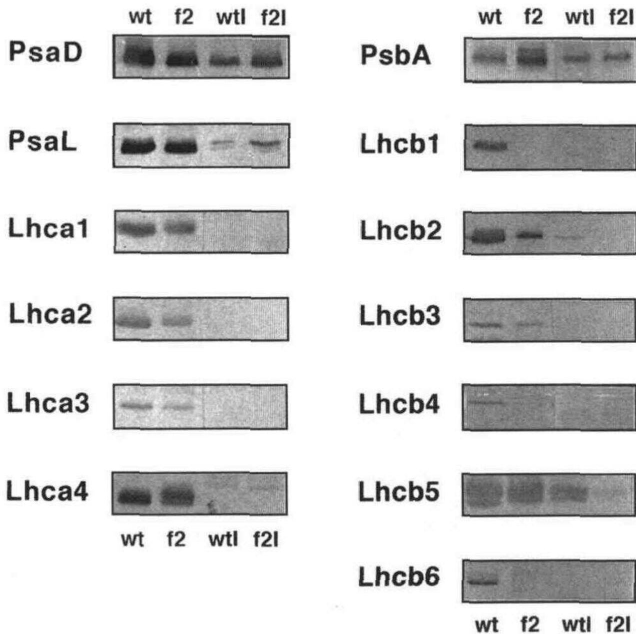

Figure 5. Western blots of thylakoid proteins prepared from wild-type and *chlorina f2* barley, grown under normal and ImL, using the antibodies listed in Table I. Legend as in Figure 2.

abundant on a protein basis in *chlorina f2* than in the wild-type sample. Since this is the case for PsaD as well, the amount of Lhca1 per PSI RC is approximately the same in the two samples. In contrast, Lhcb2 is less abundant in *chlorina f2* than in wild type, although the level of PsbA is higher. Thus, Lhcb2 is depleted in *chlorina f2*.

In *chlorina f2* grown under normal light, the Lhca proteins were found at levels fairly close to those in wild type; Lhca4 might be slightly enriched but the others were (insignificantly?) depleted. For the Lhcb proteins the situation was quite different. Lhcb1 and Lhcb6 were completely missing, Lhcb2 and Lhcb3 were reduced (Lhcb2 more than Lhcb3), and Lhcb4 occurred only in very small amounts (Table III). Only Lhcb5 was present at wild-type levels. Wild-type ImL plants contained only Lhcb5 and some

Table III. *Relative amounts of the different LHC proteins in wild-type (wt) and chlorina-f2 barley, grown under normal light and ImL*

+++, Wild-type level; ++, reduced as compared to wild type; +, small amounts; (+), trace amount; −, absent.

Protein	wt	*Chlorina f2*	wt ImL	*Chlorina f2* ImL
Lhca1	+++	+++	−	−
Lhca2	+++	+++	−	−
Lhca3	+++	+++	−	−
Lhca4	+++	+++	−	−
Lhcb1	+++	−	−	−
Lhcb2	+++	++	+	−
Lhcb3	+++	++	−	−
Lhcb4	+++	(+)	−	−
Lhcb5	+++	+++	++(+)	(+)
Lhcb6	+++	−	−	−

Lhcb2, whereas *chlorina f2* ImL plants had no LHC proteins except for trace amounts of Lhcb5.

We also measured the levels of all proteins after 1 h of HL treatment, but no change in the amount of any LHC proteins was detected (data not shown).

Quantification of ELIPs

Figure 6 shows the presence of ELIPs (detected by western blotting) in the thylakoid membranes in the barley wild type and *chlorina f2* mutant grown under different light regimes. The antibody was raised against the small type of ELIP, expressed in *Escherichia coli* from its cloned gene (Pötter and Kloppstech, 1993). In *chlorina f2*, and to a very low extent in wild-type ImL HL, there is a lower molecular mass band reacting with the antibody. We do not know whether this represents a modification of the protein or another type of ELIP. We also observed cross-reaction with a 27-kD protein, apparently Lhcb1. ELIPs were, on a PSI/PSII basis, most abundant in *chlorina f2* ImL, but a lot was also present in wild-type ImL. The levels in *chlorina f2* grown in normal light were lower, and ELIPs were barely detectable in wild type grown in normal light. The amount of ELIPs increased considerably after the HL treatment, more in wild type than in *chlorina f2*, in which the basal level on the other hand was higher. Thus, 1 h of HL treatment was sufficient to induce the accumulation of ELIPs.

Pigment Analysis

We measured by HPLC the amount of pigments in the different plant samples before and after HL treatment (Table IV). Neoxanthin is associated with the Lhcb polypeptides (Bassi et al., 1993; Thornber et al., 1993), which are severely depleted in *chlorina f2*. As a consequence, neoxanthin (and of course Chl *b*) was largely reduced in *chlorina f2* if compared to wild type, as reported by Simpson et al. (1985). In plants grown in normal light, the only prominent change in pigment composition induced by the HL treatment was de-epoxidation of violaxanthin into antheraxanthin and zeaxanthin (Table IV). Wild-type ImL plants, containing very few LHC proteins, had reduced amounts of neoxanthin, Chl *b*, and lutein but a large amount of violaxanthin. In *chlorina f2* grown under ImL, the levels of xanthophylls were even lower, e.g. neoxanthin was completely absent. There were still lutein and violaxanthin present, 45% on a Chl *a* basis as compared to wild type grown in normal light. The 1-h HL treatment induced a massive accumulation of xanthophylls in ImL plants, and lutein,

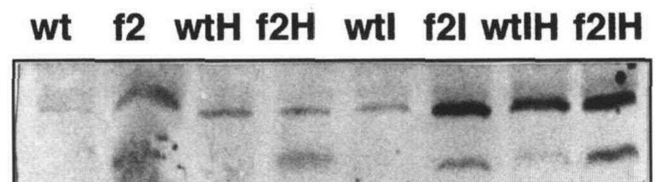

Figure 6. Western blot using an ELIP-antibody. Legend as in Figure 3, but the suffix H indicates after 1 h of HL treatment.

Table IV. *Pigment composition of wild-type (wt) and chlorina f2 barley thylakoids treated in various ways*

Figures are numbers per 1000 pigment molecules (see "Materials and Methods"). Pheophytine *a* is included in the Chl *a* figure. L, Lutein; N, neoxanthin; V, violaxanthin; A, antheraxanthin; Z, zeaxanthin; EPS, epoxidation state (see "Materials and Methods"); L epoxide, lutein epoxide (?); Car X, an unidentified carotenoid. Asterisk (*) denotes uncertain figures; accurate measurement of low amounts of Chl *b* was complicated by presence of a few minor, unidentified peaks and/or baseline variations in that region of the chromatogram. For calculation of the approximate number of PSI and PSII centers, see text.

Sample	Chl *a*	Chl *b*	Pchlide	L	N	V+A+Z	EPS	L Epoxide	Car X	PSI Centers	PSII Centers
wt	624	187	0	113	31	42	1.0	0	3	1.4	2.3
wt HL	609	208	0	108	27	45	0.78	0	2		
f2	855	3*	0	80	9	50	0.98	1	3	3.7	6.1
f2 HL	841	12*	0	78	11	54	0.53	0	0		
wt ImL	801	90	3	58	7	36	0.82	2	3	3	6
wt ImL HL	601	80	3	176	20	108	0.18	7	5		
f2 ImL	934	5*	4	27	0	28	1	0	2	4	8
f2 ImL HL	833	3*	3	73	7	69	0.27	7	5		

neoxanthin, and violaxanthin/antheraxanthin/zeaxanthin increased approximately 3-fold. In addition, the EPS of the xanthophyll pool became very low, i.e. almost all of the violaxanthin had been phototransformed to zeaxanthin.

We also wanted to relate the pigment content to the number of PSI and PSII centers. It is known that wild-type barley contains 1.7 PSI and 3 PSII centers/1000 Chl, whereas the corresponding figures for *chlorina f2* are about 3 and 10 (Harrison et al., 1993). In the ImL plants, the antenna size of PSI is probably about 100 Chl *a* and of PSII approximately 50 Chl *a*, the sizes of the core antenna. If these figures are considered, it appears as if the relative number of PSI and PSII centers loaded on our gels is in wild type 3.5 and 6, respectively, in *chlorina f2* 3 and 9, and in ImL plants 1 and 2. This corresponds well with the relative strength of the signals for PsaD and PsbA in the blots (Fig. 5). These figures were then used to estimate the number of PSI and PSII centers in the preparations. It must be pointed out that such calculations have a large degree of uncertainty and can serve only as an approximation. With this limitation in mind, there appear to be about 10 violaxanthin cycle molecules/RC in wild type, whereas *chlorina f2* contains only approximately 5. This was expected, since *chlorina f2* has fewer LHC polypeptides/RC (Table III). The wild-type ImL plants contain about four violaxanthins and six luteins/RC and the *chlorina f2* ImL plants have two or three molecules/RC of both violaxanthin and lutein. The latter figure is surprising, since no LHC proteins are present in these plants. Thus, the xanthophylls must either be bound to other proteins where phototransformation also can take place or remain free in the membrane.

Ultrastructure of the Thylakoids

The thylakoid ultrastructure has been investigated in different Chl *b*-less mutants and plants grown under ImL since the LHC polypeptides are thought to play an important role in grana stacking.

Figure 7A shows the ultrastructure of *chlorina f2* barley chloroplasts developed under ImL. The long, unbranched thylakoids arranged in parallel arrays resemble the pattern observed in the conditionally Chl *b*-deficient wheat mutant CD3, grown under restrictive conditions (Allen et al., 1988). The long thylakoids are not in close contact with each other as they are in the grana stacks, but a few primary thylakoids surrounding the large arrays are closely spaced. In contrast, no parallel arrays are found in wild-type plants grown under ImL (Fig. 7B). The primary thylakoids of these plants are found in smaller fragments, some of which are closely spaced.

DISCUSSION

The LHC Protein Composition and Chloroplast Ultrastructure of *Chlorina f2*

With our specific antibodies, we are able to quantify the relative levels of all LHC proteins in the Chl *b*-deficient mutant *chlorina f2* grown under normal light and ImL. Our results differ in some aspects from those reported before (White and Green, 1987, 1988; Morrissey et al., 1989; Harrison and Melis, 1992; Harrison et al., 1993). In *chlorina f2* grown under normal light, White and Green (1987) reported the presence of the so-called 25-kD LHC II polypeptide (Lhcb3) and CP 29, which correspond to either Lhcb4 or Lhcb5 or both. Harrison and Melis (1992) and Harrison et al. (1993) detected a single PSII LHC polypeptide, probably equivalent to Lhcb3, in *chlorina f2*. In contrast, we find that Lhcb5 is the most abundant Lhcb protein and that Lhcb2 and Lhcb3 are present, although in diminished amounts. The differences might simply be a consequence of methodology (specific antibodies versus Coomassie staining), but it is also known that the LHC protein composition of *chlorina f2* varies with growth conditions (D.J. Simpson, personal communication). We also show that the abundance of Lhca proteins in *chlorina f2* does not significantly differ from wild type, in spite of the lack of Chl *b*. We offer no explanation for why the mutation strongly affects most Lhcb protein levels but leaves the Lhca protein levels virtually unchanged. The level of depletion of a particular protein does not correlate with the Chl *b* content, as previously suggested (White and Green, 1988).

More interesting than the LHC polypeptide composition of *chlorina f2* grown under normal light is the absence of LHC proteins in this mutant grown under ImL. Even Lhcb5, which is quite abundant in wild-type barley grown

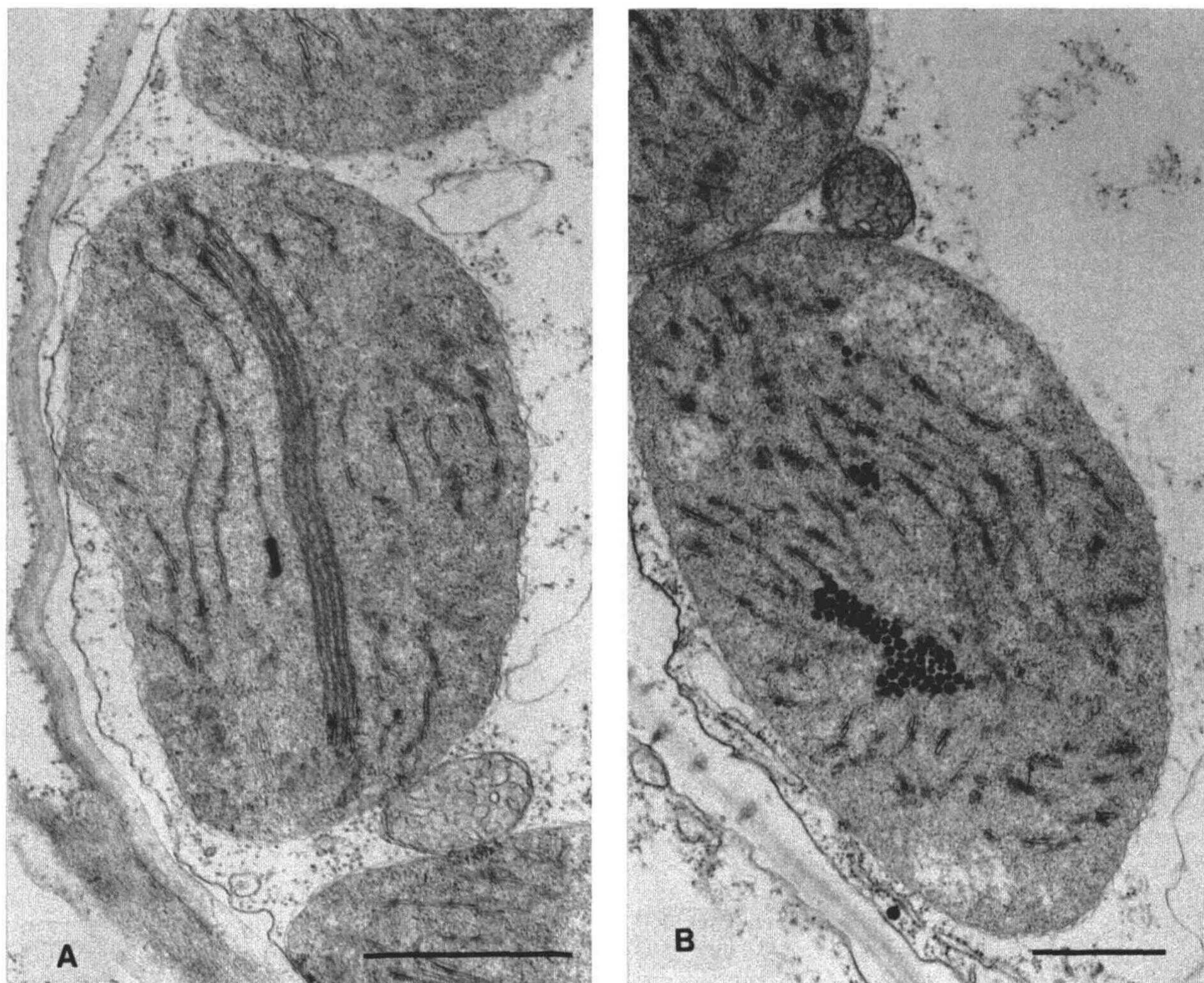

Figure 7. Electron micrograph of *chlorina f2* chloroplasts developed under ImL (A) and wild-type barley chloroplasts developed under ImL (B).

under ImL, is present in only trace amounts in our *chlorina f2* ImL plants. This is, to our knowledge, the first report in which the level of all 10 LHC polypeptides have been assayed. Thus, the *chlorina f2* ImL plants are the best verified examples of plants completely lacking LHC polypeptides. In addition to our present analysis of the xanthophyll cycle components, such plants could be useful for various studies of LHC protein function. The PsbS gene product that recently has been shown to be a Chl-binding protein (Funk et al., 1994) is present in these plants, but it is not known whether this protein also binds xanthophylls. If this should be the case, it could have some implications for our interpretations of the other results but would not change the final conclusions.

The LHC polypeptide composition of wild-type barley grown under ImL might give some hints concerning the PSII antenna structure. Only Lhcb5 and small amounts of Lhcb2 are present in these plants. Lhcb5 is an inner antenna protein but Lhcb2 is part of the outer antenna, sometimes regarded as the most peripheral Lhcb protein (Larsson et al., 1987a, 1987b). If we consider the generally accepted view that the minor LHC proteins act as linkers between the peripheral antenna and the RC, it seems that the Lhcb2 protein present in wild-type ImL plants is connected to PSII through Lhcb5, which then probably also is the case in plants developed in normal light.

The strong depletion of the PsaL polypeptide in the plants grown under ImL is worth mentioning. PsaL is an integral part of the PSI complex and tightly bound to the RC I polypeptides (Okkels et al., 1991) and should, thus, be expected to be present in all PSI centers. Our finding that the protein is virtually absent in conditions where the Lhca polypeptides are missing could perhaps indicate a role for the PsaL polypeptide in LHC I assembly, but other functions certainly are also possible.

The thylakoid ultrastructure differs quite distinctly between ImL-grown plants of wild type and *chlorina f2*, despite relatively moderate changes in LHC protein composition. *Chlorina f2* grown under ImL have long, parallel thylakoids that are not in contact with each other like the thylakoids in a grana stack. This peculiar feature has been reported before in a Chl *b*-deficient wheat mutant, which has greatly reduced levels of all LHC proteins (Allen et al., 1988). Our data indicate that this phenotype is associated

with severe (total?) depletion of LHC proteins. Presence of Lhcb5 and a very small amount of Lhcb2 gives instead short thylakoids fragments, which have the ability to adhere to each other. The minor Lhcb proteins can apparently mediate membrane appression, since an *Arabidopsis* mutant lacking Lhcb1 and Lhcb2 (Murray and Kohorn, 1991), and also *chlorina f2* grown in normal light, have grana stacks. Although the small amount of Lhcb2 present in our wild-type ImL plants could be important, we believe that our data indicate that Lhcb5 (CP 26) has a possible role in membrane adhesion.

Localization of the Xanthophyll Cycle

All photosynthetic pigments are probably bound to proteins in vivo. This implies that the photoconversion of the pigments in the xanthophyll cycle takes place on the LHC proteins, since the proteins of the core antenna bind no violaxanthin. Another implication is that changes in pigment composition can be viewed as changes in the amounts of individual pigment-binding proteins, provided that the binding sites for the different pigments on these proteins are specific. Although the xanthophyll cycle has been extensively investigated (reviewed by Demmig-Adams, 1990), little attention has been paid to the site of the photoconversion until recently. Bassi et al. (1993) suggested that photoconversion in PSII takes place only on the minor LHC proteins (Lhcb4, Lhcb5, and Lhcb6) and not on the more abundant Lhcb1 and Lhcb2 proteins. In contrast, Horton and co-workers (Horton et al., 1991; Ruban et al., 1994) showed that zeaxanthin could also be bound to LHC II (Lhcb1 and Lhcb2). The xanthophyll cycle operates in PSI as well (Thayer and Björkman, 1992), but it is not known whether all or a subset of the Lhca proteins are sites for the cycle.

In our *chlorina f2* plants grown under ImL, there are no LHC proteins present, but these plants nevertheless contain two to three phototransformable violaxanthin molecules per RC. We also found that 1 h of HL treatment induces a 3-fold increase in the content of neoxanthin, lutein, and (phototransformable) violaxanthin, without accumulation of LHC proteins. Even if the PsbS protein should turn out to bind some of the xanthophylls present in the ImL plants, they cannot account for the increase, since the level of PsbS is not affected by the HL treatment (data not shown). Thus, we have demonstrated the presence of a pool of the phototransformable xanthophyll cycle components not bound to any LHC protein. These additional xanthophylls must be associated with other proteins or exist freely in the membrane.

In contrast to Jahns and Krause (1994), who studied ImL pea plants, we think that it is unlikely that these xanthophylls are free in the thylakoid membrane. We believe that they probably have a function associated with photosynthesis and have chosen to consider yet another possibility: this phototransformable xanthophyll pool is bound to ELIPs.

It is not known whether the ELIPs bind pigments. Nevertheless, we show in this paper a correlation between xanthophylls not bound to LHC proteins and ELIPs. Thus, our data support the previous suggestion that ELIPs are pigmented proteins present under certain conditions (Levy et al., 1993; Jansson, 1994). We postulate that the ELIPs function as substitutes for the inner LHC proteins, possibly in both PSI and PSII, when plants are grown under potentially harmful light conditions. Levy et al. (1992) suggested that the β-carotene/ELIP protein is an integral part of the LHC II complex during light stress. If this is true, small amounts of co-purifying ELIPs could account for the increased carotenoid/protein ratio in the preparations of Marquardt and Bassi (1993). Our data and the fact that accumulation of ELIPs precedes accumulation of LHC proteins during normal thylakoid development indicate that ELIPs also can bind directly to the core antenna in the absence of LHC protein.

We believe that the differences in pigment composition found between different plant species and plants grown under different light environments (Demmig-Adams and Adams, 1990; Thayer and Björkman, 1990) can be explained by differences in PSI/PSII ratio and the content of the various LHC proteins, including ELIPs. Plants adapted to grow under HL should consistently have a higher level of ELIPs and lower amounts of LHC proteins. On the other hand, LHC polypeptides depleted of pigments could be stable (e.g. the LHC proteins present in *chlorina f2*), so a strict correlation should not be expected in mutants lacking specific pigments or in plants grown under ImL, where pigment synthesis might be suppressed.

In conclusion, we hypothesize that the function of the ELIPs is to act as pigmented regulators of energy transfer under conditions where there are special needs for a sink for excitation energy.

ACKNOWLEDGMENTS

We are indebted to Andrew Staehelin, Birger Lindberg Møller, Klaus Kloppstech, Bertil Andersson, and John Mullet for providing us with antibodies and R. Smith for skillful technical assistance with EM. Liselotte Eriksson and Birger Lindberg Møller are acknowledged for critical reading of the manuscript.

Received August 15, 1994; accepted November 15, 1994.
Copyright Clearance Center: 0032-0889/95/107/0873/11.

LITERATURE CITED

Adamska I, Ohad I, Kloppstech K (1992) Synthesis of the early light-inducible protein is controlled by blue light and related to light stress. Proc Natl Acad Sci USA **89:** 2610–2613

Allen KD, Duysen ME, Staehelin LA (1988) Biogenesis of thylakoid membranes is controlled by light intensity in the conditional chlorophyll b-deficient CD3 mutant of wheat. J Cell Biol **107:** 907–919

Andersen B, Koch B, Vibe Scheller H (1992) Structural and functional analysis of the reducing side of photosystem I. Physiol Plant **84:** 154–161

Bartels D, Hanke C, Schneider K, Michel D, Salamini F (1992) A desiccation-related Elip-like gene from the resurrection plant Craterostigma plantagineum is regulated by light and ABA. EMBO J **11:** 2771–2778

Bassi R, Pineau B, Dainese P, Marquardt J (1993) Carotenoid-binding proteins of photosystem II. Eur J Biochem **212:** 297–303

Britton G (1993) Carotenoids in chloroplast pigment-protein complexes. *In* C Sundqvist, ed, Pigment-Protein Complexes in Plas-

tids: Synthesis and Assembly. Cell Biology: A Series of Monographs. Academic Press, San Diego, CA, pp 447–483

Bryant D (1992) Molecular biology of photosystem I. *In* J Barber, ed, The Photosystems: Structure, Function and Molecular Biology. Elsevier, Amsterdam, pp 501–549.

Camm EL, Green BR (1989) The chlorophyll *ab* complex, CP29, is associated with the photosystem II reaction centre core. Biochim Biophys Acta **974:** 180–184

Demmig-Adams B (1990) Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. Biochim Biophys Acta **1020:** 1–24

Demmig-Adams B, Adams WW (1990) Carotenoid composition in sun and shade leaves of plants with different life forms. Plant Cell Environ **15:** 411–418

Falbel TG, Staehelin LA (1992) Species-related differences in the electrophoretic behaviour of CP29 and CP26: an immunochemical analysis. Photosynth Res **34:** 249–262

Funk C, Schröder WP, Green BR, Renger G, Andersson B (1994) The intrinsic 22 kDa protein is a chlorophyll-binding subunit of photosystem II. FEBS Lett **342:** 261–266

Gilmore AM, Yamamoto HY (1991) Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C18 high performance liquid chromatographic column. J Chromatogr **543:** 137–145

Gilmore AM, Yamamoto HY (1993) Linear models relating xanthophylls and lumen acidity to non-photochemical fluorescence quenching. Evidence that antheraxanthin explains zeaxanthin-independent quenching. Photosynth Res **35:** 67–78

Gordon RD, Fieles WE, Schotland DL, Hogue-Angeletti R, Barchi RL (1987) Topological localization of the C-terminal region of the voltage-dependent sodium channel from *Electrophprus electricus* using antibodies raised against a synthetic peptide. Proc Natl Acad Sci USA **84:** 308–312

Green BR, Pichersky E (1993) Nucleotide sequence of an *Arabidopsis thaliana Lhcb4* gene. Plant Physiol **103:** 1451–1452

Green BR, Pichersky E, Kloppstech K (1991) Chlorophyll *a/b* binding proteins: an extended family. Trends Biochem Sci **16:** 181–186

Grimm B, Kruse E, Kloppstech K (1989) Transiently expressed early light-inducible thylakoid proteins share transmembrane domains with light-harvesting chlorophyll binding proteins. Plant Mol Biol **13:** 583–593

Harrison MA, Melis A (1992) Organization and stability of polypeptides associated with the chlorophyll a-b light-harvesting complex of photosystem-II. Plant Cell Physiol **33:** 627–637

Harrison MA, Nemson JA, Melis A (1993) Assembly of the chlorophyll *a-b* light-harvesting complex of barley (*Hordeum vulgare* L.): immunochemical analysis of chlorophyll *b*-less and chlorophyll *b*-deficient mutants. Photosynth Res **38:** 141–151

Horton P, Ruban AV, Rees D, Pascal AA, Noctor G, Young AJ (1991) Control of the light harvesting function of chloroplast membranes by aggregation of the LHCII chlorophyll-protein complex. FEBS Lett **292:** 1–4

Høyer-Hansen G, Bassi R, Hønberg LS, Simpson DJ (1988) Immunological characterization of chlorophyll a/b-binding proteins of barley thylakoids. Planta **173:** 13–21

Ikeuchi M, Hirano A, Inoue Y (1991) Correspondence of apoproteins of light-harvesting chlorophyll *a/b* complexes associated with photosystem I to *cab* genes: evidence for a novel type IV apoprotein. Plant Cell Physiol **32:** 103–112

Irrgang K-D, Kablitz B, Vater J, Renger G (1993) Identification, isolation and partial characterization of a 14–15 kDa pigment binding protein complex of PS II from spinach. Biochim Biophys Acta **1143:** 173–182

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

Jansson S (1994) The light-harvesting chlorophyll a/b-binding proteins. Biochim Biophys Acta **1194:** 1–19

Jansson S, Pichersky E, Bassi R, Green BR, Ikeuchi M, Melis A, Simpson DJ, Spangfort M, Staehelin LA, Thornber JP (1992) A nomenclature for the genes encoding the chlorophyll *a/b*-binding proteins of higher plants. Plant Mol Biol Rep **10:** 242–253

Knötzel J, Svendsen I, Simpson DJ (1992) Identification of the photosystem I antenna polypeptides in barley isolation of three pigment binding antenna complexes. Eur J Biochem **206:** 209–215

Król M, Huner NPA, Williams JP, Maissan E (1988) Chloroplast biogenesis at cold-hardening temperatures. Kinetics of trans-Δ3-hexadecenoic acid accumulation and the assembly of LHC II. Photosynth Res **15:** 115–132

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685

Larsson UK, Anderson JM, Andersson B (1987a) Variations in the relative content of the peripheral and inner light-harvesting chlorophyll *a/b*-protein complex (LHC II) subpopulations during thylakoid light adaptation and developement. Biochim Biophys Acta **894:** 69–75

Larsson UK, Sundby C, Andersson B (1987b) Characterization of two different subpopulations of spinach light-harvesting chlorophyll *a/b*-protein complex (LHC II): polypeptide composition, phosphorylation pattern and association with photosystem II. Biochim Biophys Acta **894:** 59–68

Lers A, Levy H, Zamir A (1991) Co-regulation of a gene homologous to early light-induced genes in higher plants and β-carotene biosynthesis in the alga *Dunaliella bardawil*. J Biol Chem **266:** 13698–13705

Levy H, Gokhman I, Zamir A (1992) Regulation and light-harvesting complex II association of a *Dunaliella* protein homologous to early light-induced proteins in higher plants. J Biol Chem **267:** 18831–18836

Levy H, Tal T, Shaish A, Zamir A (1993) Cbr, an algal homolog of plant early light-induced proteins, is a putative zeaxanthin binding protein. J Biol Chem **268:** 20892–20896

Ljungberg U, Åkerlund HE, Andersson B (1986) Isolation and characterization of the 10-kDa and 22-kDa polypeptides of higher plant photosystem 2. Eur J Biochem **158:** 477–482

Marquardt J, Bassi R (1993) Chlorophyll-proteins from maize seedlings grown under intermittent light conditions. Planta **191:** 265–273

Morrissey PJ, Glick RE, Melis A (1989) Supramolecular assembly and function of subunits associated with the chlorophyll *a-b* light-harvesting complex II (LHC II) in soybean chloroplasts. Plant Cell Physiol **30:** 335–344

Mullet JE, Gemble Klein P, Klein RR (1990) Chlorophyll regulates accumulation of the plastid-encoded chlorophyll apoproteins CP43 and D1 by increasing apoprotein stability. Proc Natl Acad Sci USA. **87:** 4038–4042

Murray DL, Kohorn BD (1991) Chloroplasts of *Arabidopsis thaliana* homozygous for the ch-1 locus lack chlorophyll *b*, lack stable LHCPII and have stacked thylakoids. Plant Mol Biol **16:** 71–79

Okkels JS, Vibe Scheller H, Svendsen I, Lindberg Møller B (1991) Isolation and characterization of a cDNA clone encoding an 18-kDa hydrophobic photosystem I subunit (PSI-L) from barley (*Hordeum vulgare* L.). J Biol Chem **266:** 6767–6773

Peter GF, Thornber JP (1991) Biochemical composition and organization of higher plant photosystem II light-harvesting pigment proteins. J Biol Chem **266:** 16745–16754

Pötter E, Kloppstech K (1993) Effects of light stress on the expression of early light-inducable proteins. Eur J Biochem **214:** 779–786

Ruban AV, Yuong 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

Siefermann-Harms D (1988) High-performance liquid chromatography of chloroplast pigments. One step separation of carotene and xantophyll isomers, chlorophylls and pheophytins. J Chromatogr **448:** 411–416

Sigrist M, Staehelin LA (1992) Identification of type 1 and type 2 light-harvesting chlorophyll *a/b*-binding proteins using monospecific antibodies. Biochim Biophys Acta **1098:** 191–200

Sigrist M, Staehelin LA (1994) Appearance of type 1, 2 and 3 light-harvesting complex II and light-harvesting complex I proteins during light-induced greening of barley (*Hordeum vulgare*) etioplasts. Plant Physiol **104:** 135–145

Simpson DJ, Machold O, Høyer-Hansen G, von Wettstein D (1985) *Chlorina* mutants of barley (*Hordeum vulgare*) L.). Carlsberg Res Commun **50:** 223–238

Spangfort M, Andersson B (1989) Subpopulations of the main chlorophyll *a/b* light harvesting complex of photosystem II—isolation and biochemical characterization. Biochim Biophys Acta **977:** 163–170

Thayer SS, Björkman O (1990) Leaf xanthophyll content and composition in sun and shade determined by HPLC. Photosynth Res **23:** 331–343

Thayer SS, Björkman O (1992) Carotenoid distribution and de-epoxidation in thylakoid pigment-protein complexes from cotton leaves and bundle-sheath cells of maize. Photosynth Res **33:** 213–225

Thornber JP, Peter GF, Morishige DT, Gómez S, Anandan S, Welty BA, Lee A, Kerfeld C, Takeuchi T, Preiss S (1993) Light harvesting in photosystems I and II. Biochem Soc Trans **21: 15–18**

White MJ, Green BR (1987) Polypeptides belonging to each of the three major chlorophyll a+b protein complexes are present in a chlorophyll-*b*-less barley mutant. Eur J Biochem **165:** 531–535

White MJ, Green BR (1988) Intermittent-light chloroplasts are not developmentally equivalent to chlorina f2 chloroplasts in barley. Photosynth Res **15:** 195–203

Young AJ, Britton G (1989) The distribution of α-carotene in the photosynthetic pigment-protein complexes of higher plants. Plant Sci **64:** 179–183