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Thermo-optically induced reorganizations in the main light harvesting antenna of plants. II. Indications for the role of LHCII-only macrodomains in thylakoids

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Abstract

We have investigated the circular dichroism spectral transients associated with the light-induced reversible reorganizations in chirally organized macrodomains of pea thylakoid membranes and loosely stacked lamellar aggregates of the main chlorophyll *a*/*b* light harvesting complexes (LHCII) isolated from the same membranes. These reorganizations have earlier been assigned to originate from a thermo-optic effect. According to the thermo-optic mechanism, fast local thermal transients due to dissipation of the excess excitation energy induce elementary structural changes in the close vicinity of the dissipation [Cseh et al. (2000) Biochemistry 39: 15250–15257]. Here we show that despite the markedly different CD spectra in the dark, the transient (light-minus-dark) CD spectra associated with the structural changes induced by high light in thylakoids and LHCII are virtually indistinguishable. This, together with other close similarities between the two systems, strongly suggests that the gross short-term, thermo-optically induced structural reorganizations in the membranes occur mainly, albeit probably not exclusively, in the LHCII-only domains [Boekema et al. (2000) J Mol Biol 301: 1123–1133]. Hence, LHCII-only domains might play an important role in light adaptation and photoprotection of plants.

Abbreviations: CD – circular dichroism; LHC II – light-harvesting chlorophyll *a*/*b* pigment-protein complex*;* psi – polymer- and salt-induced

Introduction

Flexibility of photosynthetic membranes is essential for their ability to adjust their functions upon short- and long-term variations in external environmental conditions, such as temperature and illumination. In higher plants, a significant part of the multilevel regulatory mechanisms involves structural changes in the light harvesting system, and in LHCII, the main chlorophyll *a*/*b* complex of Photosystem II, in particular (Anderson and Andersson 1988). LHCII has been shown to exhibit a remarkable structural flexibility. It has been well established that structural changes involving LHCII and affecting photosynthetic functions can be driven by feedback mechanisms,

which are governed ultimately by the operation of the photosynthetic electron and proton transport system. These include reorganizations following the reversible phosphorylation of the polypeptides (Allen and Forsberg 2001), changes induced directly by lowering the lumenal pH (Horton et al. 1996), and flexibility changes associated with the conversion of violaxanthin to zeaxanthin (Ruban et al. 2000; Havaux et al. 2004). There are, however, reorganizations in LHCII that are largely or entirely indendent of the activity of the photochemical apparatus, and these cannot originate from photosynthetic feed back mechanisms. These structural changes include heat-induced changes in the oligomerization state of the complexes (Takeuchi and Thornber 1994; Cseh et al. 2000), heat-induced alterations in the surface electric properties (Dobrikova et al. 2002), reversible changes associated with the quenching of the fluorescence of isolated LHCII (Barzda et al. 1999; Grudzinski et al. 2002), light-induced reversible and irreversible changes in the macrodomain and trimeric organization of the complexes (Barzda et al. 1996; Garab et al. 2002; Dobrikova et al. 2003), light-induced alterations regulating the phosphorylation of LHCII at the substrate level (Zer et al. 1999, 2003), and reorganizations connected with the light-induced isomerization of violaxanthin (Grudzinski et al. 2001). These reorganizations appear to originate from the thermal instability of the (macro)assemblies of LHCII and, as concerns the light-induced reorganizations, from a novel, biological thermo-optic effect. According to the thermo-optic mechanism, fast, local thermal transients due to dissipation of the excess excitation energy induce elementary structural changes in the close vicinity of the site of internal conversion (Cseh et al. 2000; Garab et al. 2002).

The light-induced reversible and irreversible structural changes in the chiral macrodomains exhibit an approximately linear dependence on the intensity of the unused excitation energy, and thus represent a unique and potentially important regulatory mechanism in the adaptation and protection of plants against excess light (Cseh et al. 2005). The main features of these structural changes in lamellar aggregates of isolated LHCII and granal thylakoid membranes, including their temperature and light-intensity dependencies, show remarkable similarities. It thus appears that this particular structural flexibility of the thylakoid membranes, i.e. which manifests itself in light-induced reorganizations affecting the long-range chiral order of the chromophores, is closely mimicked by loosely stacked lamellar aggregates of isolated LHCII, and might be 'borrowed' from the main light harvesting complexes.

It is also noteworthy that the organization of chromphores in chiral macrodomains in thylakoid membranes depends largely on LHCII (Garab et al. 1991). Loosely or tightly stacked lamellar aggregates of isolated light harvesting complexes also contain macrodomains with similar long-range chiral order as the native membranes (Faludi-Dániel and Mustárdy 1983; Garab et al. 1988a). This is evidently determined by the high self-aggregation capability of LHCII, which thus readily assemble into highly ordered macroarrays both *in vitro* and *in vivo*. They give rise to psi-type CD (Garab 1996), and to intense circularly polarized luminescence emission (Gussakovsky et al. 2000). These macroassemblies resemble each other also with regard to their anisotropic molecular architecture and electric and magnetic parameters (Garab 1987). The self-aggregation of LHCII in the lamellae plays an important role in the 'sorting' of complexes, and thus in the lateraral heterogeneity of the thylakoid membranes (Garab and Mustárdy 1999); these complexes also play a key role in the stacking of membranes (Arntzen 1978; Barber 1982). Hence, stacking and macro-organization of LHCII play important roles in the self-assembly and stabilization of the granal ultrastructure. At the same time, as concluded from an analysis of electron micrographs of freeze-fractured chloroplasts, LHCII trimers and lipids contribute significantly to randomizing the supramolecular arrangement of the LHCII–PS II supercomplexes (Kirchhoff et al. 2004). (For the role of lipids, see also (Simidjiev et al. 1998).

With regard to the origin of these close similarities in the 'static and dynamic' features of loosely stacked lamellar aggregates of LHCII and granal thylakoid membranes, recent models of the organization of LHCII and LHCII–PS II particles in granal thylakoids are of special interest (Boekema et al. 2000; Ford et al. 2002; Dekker and Boekema 2005). These models, derived from electron microscopy analysis, depict the granum thylakoid membranes to contain two large domains, which are adjacent to each other: an

extended area containing ordered arrays of LHCII–PS II supercomplexes, which however contain substoichiometric amounts of LHCII, and an LHCII-only macrodomain, which contains, also in highly ordered arrays, the 'missing' light harvesting complexes. The role of these LHCII-only domains is yet unknown, but can be hypothesized that they play a significant role in the regulation of the light energy utilization. The composition and structure of these macrodomains are expected to be more readily adjustible than those of the supercomplexes. In other terms, it can be assumed that the LHCII-only macrodomains are structurally more flexible than the LHCII-PSII supercomplexes and their domains. Our data, obtained from CD spectroscopy, are in line with this hypothesis. Although isolated thylakoid membranes and lamellar aggregates of LHCII exhibit markedly different CD spectra in the dark, the transient spectra belonging to the structural changes induced by high light are virtually indistinguishable. These strongly suggest that the gross short-term, thermo-optically induced structural reorganizations in the membranes occur mainly, albeit probably not exclusively, in the LHCII-only domains. These macrodomains thus appear to play an important role in light adaptation and photoprotection of plants.

Materials and methods

Plant material

Thylakoid membranes were isolated as previously described (Barzda et al. 1996) from dark adapted 2-week-old pea leaves grown in the greenhouse, and were suspended, stored and measured in a medium containing 20 mM Tricine (pH 7.5), 400 mM sorbitol, 5 mM $MgCl_2$ and 5 mM KCl. Type II, loosely stacked lamellar aggregates of LHCII, which retain substantial amounts of lipids and are capable of undergoing light-induced reversible structural changes, were prepared from pea as described earlier (Simidjiev et al. 1997).

Circular dichroism spectroscopy

CD spectra were recorded in a Jobin-Yvon CD6 dichrograph, which was equipped with a side illumination attachment (Barzda et al. 1996). The chlorophyll concentration was adjusted to approx. 10 μg/ml with thylakoid membranes and 20 μg/ml with LHCII. The optical pathlength of the cuvette was 1 cm. All measurements were done at room temperature. The same illumination and measuring protocol was used for thylakoid membranes and LHCII: (i) prior to the measurement, the sample was incubated for 5 min in the sample compartment of the dichrograph with the 400 nm measuring beam on; (ii) a CD spectrum was recorded, referred to as the dark spectrum; (iii) the sample was illuminated in the sample compartment via fiber optics for 5 min with heat-filtered white light of approx. 1500 μmol m^{-2} s^{-1}; (iv) immediately after the illumination a CD spectrum was measured (light spectrum); (v) the re-dark spectrum was recorded after 15 min incubation in the dark. One scan, between 400 and 720 nm, was performed in less than 2 min. Light-*minus*-dark spectra represent average spectra from three consecutive dark–light cycles. CD is plotted in absorbance units. The reversible and irreversible components were calculated by averaging the difference spectra between the dark spectra and the preceding light spectra, and between consecutive dark spectra, respectively.

Results and discussion

Figure 1 shows typical CD spectra of isolated granal thylakoid membranes exposed to light–dark cycles of a preillumination with intense light. These data are in reasonably good agreement with earlier results. The spectrum of the thylakoid membranes following a dark-adaptation is similar to those measured under similar conditions (Barzda et al. 1994; Istokovics et al. 1997). It has been shown earlier that intact thylakoid membranes are capable of undergoing light-induced reversible structural changes that affect the long-range chiral organization of the chromophores. The previously applied preilluminations, however, were somewhat shorter and the light intensities were usually lower than here. The conditions for these experiments were chosen to result in somewhat longer-lasting effects, which thus can yet be picked up following the preillumination, while mild enough to ensure a significant degree of reversibility in the dark (see also Materials and methods). Hence, under these

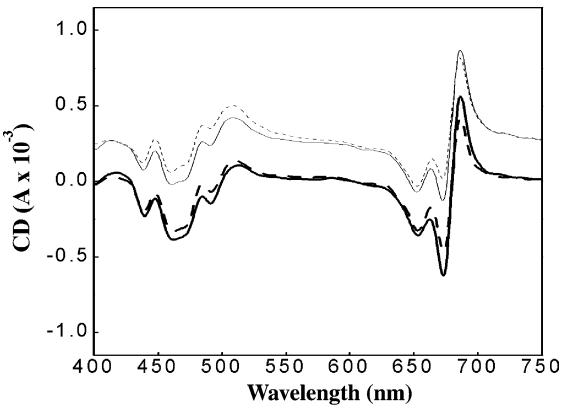

Figure 1. Typical CD spectra of dark-adapted (lower pair of curves) and preilluminated (upper pair of curves) thylakoid membranes from two consecutive 15 min dark–5 min light cycles. The preillumination was performed in the sample holder of the dichrograph with heat-filtered white light of approx. 1500 μmol m^{-2} s^{-1}. For clarity, the spectra of preilluminated thylakoids are shifted up by 2×10^{-4} units. Continouos and dashed lines represent spectra from the second and third cycle, respectively.

conditions, in contrast to previous reports for faster and fully reversible (Garab et al. 1988b) and largely irreversible changes (Gussakovsky et al. 1997), we selectively detect the slowly reversible components of the reorganizations.

It can be seen that, as expected, preillumination affected the main, psi-type CD bands and the long tails associated with differential scattering of left and right circularly polarized light (Figure 2a). These signals originate from LHCII-containing chiral macrodomains (Garab 1996). The strongest variation could be seen at around 675 nm. The diminishment of this negative psi-type band also affected the positive amplitudes, in accordance with the previously established composite character of the CD signal of thylakoid membranes (Finzi et al. 1989). Although upon the repetition of the light treatment the CD changes were not entirely reversible, the basic character of each light treatment remained essentially the same, as also verified by the invariance of the basic features of the transient spectra on the number of cycles. It also appeared that the first dark state differed most from all other consecutive dark states; this might be accountable for a residual delta pH and transmembrane electric field following the illuminations, and the absence of this component after long dark adaptation in the stock suspension (data not shown). Although the changes are largely independent of the photochemical activity of the

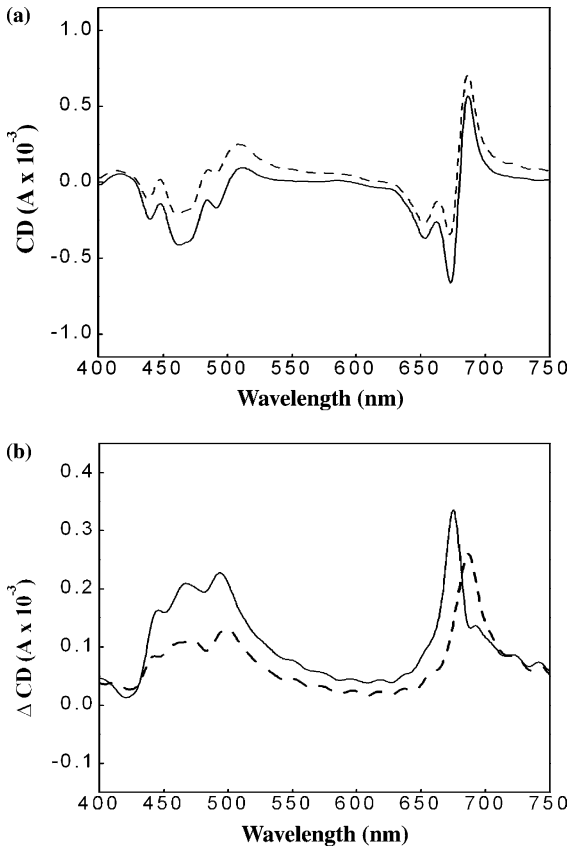

Figure 2. CD spectra (a) associated with the dark (continuous line) and preilluminated states (dashed line) of isolated thylakoid membranes, and light-minus-dark difference spectra obtained from the corresponding averaged light and dark spectra (continuous line) as well as its reversible component using the dark spectra and the preceding light spectra (b). The spectra in (a) were obtained from averaging three spectra of dark-adapted and preilluminated thylakoid membranes from a series as in Figure 1.

membranes the effect of ΔpH has been shown to modulate the reversible ΔCD (Istokovics et al. 1997).

The transient spectra (Figure 2b) exhibited maxima at around 675 and 495 nm for the averaged light *minus* dark difference spectrum (full line), and at 685 and 495 nm for the reversible component (dashed curve). This latter was extracted by using the difference spectra between the dark spectrum and the preceding light spectrum. These transients differ somewhat from those recorded earlier. The light-minus dark spectra obtained with 'light' spectra recorded during the illumination periods contained more evenly all CD bands associated with the chiral macrodomains, i.e. also involved the main, (+) 690 nm psi-type band

(Garab et al. 1988b). In contrast, under the presently used experimental conditions, which favor the detection of slowly reversible changes, the transients were dominated by the (−) 675 nm band. Although the amplitude changes of the (−) 675 nm band were not fully reversible, each consecutive light *minus* dark difference spectra were dominated by this band. Also, it was observed that the decrease of the (−) 675 nm band was accompanied with an apparent increase in the (+) 690 nm band. The CD spectrum in the red has earlier been shown to originate from two largely independent psi-type bands of opposite sign (Finzi et al. 1989). The reversible component of the transients peaking at 685 nm (Figure 2b, dashed line) appears to originate mainly from the variation of the broad negative psi-type band. The irreversible component, obtained from difference spectra of consecutive dark spectra also contained somewhat larger contribution from the (−) 675 nm band but it could be characterized as an overall decrease of the psi-type bands (data not shown). This is in agreement with data reported earlier for prolonged preillumination periods (Cseh et al. 2000).

The (−) 675 nm CD band has been shown to exhibit a dependency on the concentration of Mg-ions, and thus it has been proposed to be associated with the stacking of membranes (Garab et al. 1991). We have earlier shown that upon preillumination granal thylakoid membranes the structural reorganizations begin with unstacking, and are followed by lateral desorganizations of the macrodomains and monomerization of LHCII trimers (Dobrikova et al. 2003). (This lattest component, i.e. the monomerization of LHCII trimers, has been shown to occur upon prolonged illumination with high light, and thus probably does not occur in sizeable quantities under the presently used experimental conditions.) Hence, these data suggest that re-stacking and/or the re-binding of Mg-ions and the consecutive restoration of the chiral macrostructure occur relatively more slowly and somewhat less reversibly than the lateral desorganization of the macrodomains.

In the Soret region, the transients exhibited a broad CD band and some well discernible minor bands. The meanings of the variations in the minor bands remain to be determined but probably reflect changes in the organization and excitonic interactions of carotenoids in the antenna.

Figure 3 shows that, similar to thylakoids, loosely stacked lamellar aggregates of LHCII are also capable of undergoing light-induced reversible reorganizations. The dark and light (preilluminated) states can readily be discriminated in these LHCII macroassemblies, too. Also, in contrast to thylakoids, the changes were almost fully reversible, and the irreversible component contained no easily discernible bands. Hence, the averaging of the spectra and calculation of the difference spectrum were straight forward (Figure 4). Again, as it will be reported elsewhere, the transient spectra recorded with light spectra during the illumination period, or under different illumination conditions differ somewhat from those shown in Figure 4. Nonetheless, as it is clear from a comparison of Figures 2b and 4b, thylakoids and lamellar aggregates of isolated LHCII under comparable experimental conditions exhibit very similar transient spectra both in the red and in the Soret region. This is somewhat surprising because the steady state spectra (i.e. the spectra in the dark or in the light) differ markedly from each other for thylakoids and LHCII (cf. Figures 2a and 4a).

This close similarity in the transients of the two systems, together with other similarities, most notably the existence of chiral macrodomains and their thermo-optically inducible reorganizations, are remarkable. Although thermo-optically induced reorganizations have also been observed

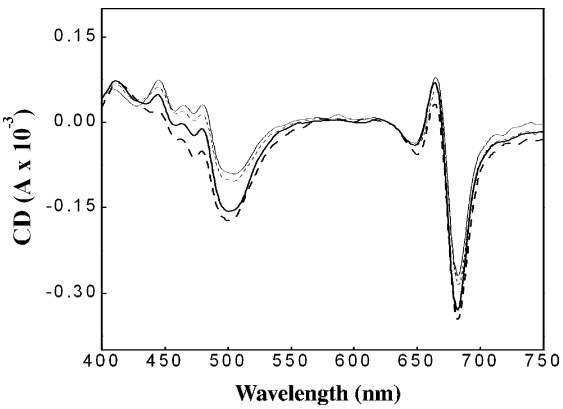

Figure 3. Typical CD spectra of dark-adapted (thick lines) and preilluminated (thin lines) loosely stacked lamellar aggregates of isolated LHCII from two consecutive 15 min dark–5 min light cycles. The preillumination was performed in the sample holder of the dichrograph with heat-filtered white light of approx. 1500 μmol m^{-2} s^{-1}. Continuous and dashed lines represent spectra from the second and third cycle, respectively.

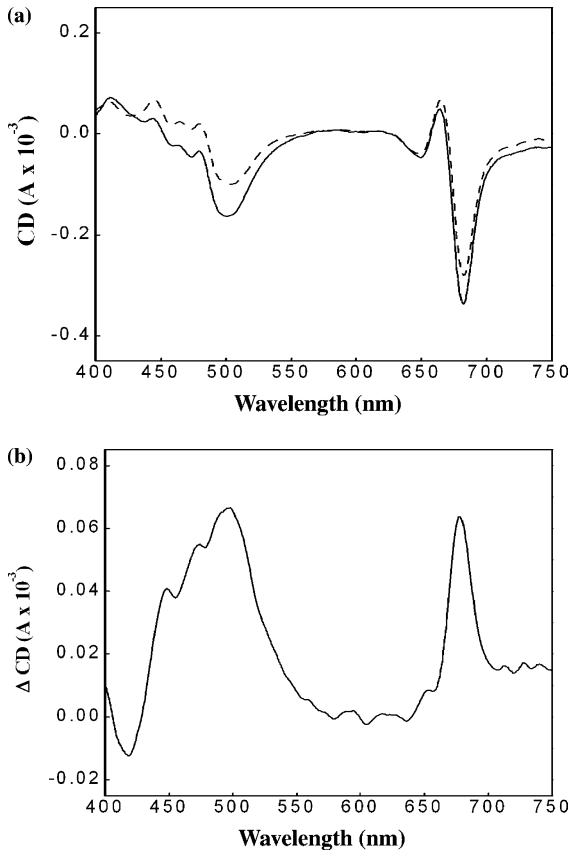

Figure 4. CD spectra (a) associated with the dark (continuous line) and preilluminated states (dashed line) of loosely stacked lamellar aggregates of isolated LHCII, and the light-minus-dark difference spectrum (b). The spectra in (a) were obtained from averaging three spectra of dark-adapted and preilluminated samples from a series as in in Figure 3.

in the absence of chiral macrodomains (Dobrikova et al. 2003) the above data strongly suggest a specific role of LHCII-only domains in thylakoids. The simplest explanation for all these data is if we assume that the changes in thylakoids originate from LHCII-only macrodomains, rather than from the regions of PSII supercomplexes.

The hypothesis concerning the role of LHCII-only domains in the structural flexibility of membranes is plausible because LHCII trimers are probably attached to the PSII core more firmly than to each other, and thus PSII particles are likely to be more rigid than the LHCII-only domains. In general, it is tempting to speculate that LHCII-only domains play important regulatory roles: (i) in mature membranes, the incorporation of the newly synthesized LHCII trimers might be easier into the LHCII-only macrodomains than into the PSII supercomplexes; LHCII accumulates readily e.g. in low light (Larsson et al. 1987) or diurnally (Busheva et al. 1991); (ii) *vice versa*, the proteolytic removal of LHCII might also be easier from these domains than from the supercomplexes; in addition, this latter also requires the monomerization of the complexes (Yang et al. 2000), which seems more difficult to satisfy for the case of PSII supercomplexes; (iii) it seems plausible that the reorganizations upon short-term variations in the environmental conditions are confined to LHCII-only macrodomains, and probably do not involve the supercomplexes. It is also to be noted that this proposal is perfectly in line with earlier data showing that there is a fraction of LHCII, the peripheral antenna complexes, that exhibit a relatively high mobility in the membrane (Anderson and Andersson 1988).

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References

Allen JF and Forsberg J (2001) Molecular recognition in thylakoid structure and function. Trends Plant Sci 6: 317–326

Anderson JM and Andersson B (1988) The dynamic photosynthetic membrane and regulation of solar energy conversion. Trends Biochem Sci 13: 351–355

Arntzen CJ (1978) Dynamic structural features of chloroplast lamellae. Curr Top Bioenerg 8: 111–160

Barber J (1982) Influence of surface charges on thylakoid structure and function. Annu Rev Plant Physiol 33: 261–295

Barzda V, Istokovics A, Simidjiev I and Garab G (1996) Structural flexibility of chiral macroaggregates of light-harvesting chlorophyll *a*/*b* pigment–protein complexes. Light-induced reversible structural changes associated with energy dissipation. Biochemistry 35: 8981–8985

Barzda V, Jennings RC, Zucchelli G and Garab G (1999) Kinetic analysis of the light-induced fluorescence quenching in light-harvesting chlorophyll *a*/*b* pigment–protein complex of Photosystem II. Photochem Photobiol 70: 751–759

Barzda V, Mustardy L and Garab G (1994) Size dependency of circular dichroism in macroaggregates of photosynthetic pigment–protein complexes. Biochemistry 33: 10837–10841

Boekema EJ, van Breemen JF, van Roon H and Dekker JP (2000) Arrangement of Photosystem II supercomplexes in

crystalline macrodomains within the thylakoid membrane of green plant chloroplasts. J Mol Biol 301: 1123–1133

Busheva M, Garab G, Liker E, Szell M and Nagy F (1991) Diurnal fluctuations in the content and functional properties of the light harvesting chlorophyll *a*/*b* complex in thylakoid membranes. Plant Physiol 95: 997–1003

Cseh Z, Rajagopal S, Tsonev T, Busheva M, Papp E and Garab G (2000) Thermooptic effect in chloroplast thylakoid membranes. Thermal and light stability of pigment arrays with different levels of structural complexity. Biochemistry 39: 15250–15257

Cseh Z, Vianelli A, Rajagopal S, Krumova S, Kovács L, Papp E, Barzda V, Jennings R and Garab G (2005) Thermo-optically induced reorganizations in the main light harvesting antenna of plants. I. Non-Arrhenius type of temperature dependence and linear light-intensity dependencies. Photosynth Res 86: 263–273 (this issue)

Dekker JP and Boekema EJ (2005) Supramolecular organization of thylakoid membrane proteins in green plants. Biochim Biophys Acta 1706: 12–39

Dobrikova A, Petkanchin I and Taneva SG (2002) Temperature-induced changes in the surface electric properties of thylakoids and Photosystem II membrane fragments. Coll Surf A 209: 185–192

Dobrikova AG, Krumova SB, Kovács L, Kostov GK, Todinova SJ, Busheva MC, Taneva SG and Garab G (2003) Structural rearrangements in chloroplast thylakoid membranes revealed by differential scanning calorimetry and circular dichroism spectroscopy. Thermo-optic effect. Biochemistry 42: 11272–11280

Faludi-Dániel Á and Mustárdy L (1983) Organization of chlorophyll a in the light-harvesting chlorophyll *a*/*b* protein complex as shown by circular dichroism. Plant Physiol 73: 16–19

Finzi L, Bustamante C, Garab G and Juang CB (1989) Direct observation of large chiral domains in chloroplast thylakoid membranes by differential polarization microscopy. Proc Natl Acad Sci USA 86: 8748–8752

Ford RC, Stoylova SS and Holzenburg A (2002) An alternative model for Photosystem II/light harvesting complex II in grana membranes based on cryo-electron microscopy studies. Eur J Biochem 269: 326–336

Garab G (1987) Liquid crystal-like domains of the light harvesting chlorophyll *a*/*b* protein complex in chloroplast thylakoid membranes. Mol Cryst Liq Cryst 152: 259–266

Garab G (1996) Linear and circular dichroism. In: Amesz J and Hoff AJ (ed) Biophysical Techniques in Photosynthesis, Advances in Photosynthesis, Vol. 3, pp 11–40. Kluwer Academic Publishers, Dordrecht/Boston/London

Garab G and Mustárdy L (1999) Role of LHCII-containing macrodomains in the stucture function and dynamics of grana. Aust J Plant Phys 26: 649–658

Garab G, Faludi-Dániel Á, Sutherland JC and Hind G (1988a) Macroorganization of chlorophyll *a*/*b* light-harvesting complex in thylakoids and aggregates:Information from circular differential scattering. Biochemistry 27: 2425–2430

Garab G, Leegood RC, Walker DA, Sutherland JC and Hind G (1988b) Reversible changes in macroorganization of the light-harvesting chlorophyll *a*/*b* pigment–protein complex detected by circular dichroism. Biochemistry 27: 2430–2434

Garab G, Kieleczawa J, Sutherland JC, Bustamante C and Hind G (1991) Organization of pigment–protein complexes into macrodomains in the thylakoid membranes of wild-type and chlorophyll *b*-less mutant of barley as revealed by circular dichroism. Photochem Photobiol 54: 273–281

Garab G, Cseh Z, Kovács L, Rajagopal S, Wentworth M, Mustárdy L, Dér A, Ruban AV, Papp E, Holzenburg A and Horton P (2002) Light-induced trimer to monomer transition in the main light-harvesting antenna complex of plants: thermo-optic mechanism. Biochemistry 41: 15121–15129

Grudzinski W, Krupa Z, Garstka M, Maksymiec W, Swartz TE and Gruszecki WI (2002) Conformational rearrangements in light-harvesting complex II accompanying light-induced chlorophyll *a* fluorescence quenching. Biochim Biophys Acta 1554: 108–117

Grudzinski W, Matula M, Sielewiesiuk J, Kernen P, Krupa Z and Gruszecki WI (2001) Effect of 13-*cis* violaxanthin on organization of light harvesting complex II in monomolecular layers. Biochim Biophys Acta 1503: 2091–302

Gussakovsky EE, Barzda V, Shahak Y and Garab G (1997) Irreversible disassembly of chiral macrodomains in thylakoids due to photoinhibition. Photosynth Res 51: 119–126

Gussakovsky EE, Shahak Y, Amerongen Hvan and Barzda V (2000) Circularly polarized chlorophyll luminescence reflects the macro-organization of grana in pea chloroplasts. Photosynth Res 65: 83–92

Havaux M, Dall'Osto L, Cuine S, Giuliano G and Bassi R (2004) The effect of zeaxanthin as the only xanthophyll on the structure and function of the photosynthetic apparatus in *Arabidopsis thaliana*. J Biol Chem 279: 13878–13888

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

Horton P, Ruban AV and Young AJ (1999) Regulation of the structure and function of the light-harvesting complexes of Photosystem II by the xanthophyll cycle. In: Frank HA (ed) Advances in Photosynthesis. The Photochemistry of Carotenoids, pp 271–291. Kluwer Academic Publishers, Dordrecht/Boston/London

Istokovics A, Simidjiev I, Lajkó F and Garab G (1997) Characterization of the lightüinduced reversible changes in the chiral macroorganization of the chromophores in chloroplast thylakoid membranes. Temperature dependence and effect of inhibitors. Photosynth Res 54: 45–53

Jennings RC, Garlaschi FM and Zucchelli G (1991) Light-induced fluorescence quenching in hte light-harvesting chlorophyll *a*/*b* protein complex. Photosynth Res 27: 57–64

Kirchhoff H, Tremmel I, Haase W and Kubitscheck U (2004) Supramolecular Photosystem II organization in grana thylakoid membranes: evidence for a structured arrangement. Biochemistry 43: 9204–9213

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

Ruban AV, Walters RG and Horton P (1992) The molecular mechanism of the control of excitation energy dissipation in chloroplast membranes. Inhibition of delta pH-dependent quenching of chlorophyll fluorescence by dicyclohexylcarbodiimide. FEBS Lett 309: 175–179

Ruban AV, Pascal AA and Robert B (2000) Xanthophylls of the major photosynthetic light-harvesting complex of plants: identification, conformation and dynamics. FEBS Lett 477: 181–185

Simidjiev I, Barzda V, Mustárdy L and Garab G (1997) Isolation of lamellar aggregates of the light-harvesting chlorophyll *a*/*b* protein complex of Photosystem II with

long-range chiral order and structural flexibility. Anal Biochem 250: 169–175

Simidjiev I, Barzda V, Mustárdy L and Garab G (1998) Role of thylakoid lipids in the structural flexibility of lamellar aggregates of the isolated light-harvesting chlorophyll *a*/*b* complex of Photosystem II. Biochemistry 37: 4169–4173

Takeuchi TS and Thornber JP (1994) Heat-induced alterations in thylakoid membrane-protein composition in barley. Aust J Plant Physiol 21: 759–770

Yang DH, Paulsen H and Andersson B (2000) The N-terminal domain of the light-harvesting chlorophyll *a*/*b*-binding protein complex (LHCII) is essential for its acclimative proteolysis. FEBS Lett 466: 385–388

Zer H, Vink M, Keren N, Dilly-Hartwig HG, Paulsen H, Herrmann RG, Andersson B and Ohad I (1999) Regulation of thylakoid protein phosphorylation at the substrate level: reversible light-induced conformational changes expose the phosphorylation site of the light-harvesting complex II. Proc Natl Acad Sci USA 96: 8277–8282

Zer H, Vink M, Shochat S, Hermann RG, Andersson B and Ohad I (2003) Light affects the accessibility of the thylakoid light harvesting complex II (LHCII) phosphorylation site to the membrane protein kinase(s). Biochemistry 42: 728–738