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Effect of 13-*cis* violaxanthin on organization of light harvesting complex II in monomolecular layers[1]

Wojtek Grudziński [a], Magda Matuła [a], Jan Sielewiesiuk [a], Peter Kernen [b,2], Zbigniew Krupa [c], Wiesław I. Gruszecki [a,*]

[a] *Department of Biophysics, Institute of Physics, Maria Curie-Skłodowska University, Pl. M. Curie-Skłodowskiej 1, 20-031 Lublin, Poland*
[b] *EMPA, Swiss Federal Laboratories for Materials Testing and Research, 9014 St. Gallen, Switzerland*
[c] *Department of Plant Physiology, Institute of Biology, Maria Curie-Skłodowska University, 20-031 Lublin, Poland*

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Abstract

Lutein, neoxanthin and violaxanthin are the main xanthophyll pigment constituents of the largest light-harvesting pigment–protein complex of photosystem II (LHCII). High performance liquid chromatography analysis revealed photoisomerization of LHCII-bound violaxanthin from the conformation all-*trans* to the conformation 13-*cis* and 9-*cis*. Maximally, the conversion of 15% of all-*trans* violaxanthin to a *cis* form could be achieved owing to the light-driven reactions. The reactions were dark-reversible. The all-*trans* to *cis* isomerization was found to be driven by blue light, absorbed by chlorophylls and carotenoids, as well as by red light, absorbed exclusively by chlorophyll pigments. This suggests that the photoisomerization is a carotenoid triplet-sensitized reaction. The monomolecular layer technique was applied to study the effect of the 13-*cis* conformer of violaxanthin and its de-epoxidized form, zeaxanthin, on the organization of LHCII as compared to the all-*trans* stereoisomers. The specific molecular areas of LHCII in the two-component system composed of protein and exogenous 13-*cis* violaxanthin or 13-*cis* zeaxanthin show overadditivity, which is an indication of the xanthophyll-induced disassembly of the aggregated forms of the protein. Such an effect was not observed in the monomolecular layers of LHCII containing all-*trans* conformers of violaxanthin and zeaxanthin. 77 K chlorophyll *a* fluorescence emission spectra recorded from the Langmuir–Blodgett (L–B) films deposited to quartz from monomolecular layers formed with LHCII and LHCII in the two-component systems with all-*trans* and 13-*cis* isomers of violaxanthin and zeaxanthin revealed opposite effects of both conformers on the aggregation of the protein. The *cis* isomers of both xanthophylls were found to decrease the aggregation level of LHCII and the all-*trans* isomers increased the aggregation level. The calculated efficiency of excitation energy transfer to chlorophyll *a* from violaxanthin assumed to remain in two steric conformations was analyzed on the basis of the chlorophyll *a* fluorescence excitation spectra and the mean orientation of violaxanthin molecules in LHCII (71° with respect to the normal to the membrane), determined recently in the linear dichroism experiments [Gruszecki et al., Biochim. Biophys. Acta 1412 (1999) 173–183]. The calculated efficiency of excitation energy transfer from the violaxanthin pool assumed to remain in conformation all-*trans* was found to be almost independent on the orientation angle within a variability range. In contrast the calculated efficiency of energy transfer from the form *cis* was found to be strongly dependent on the orientation and varied between 1.0 (at 67.48°) and 0 (at 70.89°). This is consistent with two essentially different, possible functions of the *cis* forms of violaxanthin: as a highly efficient excitation donor (and

* Corresponding author. Fax: +48-81-537-6252; E-mail: wieslaw@tytan.umcs.lublin.pl

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[2] Present address: Zyomyx Inc., 3911 Trust Way, Hayward, CA 94545, USA.

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possibly energy transmitter between other chromophores) or purely as a LHCII structure modifier.

Keywords: Xanthophyll cycle; Violaxanthin; Photoisomerization; LHCII

1. Introduction

Functioning of the photosynthetic apparatus under conditions characterized by extreme light intensity levels requires the operation of several regulatory processes, at different organization levels of a plant. In particular, overexcitation to the photosynthetic apparatus is known to be associated with activation of the molecular mechanisms to protect the reaction centers and the antenna systems against photo-degradation. The rearrangement of the light-harvesting pigment proteins of photosystem (PS) II, including the largest one (LHCII) is considered as one of the most important mechanisms operative under overexcitation conditions at the molecular level (see [1] for a review). According to the current state of knowledge, light stress is associated with the aggregation of LHCII, a process correlated with an increased rate of non-radiative excitation energy dissipation. The aggregation of LHCII [2] and the excitation quenching [3] was demonstrated to be enhanced by the presence of the xanthophyll pigment zeaxanthin and the opposite effect was observed in the case of violaxanthin, the epoxidated form of zeaxanthin. Such a mechanism was attributed to indirect structural effects and not to direct excitation energy quenching by zeaxanthin, operating via the singlet–singlet energy transfer from the Q_y level of chlorophyll *a* to the S1 (A_g^1) level of the xanthophyll [4]. Zeaxanthin is a xanthophyll pigment synthesized in the photosynthetic apparatus of plants within the so-called xanthophyll cycle upon illumination exceeding the saturation level [5]. Accumulation of zeaxanthin is realized via the two-step de-epoxidation of violaxanthin, the xanthophyll pigment distributed heterogeneously in the photosynthetic apparatus [6], but associated mainly with the accessory pigment–protein complexes [7]. Violaxanthin seems to be a unique xanthophyll in a sense that it is an accessory pigment bound relatively weakly to the proteins and present, at least in LHCII, in sub-stoichiometric concentrations [7]. The fact that monomeric LHCII does not bind any violaxanthin molecules [8] suggests a structural role for this xanthophyll in stabilizing the trimeric structures of the protein or/and a role of the organization of LHCII in trapping violaxanthin unspecifically attached to a protein bed. Recently [9], very mild isolation methods allowed isolation of the oligomeric forms of the LHCII trimers from spinach comprising a relatively high number of violaxanthin molecules (almost one violaxanthin per one protein monomer). A recent study from our laboratory revealed a 54% yield of the singlet excitation energy transfer from violaxanthin to chlorophyll *a* in a model system [10]. This yield is less than in the case of lutein (62%) and neoxanthin (85%) in the same system. Such a difference may be directly associated with the postulated structural function of violaxanthin with respect to LHCII in addition to light harvesting. Also the orientation angle of the violaxanthin molecules in LHCII ($71 \pm 3°$ with respect to the axis normal to the plane of the membrane) was determined to be clearly greater than the ones reported for neoxanthin ($64 \pm 4°$, [10]; $57 \pm 1.5°$, [11]) and lutein as concluded on the basis of the crystallographic data (orientation of the molecule 45–50°, [12]) or on the basis of linear dichroism measurements (orientation of the transition dipole moment $66 \pm 1°$, [10]). Both orientation angle values determined for lutein by different techniques are in good agreement, considering the difference between the transition dipole moment and the molecular axis (ca. 15°) as discussed in detail previously [10]. In the present report we present the results of the experiments which further show the importance of violaxanthin in regulating the structural organization of LHCII.

2. Materials and methods

The largest light-harvesting pigment–protein complex of PS II (LHCII) was isolated from 10-day-old rye leaves *Secale cereale* L. cv Pastar, according to

the procedure described previously [13,14]. The chlorophyll *a* to chlorophyll *b* molar ratio in our present preparation is close to the value 1.2, which suggests that 7–8 molecules of chlorophyll *a* and 6 molecules of chlorophyll *b* were present per single monomer of LHCII. The concentration of the xanthophyll pigments per LHCII in our preparation was the following: 2 lutein, 0.13 violaxanthin and 0.9 neoxanthin molecules. All-*trans* zeaxanthin was a generous gift from Hoffmann-La Roche (Basel) and all-*trans* and 13-*cis* violaxanthin were isolated from blossoms of *Viola tricolor* [15]. 13-*cis* zeaxanthin was obtained as a product of iodine-catalyzed photoconversion of the all-*trans* form following the procedure described by Molnar et al. [16]. Xanthophyll pigments extracted from LHCII and the isomeric forms of xanthophylls were separated chromatographically on a C-30-coated high performance liquid chromatography (HPLC) column (YMC Europe, length 25 cm, internal diameter 4.6 mm). A solvent mixture of acetonitrile:methanol:water (72:8:3, by volume) was used as a mobile phase. The HPLC elution rate was 0.7 ml/min. A diode-array Hewlett-Packard spectrophotometer, model HP 8453, was used to record absorption spectra between 280 and 800 nm in 5 s intervals. Xanthophyll pigments were extracted in darkness. Monomolecular layers of LHCII were formed at the surface of doubly distilled water and then distilled a third time with $KMnO_4$ to eliminate any organic impurities. LHCII was deposited at the argon–water interface from 25% (v/v) isopropanol solution in water. Such a system was found to dissolve large molecular aggregates of the protein but without pigment extraction or disruption of the excitation energy transfer from chlorophyll *b* to chlorophyll *a* and from the xanthophyll pigments to chlorophyll *a* within the complex [10]. In the case of two-component LHCII–xanthophyll monolayers, a pigment was deposited first in 50 μl of benzene:ethanol mixture (9:1, v:v) and LHCII was deposited after 15 min required for the organic solvent evaporation. Surface pressure was monitored with a Wilhelmy plate-based tensiometer, produced by Nima Technology (model PS3, Coventry, UK). Monolayers of LHCII were deposited onto a quartz support using the Langmuir–Blodgett technique with the FL-1E film-lift produced by Lauda Film (Koenigshofen, Germany). LHCII films were deposited at a constant computer-controlled surface pressure at the speed of 1 cm/min. Monolayers were transferred to a quartz support at the surface pressure of 20 mN/m. The deposition ratio was close to 1 under such conditions. In order to avoid pigment–protein destruction, monolayer compression and deposition to a quartz support were carried out in darkness and under argon atmosphere. Other details of the preparation of LHCII monolayers are described in our previous reports [10,17]. All spectroscopic measurements of deposited LHCII monolayers were carried out directly after the deposition of the LB-films. Absorption spectra were recorded with a Shimadzu 160A-PC spectrophotometer, and fluorescence spectra were recorded with a Shimadzu RF 5001-PC spectrofluorometer. Absorption and fluorescence measurements were carried out at 21 ± 1°C. 77 K fluorescence emission and excitation spectra of a single monolayer were recorded from an LB-film placed in a special holder of a Dewar dish filled with liquid nitrogen. The excitation and emission light beams were guided by optical fiber in order to excite a sample perpendicular to its surface. Fluorescence spectra were corrected for the lamp intensity.

3. Results and discussion

The relatively large orientation angle determined for violaxanthin in LHCII with respect to the normal to the plane of the membrane (71°) evoked a discussion concerning the localization of this particular xanthophyll pigment and its conformation in the complex [10]. The orientation angle of violaxanthin, which is greater than the two other LHCII-bound xanthophyll pigments, lutein and neoxanthin, was interpreted as an indication of the existence of a certain pool of violaxanthin parallel with respect to the plane of the membrane [10]. According to the general rules of orientation and localization of carotenoid pigments in membranes [18], the horizontal orientation of the all-*trans* conformer would be rather improbable, and therefore the appearance of a certain fraction of violaxanthin in LHCII in a conformation *cis* has been postulated [10]. The appearance of a central *cis* isomer of violaxanthin in LHCII was also suggested on the basis of the resonance Raman scattering and electronic absorption measure-

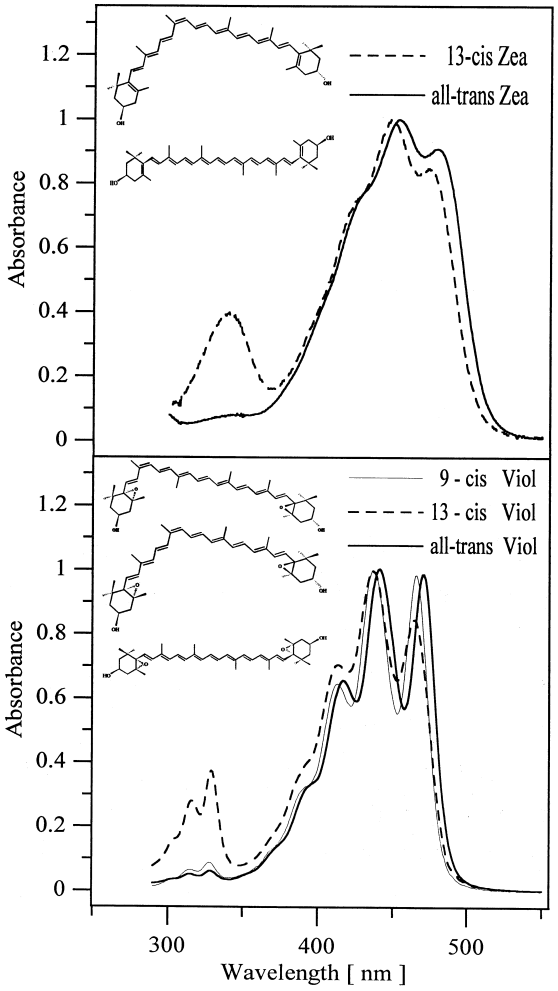

Fig. 1. Absorption spectra of the isomeric forms of violaxanthin isolated from LHCII and all-*trans* and 13-*cis* zeaxanthin used for preparation of the monomolecular layers (indicated). The spectra were normalized at the absorption maxima. Spectra were taken in the elution solvent mixture by the diode array detector. Insets contain chemical structures of the isomeric forms of violaxanthin and zeaxanthin.

ments [19]. Light-induced formation of the 13-*cis* form of violaxanthin in leaves of *Hordeum vulgare* was very recently reported by Phillip et al. [20]. We were not able to detect any steric forms of violaxanthin in our LHCII preparation, using the conventional C-18-coated, reversed-phase column. The application of the C-30-coated, reversed-phase column, designed especially for the separation of isomeric forms of C-40 carotenoids enabled the separation of different isomeric forms of violaxanthin in LHCII, including mainly the conformer all-*trans* but also the conformers 13-*cis* and 9-*cis*. The column was calibrated with the extract from the *V. tricolor* containing different isomers of violaxanthin [15].

The absorption spectra of three different isomeric forms of violaxanthin isolated from LHCII are presented in Fig. 1. Proportions of the isomeric forms of violaxanthin in the dark-incubated LHCII samples were different with respect to the proportions found in the preparations preilluminated prior to the pigment analysis (see Table 1). The preilluminated samples were characterized by a higher level of *cis* forms of violaxanthin at the expense of the all-*trans* form present predominantly in the dark-adapted samples. The illumination of the complex with red light absorbed exclusively by the chlorophyll pigments and by blue light absorbed by both chlorophylls and carotenoids yielded very similar fractions of violaxanthin isomers. This finding is consistent with the interpretation of the mechanism of photoisomerization of violaxanthin mediated by the triplet state of a carotenoid activated via the excitation energy transfer from a triplet-excited chlorophyll. Indeed, a 100% efficiency of transfer of the chlorophyll triplet excitations to the LHCII-bound xanthophyll pigments was determined in the isolated complex at room temperatures [21–24]. This energy transfer is considered to be one of the main mechanisms, operating to protect photosynthetic structures against photosensitization of active oxygen species, including the singlet oxygen, potentially harmful to the photosynthetic apparatus. Carotenoid triplet formation, a very efficient process in isolated LHCII, is, however, not expected to be that efficient in the photosynthetic apparatus under the physiological conditions characterized by a fast, singlet–singlet excitation energy transfer from the an-

Table 1
Light-induced isomerization of violaxanthin in LHCII

Violaxanthin isomers in LHCII	Light quality (% of the pool ± S.D.)		
	dark	blue	red
9-*cis*	5.4 ± 2.7	9.0 ± 0.2	10.6 ± 1.2
13-*cis*	1.2 ± 0.6	4.0 ± 0.6	4.2 ± 1.2
All-*trans*	93.5 ± 3.5	87.0 ± 0.8	85.3 ± 2.4

Results are based on the integration of HPLC chromatograms. Data are the mean of three determination ± S.D. Samples were illuminated with the light beam of the spectrofluorometer: red light, 670 nm; blue light, 450 nm. Light intensities were 250 μmol $m^{-2}s^{-1}$, temperature 21°C and illumination time 2 min.

tenna pigments arranged in the pigment–protein complexes to the photosynthetic reaction centers. It means that the photoisomerization of violaxanthin, observed at present in isolated LHCII, would take place in the photosynthetic apparatus subjected to light stress. Therefore, it is possible that light-induced isomerization of violaxanthin from the conformation all-*trans* to a conformation *cis* plays a role in a complex regulatory response of the photosynthetic apparatus to overexcitation. The extent of the photoisomerization of violaxanthin in LHCII is close to that observed in intact leaf [20], which suggests that this reaction takes place in the PS II antenna complexes in vivo. The fact that only a certain small fraction of the all-*trans* violaxanthin undergoes the photo-conversion [20] seems to support the concept concerning a regulatory function of this process. As may be seen from the electronic absorption spectra (Fig. 1), the photoisomerization does not change essentially the energy level of the B_u^1 state. A very similar situation may be expected with regards to the first singlet excited state A_g^1, responsible, most probably, for the energy exchange with chlorophyll *a* [25,26], but hardly measurable directly by electronic absorption and fluorescence techniques due to reasons of symmetry [27].

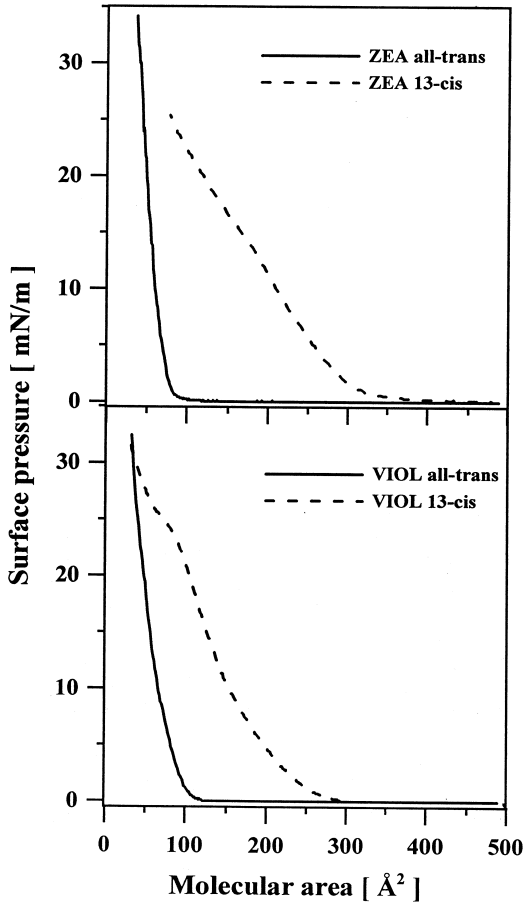

Fig. 2. Isotherms of compression of monomolecular layers of zeaxanthin and violaxanthin in the conformation all-*trans* and 13-*cis* (indicated).

The all-*trans* and *cis* stereoisomers of violaxanthin are very different in terms of their molecular shape (see inset to Fig. 1). This may suggest a structural effect of violaxanthin on LHCII, accompanying pigment photoisomerization. A possible effect of both *trans* and 13-*cis* isomers of violaxanthin on the organization of LHCII was studied applying the Langmuir–Blodgett technique for the preparation of supported monomolecular layers. Effects of all-*trans* and 13-*cis* zeaxanthin on the organization of LHCII were examined, in parallel, in order to find possible effects induced by the de-epoxidation of violaxanthin within the xanthophyll cycle.

Fig. 2 presents the isotherms of compression of the monomolecular layers formed with zeaxanthin and violaxanthin in the conformations all-*trans* and 13-*cis*. The specific molecular areas of the *cis* isomers are much larger than in the case of the all-*trans* isomers (by ca. 200 Å^2 for zeaxanthin and 120 Å^2 for violaxanthin at the surface pressure of 5 mN/m). This is an indication of the possibility of *cis* conformers of violaxanthin and zeaxanthin to be oriented horizontally at the argon–water interface, even under relatively high surface pressures (in contrast to most of the all-*trans* xanthophylls, see [28]). Fig. 3 presents the isotherms of compression of the monomolecular layers formed with LHCII and two-component monolayers formed with LHCII and violaxanthin and zeaxanthin in the conformations all-*trans* and 13-*cis* (one exogenous xanthophyll per one LHCII monomer). The specific molecular area of LHCII in the monocomponent monolayers is close to 1400 Å^2 [10,17] and therefore corresponds well to the elliptical cross-section of this protein determined on the basis of crystallography [12]. The presence of the all-*trans* violaxanthin and the all-*trans* zeaxanthin in the LHCII monolayers does not affect the organization of the complex, and the molecular areas of the two-component monolayers follow practically the additivity rule. In contrast, the presence of the 13-

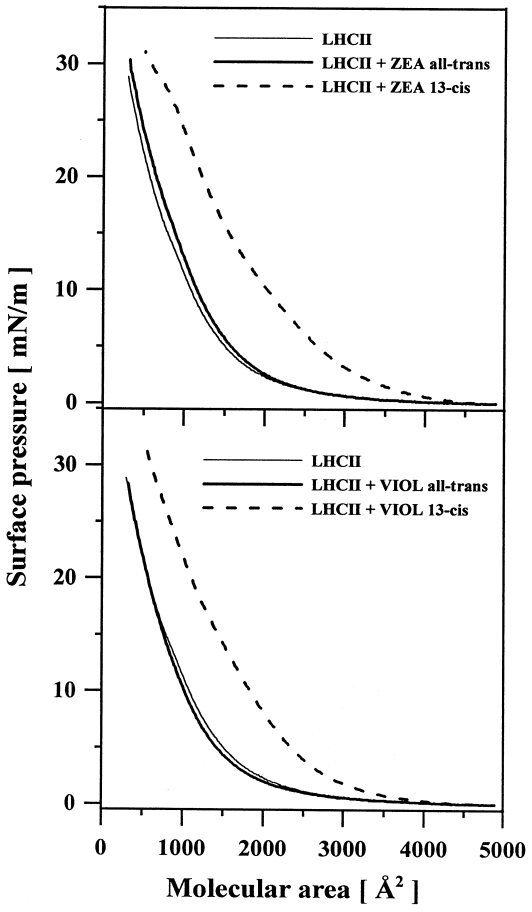

Fig. 3. Isotherms of compression of monomolecular layers of LHCII and two-component monolayers of LHCII and zeaxanthin or LHCII and violaxanthin in the conformation all-*trans* or 13-*cis* (indicated).

cis isomers of both xanthophylls in the monomolecular layers shifts the molecular areas by 1000–1200 Å^2 (at 5 mN/m, Fig. 3). Such a shift is much larger than may be expected, considering the differences in the molecular areas of the pure xanthophyll components (Fig. 2). This is an indication that the *cis* forms of exogenous xanthophylls affect the organization of LHCII in monolayers. The shift of the molecular areas towards greater values is indicative of some kind of disassembly of well-ordered LHCII layers.

An analysis of the isotherms of compression is not able to provide detailed information concerning the interaction of monolayer components. Spectroscopic techniques applied to a single LHCII monolayer can help to answer some specific questions related to the organization of the complex [10,17]. In particular, the low temperature chlorophyll *a* fluorescence emission spectroscopy provides information on the aggregation state of LHCII [29]. Fig. 4 presents the 77 K chlorophyll *a* fluorescence emission spectrum along with the Gaussian deconvolution components that correspond to the main spectroscopic features. The intensity of the band centered at 700 nm relative to the intensity of the band centered at 680 nm is a measure of the aggregation level of LHCII in a system [29]. Fig. 5 presents this ratio of the intensity levels in the 77 K fluorescence emission spectra (F_{700}/F_{680}) recorded from the LHCII-LB films containing exogenous violaxanthin or zeaxanthin, in the conformation all-*trans* or 13-*cis*. Clearly, the *cis* forms of both xanthophyll pigments have an opposite effect on LHCII to the effect of all-*trans* isomers, in terms of protein aggregation. The dissaggregation effect of 13-*cis* violaxanthin is in sharp contrast to the effect of the all-*trans* conformer of the same pig-

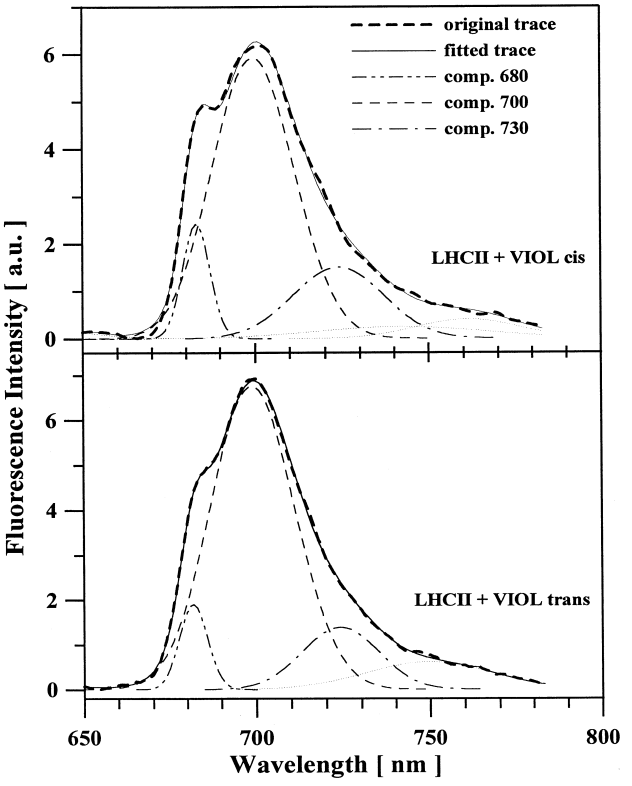

Fig. 4. 77 K chlorophyll *a* fluorescence emission spectra recorded from the Langmuir–Blodgett films deposited of monomolecular layers formed with LHCII and violaxanthin in the conformation 13-*cis* and all-*trans* (indicated). Excitation at 440 nm. The original spectra, the Gaussian components and the fitted spectra are indicated.

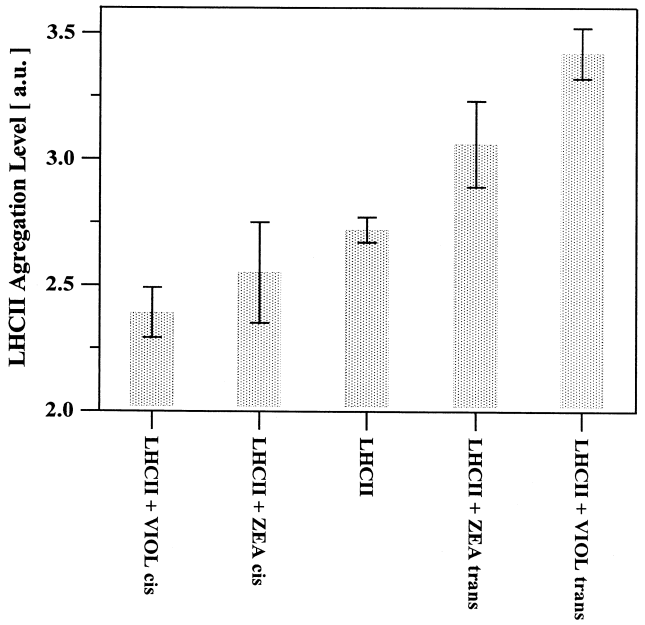

Fig. 5. LHCII aggregation level expressed as a ratio of the intensity of the Gaussian components centered at 700 and 680 nm (see Fig. 4) corresponding to the 77 K chlorophyll *a* fluorescence emission spectra recorded from the Langmuir–Blodgett films deposited of the monocomponent LHCII monolayers and the two-component monomolecular layers formed with LHCII and different xanthophyll pigments, indicated. The height of the bars correspond to the mean values of three experiments ± S.D.

ment. This behavior of violaxanthin along with the fact that part of the pool of this pigment in LHCII remains in a conformation *cis* seems to explain the dissaggregation effect of violaxanthin versus the pro-aggregation effect of zeaxanthin with respect to the antenna proteins [1].

It seems interesting to analyze how two potential roles of violaxanthin in LHCII referred to above (as an accessory pigment and protein structure modifying agent) may be affected by the pigment photoisomerization reported in this work. The efficiency of the excitation energy transfer from violaxanthin to chlorophyll *a* (determined on the basis of the 100-transmission spectra as compared to the fluorescence excitation spectra) was calculated as 0.56, following the procedure applied previously [10]. This is less than in the case of lutein (0.66), neoxanthin (0.85) and chlorophyll *b* (0.94).

Assuming that *cis* conformers of violaxanthin are oriented parallel with respect to the plane of the membrane [10] and based on the fraction of 15% violaxanthin remaining in LHCII in a conformation *cis* upon illumination (Table 1), one is able to calculate the fractional efficiency levels of excitation energy transfer to chlorophyll *a* from violaxanthin in these two differently oriented pools as a function of the mean orientation of molecules remaining in the conformation all-*trans* (see Appendix A). Such a dependency is presented in Fig. 6. As may be seen, the calculated efficiency of energy transfer from the pool of the pigment assumed to remain in the conformation all-*trans* is not very sensitive to the adopted orientation. This pool is responsible, to a large extent, for the excitation energy transfer to chlorophyll. In contrast, the calculated efficiency of excitation energy transfer from the violaxanthin molecules in a conformation *cis* depends very strongly on the actual orientation of the molecules in the conformation all-*trans*, in the samples in which the mean energy transfer efficiency was determined. Assuming that the orientation of all-*trans* violaxanthin is close to that of

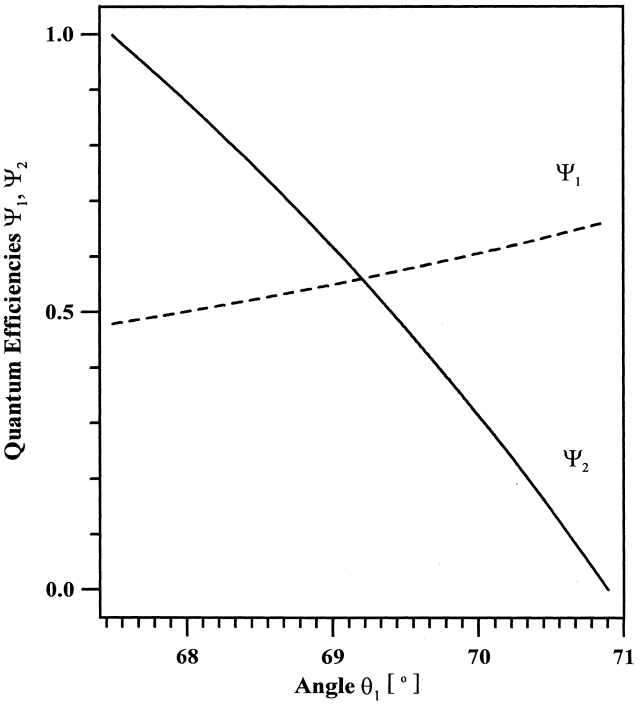

Fig. 6. Dependence of quantum efficiency of excitation energy transfer from violaxanthin in the conformation all-*trans* (ψ_1) and violaxanthin in the conformation *cis* (ψ_2) to chlorophyll *a* on the orientation angle of violaxanthin in the conformation all-*trans* with respect to the axis normal to the plane of the membrane. See Appendix A for details.

lutein (less than 68°), one obtains very high energy transfer efficiencies that are close to 1. In such a case, a special function for the *cis* violaxanthin as an 'excitation wire' might be expected, considering a small number of molecules in this particular fraction combined to a very strong energetic coupling to the chlorophyll molecules, possibly in the different monomers of LHCII in the same trimeric structure [10]. The dependence presented in Fig. 6 is very sharp and therefore it is also possible that the *cis* violaxanthin does not efficiently transfer excitation energy to chlorophyll and plays in LHCII mostly a structural role.

A potential of violaxanthin to function as an LHCII structure modifying agent follows directly from the results of the experiments presented in this report. In particular, the light-dependent isomerization of the all-*trans* form to the 13-*cis* and 9-*cis* conformers provides an efficient molecular mechanism, sensitive to the presence of chlorophyll triplet excitations, able to influence the LHCII aggregation level. The fact that chlorophyll triplet excitations appear in the thylakoid membranes only under over-excitation conditions, very likely in the assemblies of antenna complexes separated from the photosynthetic reaction centers in consequence of the protein phosphorylation, makes the violaxanthin isomerization a probable molecular mechanism of a regulatory response of the photosynthetic apparatus to strong illumination. Under such conditions, accumulation of *cis* forms of violaxanthin will induce disassembly of large LHCII aggregates [30]. A probable, physiologically important, consequence of such a disassembly allows LHCII-bound, all-*trans* violaxanthin to diffuse relatively free within the thylakoid membrane and therefore to be available for the enzymatic de-epoxidation (see the model in Fig. 7). All-*trans* zeaxanthin accumulated in the thylakoid membranes facilitates formation of large LHCII aggregates, characterized by the high rate of protectional quenching of chlorophyll *a* singlet excitations [1]. The availability of violaxanthin for de-epoxidation ranges from 60% [31] to almost 100% [32] and depends on plant species and experimental conditions. The all-*trans* conformation of violaxanthin was shown to be a sole specific substrate for the enzymatic de-epoxidation [33,34], therefore the role of the small fraction of *cis* forms, not subjected to de-epoxidation, would be limited to the regulation of the pigment availability to interconversion within the xanthophyll cycle. The fact that under some conditions availability of violaxanthin to de-epoxidation ranges up to 100% [32] indicates that a back reaction from the *cis* to *trans* conformation takes place in the system at a certain

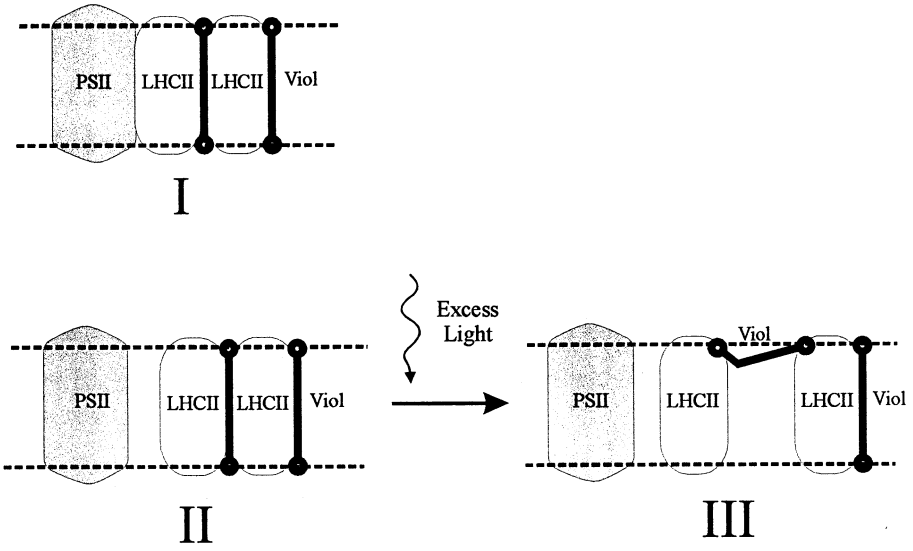

Fig. 7. Schematic representation of the organization of LHCII complexes in the thylakoid membrane as influenced by the photoisomerization of violaxanthin. The transition from state I to state II corresponds to the energetic uncoupling of the LHCII complexes from the reaction centers of PS II and the transition from state II to state III corresponds to dissaggregation of the oligomeric structures of LHCII induced by formation of *cis* forms of violaxanthin.

rate and that *trans* conformers are gradually converted into zeaxanthin. The rate constant of the isomerization of 13-*cis* to all-*trans* conformer is higher by a factor of three in the case of β-carotene [36]. This suggests, that also in the case of violaxanthin, the *cis*→*trans* isomerization operates efficiently as a back reaction with respect to the photo-isomerization observed (*trans*→*cis*) and explains the total de-epoxidation of violaxanthin. The fact that the strength of binding violaxanthin to the protein depends on the aggregation state of LHCII [9] supports our concept of a disaggregation-related diffusional freedom of the pigment within the thylakoid membrane. A structural organization of LHCII regulated by violaxanthin isomerization, as proposed in the present report, seems also to be consistent with our previous report concerning the light-induced and thermally induced conformational transition of this antenna protein [35]. The transition, which was pronounced in the increase of the specific molecular area of LHCII in the monomolecular layer, can be linked to the *cis* isomerization of violaxanthin in the system. Indeed, the incubation of the LHCII preparation in darkness for 10 min at 27°C (the temperature shown to affect the organization of LHCII [35]) has an effect in the conversion of the all-*trans* violaxanthin to the final level of 11% of the pigment in a conformation *cis* (2% of the 13-*cis* violaxanthin and 9% of the 9-*cis* violaxanthin). The steric energies of the same isomeric forms of β-carotene are below the level of kT corresponding to room temperatures [36], which may explain relatively efficient conversion to these particular isomeric forms, also in the case of violaxanthin.

Acknowledgements

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Appendix A. Formula derivations for the efficiency of excitation transfer from violaxanthin to chlorophyll

Let us assume that the plane of the layer coincides with the plane x, y of our coordinate system. The axis z is perpendicular to the plane of the layer. A vector under consideration forms the angle θ with the z-axis, and its projection on the plane x, y forms the angle φ with the x-axis. In such coordinates, the components of any unit vector ($\overline{\mu}$) parallel to the absorbing moment can be expressed as:

$$\overrightarrow{\mu} = (\sin\theta\cos\varphi,\ \sin\theta\sin\varphi,\ \cos\theta) \tag{1}$$

The unit vector parallel to the electric field of the light incident on the layer at the angle 45° can be represented as:

$$\overrightarrow{E} = \left(\frac{\cos\delta}{\sqrt{2}},\ \sin\delta,\ \frac{\cos\delta}{\sqrt{2}}\right) \tag{2}$$

where δ is the angle between the vector $\overline{E}$ and the plane of incidence (x, z). The absorption probability is proportional to the squared cosine of the angle between vectors $\overline{\mu}$ and $\overline{E}$. In other words, it is proportional to the squared scalar product of these two vectors. After averaging in respect to the angle φ, we obtain:

$$\langle(\overrightarrow{\mu}\overrightarrow{E})^2\rangle = \frac{\cos^2\delta\sin^2\theta}{4} + \frac{\sin^2\delta\sin^2\theta}{2} + \frac{\cos^2\delta\cos^2\theta}{2} \tag{3}$$

Eq. 3 gives the relative probability of the absorption of nonpolarized light after averaging over the angle δ:

$$a_{\mathrm{n}} = \frac{3}{8}\sin^2\theta + \frac{1}{4}\cos^2\theta = \frac{3-\cos^2\theta}{8} \tag{4}$$

In order to obtain respective probabilities for the light polarized in the plane of incidence or perpendicularly to this plane, one should insert into Eq. 3 $\delta=0$ or $\delta=90°$:

$$a_{\parallel} = \frac{\sin^2\theta}{4} + \frac{\cos^2\theta}{2} = \frac{1+\cos^2\theta}{4} \tag{5}$$

$$a_{\perp} = \frac{\sin^2\theta}{2} = \frac{1-\cos^2\theta}{2} \tag{6}$$

Let us now assume that the absorbing moments consist of two pools. The first of these pools (violaxanthin all-*trans* molecules) forms with the z-axis an angle θ_1 and the second one (the pool of violaxanthin *cis* molecules) forms with the z-axis the angle $\theta_2 = 90°$. Having inserted θ_1 and θ_2 into Eqs. 5 and

6 one can express the dichroic ratio of the fluorescence excitation as:

$$r = \frac{N_1\psi_1 a_{\parallel 1} + N_2\psi_2 a_{\parallel 2}}{N_1\psi_1 a_{\perp 1} + N_2\psi_2 a_{\perp 2}} = \frac{\frac{N_1}{N_2}\psi_1(1+\cos^2\theta_1) + \psi_2}{2\left[\frac{N_1}{N_2}\psi_1(1-\cos^2\theta_1) + \psi_2\right]} \quad (7)$$

where N_1, N_2, ψ_1 and ψ_2 are the numbers of molecules and efficiencies of excitation transfer from violaxanthin to fluorescing chlorophyll molecules. Inferior indices 1 and 2 refer to *trans* and *cis* pools, respectively. Taking into account experimental values of $r = 0.62$ and $N_1/N_2 = 17/3$, we obtain Eq. 8 from Eq. 7:

$$\frac{17\psi_1(1+\cos^2\theta_1) + 3\psi_2}{17\psi_1(1-\cos^2\theta_1) + 3\psi_2} = 1.24 \quad (8)$$

The overall efficiency of the excitation transfer from violaxanthin to chlorophyll (or the ratio of the fluorescence quantum yields for the light absorbed by violaxanthin and chlorophyll) in nonpolarized light can be expressed as:

$$\psi = \frac{N_1\psi_1 a_{n1} + N_2\psi_2 a_{n2}}{N_1 a_{n1} + N_2 a_{n2}} = \frac{\frac{N_1}{N_2}\psi_1(3-\cos^2\theta_1) + 3\psi_2}{\frac{N_1}{N_2}(3-\cos^2\theta_1) + 3} \quad (9)$$

Using the experimental value of N_1/N_2, we arrive at:

$$\frac{17\psi_1(3-\cos^2\theta_1) + 9\psi_2}{60-17\cos^2\theta_1} = \psi \quad (10)$$

It follows from Eqs. 8 and 10 that:

$$\psi_1 = \frac{60-17\cos^2\theta_1}{459\cos^2\theta_1}\psi \quad (11)$$

and

$$\psi_2 = \frac{(476\cos^2\theta_1 - 51)(60-17\cos^2\theta_1)}{4131\cos^2\theta_1}\psi \quad (12)$$

Due to the physical meaning of the variables ψ_1, ψ_2 and ψ, their values must belong to the interval (0, 1). In Fig. A1 the curves $\psi_2 = 0$ (portion AB), $\psi_1 = 1$ (BC) and $\psi_2 = 1$ (CDE) are traced. Only the values of the angle θ_1 and those of the overall efficiency ψ falling into the area bounded by the curve ABCDE and the coordinate axes can result in meaningful values of ψ_1 and ψ_2. Thus, the orientation of the pool of all-*trans* isomers can be determined at sufficiently high quantum efficiency ψ.

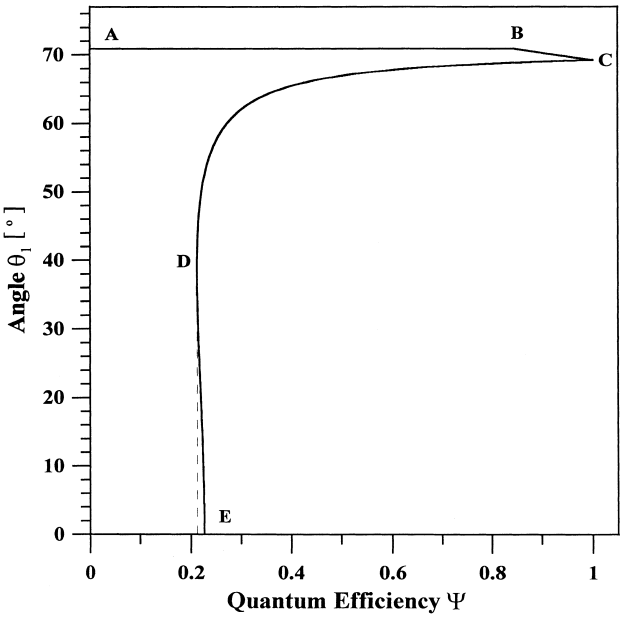

Fig. A1. The relationship between the mean angle formed by violaxanthin molecules in the conformation all-*trans* (θ_1) and overall quantum efficiency of energy transfer from violaxanthin to chlorophyll *a* (ψ).

In the case of our experimental value of $\psi = 0.56$ the allowed values of θ_1 belong to a quite narrow interval (67.48°, 70.89°). Fig. 6 presents possible values of ψ_1 and ψ_2, as predicted by Eqs. 11 and 12, at θ_1 changing from 67.48 to 70.89°. The efficiency of excitation transfer from violaxanthin all-*trans* isomers to chlorophyll changes from 0.48 to 0.66 in this interval of θ_1. However, nothing can be said about the efficiency of the excitation transfer from the pool of violaxanthin *cis* isomers to chlorophyll, because the value of ψ_2 changes in the allowed interval of the angle θ_1 from 1 to 0.

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