ELSEVIER

Biochimica et Biophysica Acta 1188 (1994) 235–242

BBA Biochimica et Biophysica Acta

Involvement of xanthophyll pigments in regulation of light-driven excitation quenching in light-harvesting complex of Photosystem II

Wiesław I. Gruszecki [a,*], Peter Kernen [b], Zbigniew Krupa [c], Reto J. Strasser [b]

[a] *Institute of Physics, Maria Curie-Skłodowska University, 20-031 Lublin, Poland*
[b] *Bioenergetics Laboratory, University of Geneva, Jussy / Geneva, Switzerland*
[c] *Department of Plant Physiology, Maria Curie-Skłodowska University, 20-031 Lublin, Poland*

Received 30 June 1994

Abstract

Light-driven chlorophyll *a* fluorescence quenching and dark reversibility of this process was measured in LHC II – the major light-harvesting pigment–protein complex of Photosystem II. Two preparations of LHC II, complex isolated from dark-adapted (LHC II-D) and light-pretreated (LHC II-L) rye leaves, differed significantly in the level of the xanthophyll pigment violaxanthin, being enriched in LHC II-D. LHC II-L contained only trace amounts of zeaxanthin but additionally antheraxanthin. LHC II-D suspended in buffer showed similar fluorescence quenching kinetics. Chlorophyll fluorescence quenching in LHC II-L was almost fully reversible after 2 h of dark-adaptation whereas only 37% of the initial fluorescence level was recovered under identical conditions. Transfer of LHC II as protein–lipid complex (PLC) in a reverse micellar solution composed of isooctane, phospholipids and limited amount of water, considerably decreased xanthophyll pigment concentrations. Under these conditions, light-driven fluorescence chlorophyll a quenching was the same for LHC II-L and LHC II-D and fluorescence level in both preparations was fully reversible within the dark period. Kinetics of light-induced fluorescence quenching and reversibility of LHC II-L reconstituted in asolectin liposomes was faster than in buffer. However, reversibility of LHC II-L in liposomes was markedly slowed down by addition of exogenous xanthophyll pigments in the following sequence: violaxanthin > zeaxanthin > nonpigmented liposomes. Remarkable light-dependent changes in chlorophyll *a* fluorescence excitation spectra of LHC II-L in liposomes were observed. These changes are ascribed to energetic uncoupling caused by physical detachment of violaxanthin from the antenna complex – this process being discussed in terms of violaxanthin availability to de-epoxidation in thylakoid membrane. The possible involvement of the xanthophyll cycle pigments violaxanthin and zeaxanthin in the regulation of light-driven excitation quenching in LHC II is also discussed.

Keywords: Xanthophyll cycle; Excitation quenching; Zeaxanthin; Violaxanthin; Fluorescence quenching; LHC II; Photosynthesis

1. Introduction

Illumination of plants with light intensities high enough to saturate photosynthesis or higher lead to an increased level of the xanthophyll pigment zeaxanthin [1–4]. Accumulation of zeaxanthin is a result of the light-triggered enzymatic de-epoxidation of the other xanthophyll pigment violaxanthin (diepoxyzeaxanthin) via antheraxanthin (monoepoxyzeaxanthin). The back reaction, epoxidation of zeaxanthin, is a light-independent process. This dark activity restores the violaxanthin pool in parallel to the de-epoxidation process upon illumination [1–4]. These reactions are known as violaxanthin or xanthophyll cycle. The exact physiological role of the xanthophyll cycle is still not clear but it is generally accepted that it is involved in the protectional response of the photosynthetic apparatus under overexcitation conditions [4–6]. The main mechanism protecting the photosynthetic complexes against strong light-induced destruction seems to be a non-radiative excitation energy dissipation [4–7]

This mechanism may be followed by analysis of a decrease in chlorophyll *a* fluorescence emission [7]. There is increasing evidence that part of the protection

* Corresponding author. E-mail: BIOPHYS@PLUMCS11.bitnet. Fax: +48 81 376191.

0005-2728/94/$07.00 © 1994 Elsevier Science B.V. All rights reserved
SSDI 0005-2728(94)00129-4

mechanism by in vivo chlorophyll *a* fluorescence quenching originates from the largest pigment–protein antenna complex of Photosystem II – LHC II [8–12]. Isolated LHC II seems to be a reasonable model of an antenna complex in the photosynthetic apparatus under overexcitation conditions. In such a model any light, independently of its intensity, should be recognized by the system as excess energy since there is no exciton transfer from the antenna to the reaction center.

Jennings et al. [10] reported that chlorophyll fluorescence quenching in isolated LHC II linearly depends on light intensity. Since excitation quenching and xanthophyll cycle operate essentially under identical conditions, the question may arise whether there exists any causative relationship between these two processes. On the one hand it was reported that zeaxanthin synthesized in the xanthophyll cycle is a direct quencher of chlorophyll-excited states [4] and on the other hand chlorophyll fluorescence quenching induced by overexcitation of the photosynthetic apparatus seems to occur without the presence of zeaxanthin [13–16]. A model was presented where the xanthophyll cycle has no direct effect on the extent of quenching but is active as quenching amplifier [5]. It was recently observed [17] that plants lacking a major antenna complex LHC II, demonstrate an even higher extent of non-photochemical fluorescence quenching. This finding was interpreted as an indication that excitation quenching in LHC II alone may not be attributed with certainty to the entire quenching effect in photosynthetic apparatus. On the other hand excitation quenching in all antenna complexes including LHC II seems to be very important under overexcitation conditions when a ratio of exciton trapping by reaction centers is a limiting factor, so chlorophyll triplet formation may result in photosensitization and photooxidation of antenna pigments and other biomolecules.

In this work we have studied effects of the xanthophyll cycle pigments on the light-driven chlorophyll fluorescence quenching in LHC II in different model systems. Results obtained by measurements of chlorophyll *a* fluorescence changes, monitoring the process of singlet excitation quenching, may show the involvement of the xanthophyll cycle in regulation of the light-driven chlorophyll excitation quenching in LHC II.

2. Materials and methods

Light-harvesting complex of Photosystem II (LHC II) was isolated from winter rye according to the procedure described previously [18,19]. Preparations contain mainly the major LHC II complex (*b*) with minor amounts of other antenna proteins of Photosystem II. LHC II was prepared from leaves illuminated for 30 min with 500 μmol $m^{-2} s^{-1}$ (white, photosynthesis supersaturating light of the halogen lamp) before isolation (LHC II-'light', LHC II-L) or was isolated from leaves dark-adapted for 30 min (LHC II-'dark', LHC II-D). Both preparations were stored in 300 mM sorbitol and 50% glycerol at 77 K. Samples were washed in 25 mM tricine-KOH (pH 8.0) before use and diluted in the same buffer if necessary to adjust chlorophyll concentrations. LHC II in a buffer medium remained in an oligomeric form as judged by spectroscopy, gel analysis and centrifugation experiments.

LHC II was transferred into a low water system following Ayala et al. [20]. Usually 1 mg of protein was added to 1 ml of isooctane containing 10 mg partially purified asolectin. After sonication and addition of 0.1 M $MgCl_2$ an organic fraction containing LHC II protein as protein–lipid complexes (PLC) was collected. This fraction was further fractioned by a $15000 \times g$ centrifugation in a light fraction (supernatant containing mostly free pigments) and a pellet. This heavy fraction (PLC-heavy) was either dissolved in isooctane alone or containing 10 mg asolectin.

Asolectin liposomes were prepared by sonication of 1 ml of buffer in a tube containing a thin film of 10 mg asolectin. LHC II was incorporated to asolectin liposomes containing 0.6 mol% or no exogenous violaxanthin or zeaxanthin by short sonication of equal volumes of LHC II in buffer and previously prepared liposome suspension. Liposomes were used after removal of non-incorporated LHC II by centrifugation at $15000 \times g$. The ratio of total chlorophyll to exogenous carotenoids was about 6 in LHC II-liposomes.

Absorption spectra were taken on a Perkin-Elmer Lambda 3 UV/VIS spectrophotometer and corrected fluorescence excitation spectra were recorded with a Shimadzu RF5000 spectrofluorometer. Fluorescence quenching was measured with a PEA fluorometer (Plant Efficiency Analyzer, Hansatech). Samples of 200 μl were illuminated for 60 s with red light (light-emitting diodes at 650 nm with 220 Wm^{-2}) and chlorophyll fluorescence was recorded above 700 nm.

Xanthophyll pigments from LHC II were extracted in ethanol and analyzed by thin layer chromatography (TLC) on silica gel 60 (Merck) with the solvent system composed of benzene/ethyl acetate/methanol (75:20:5, v/v) [21]. Separated pigments were extracted from the gel with ethanol and pigment concentrations were calculated according to published extinction coefficients [21]. Violaxanthin and zeaxanthin to be incorporated to asolectin liposomes were isolated from pea leaves and purified by TLC as described above.

All experiments were performed at room temperature and repeated at least three times. Fluorescence quantum yield was checked to be stable at darkness at least for 3 h after sample preparation.

3. Results

The present study describes the behavior of light-induced chlorophyll *a* fluorescence quenching of the major light-collecting antenna of Photosystem II, the light-harvesting complex II (LHC II). Fluorescence quenching was either measured in LHC II in buffer (Fig. 1A) in aggregated form, most probably in monomeric form as protein–lipid complex (PLC) in an organic solvent containing phospholipid under minimal (less than 1.5%, v/v) availability of water (Fig. 1B) and as complex incorporated in a liposomal bilayer (Fig. 1C). Both LHC II preparations, LHC II-D from dark-adapted and LHC II-L from preilluminated leaves, showed a similar concentration ratio of chlorophyll *a* to chlorophyll *b* of 1.09 ± 0.01 (arithmetic mean of four different LHC II-L and LHC II-D preparations ± standard error). LHC II-L and LHC II-D differed in composition of xanthophyll pigments. Molar fractions of these pigments were following in LHC II-L: lutein 61.0%, neoxanthin 23.8%, violaxanthin 5.8%, antheraxanthin 9.4% and in LHC II-D: lutein 67%, neoxanthin 22.8%, violaxanthin 9.9% (mean value of four determinations from different preparations). LHC II-L contained antheraxanthin (this pigment was lacking in LHC II-D) and showed a decreased level of violaxanthin. Whereas the molar ratio of violaxanthin to neoxanthin equaled 0.43 for LHC II-D, only a ratio of 0.24 was found in LHC II-L. Total carotenoid level was identical in both preparations with a molar ratio of carotenoid to chlorophyll of 0.18. The light-dependent interconversion of violaxanthin to antheraxanthin due to the operation of the xanthophyll cycle in intact leaves before LHC II isolation is most probably responsible for differences in xanthophyll pigment composition of the 'light' and 'dark' preparation. Interestingly only trace amounts of zeaxanthin were detected in all examined LHC II-L samples. Apparent lower ratio of lutein in the xanthophyll pool of LHC II-L may be explained as an effect of the additional presence of antheraxanthin in this preparation. Total ratio of violaxanthin and antheraxanthin in LHC II-L is higher than the ratio of violaxanthin in LHC II-D. As a result of this, lutein, being in the same concentration per chlorophyll in both preparations, has a higher molar ratio in total xanthophylls of LHC II-L.

The most pronounced differences of LHC II-L and LHC II-D in buffer were found in the dark reversibility of light-dependent chlorophyll *a* fluorescence quenching (compare Fig. 2A and B). Whereas the fluorescence quenching in LHC II-L induced by 60 s of illumination by red light (220 W m^{-2}) was fully reversible after 2 h of dark (about 91% reversible after 1 h, as shown in Fig. 2A) only 50% reversibility (34% after 1 h, see Fig. 2B) was found in LHC II-D under identical conditions. The slow reversibility of fluorescence quenching in LHC II-D in water medium was markedly accelerated after transfer of the antenna into asolectin-isooctane-water system as PLC (see Section 2). Under these conditions chlorophyll *a* fluorescence quenching in LHC II-D was fully reversible after 2 h of dark (88% reversibility after 1 h, see Fig. 2C). Absorption and fluorescence spectra of LHC II-D transferred to PLC were very similar to the spectra found in buffer (no changes in spectral red region, data not shown) suggesting that native structures of pigment–protein complexes were preserved. But the transfer process washed out some xanthophyll pigments from the complex leading to a 8-fold decrease in the xanthophyll to total chlorophyll molar ratio (from 0.19 before transfer to 0.023 in PLC). Results obtained with LHC II-L and LHC II-D in buffer and LHC II-D as PLC may suggest that the xanthophyll pigment composition, and in particular violaxanthin is responsible for the pronounced differences in chlorophyll *a* fluorescence quenching reversibility. In order to test such a hypothesis, the quenching and reversibility process was measured after LHC II incorporation to asolectin liposomes (see Fig. 3A) and LHC II-containing liposomes pigmented with violaxanthin (Fig. 3B) or zeaxanthin (Fig. 3C). Orientation of the LHC II apoprotein and the xanthophyll pigments in the liposomal lipid bilayer (see discussion in Ref. [22]) may allow a similar interaction of the complex as in the native thylakoid membrane.

Surprisingly, the reversibility of fluorescence quenching in membrane-bound LHC II-L was 'more' than 100% after 1 h of dark adaptation (Fig. 3A). Fluorescence quenching was also much faster in a lipid phase than in other systems. Compared to LHC II incorporated in pure asolectin liposomes, additionally added exogenous xanthophyll pigments slowed down the reversibility of fluorescence quenching. A much more evident effect was found for liposomes pig-

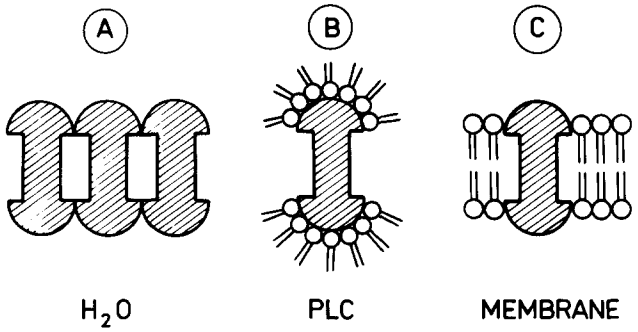

Fig. 1. Schematic presentation of light-harvesting complex of Photosystem II (LHC II) in different model systems studied. (A) As an aggregate in water medium; (B) as protein–lipid complex (PLC) in isooctane; (C) incorporated into lipid bilayer in liposomes. Small circles with two tails represent lipid molecules. The models are based on polarity analysis of molecules considered and on literature data referred to in the text.

mented with violaxanthin (about 57% reversibility in 1 h of dark, see Fig. 3B) than with zeaxanthin (87% reversibility in 1 h of dark, Fig. 3C). Besides changing the dark reversibility in LHC II-containing liposomes, exogenous xanthophyll pigments influenced also the kinetics of the light-induced fluorescence quenching process (Fig. 4). Analyzing the kinetics in the first few seconds may suggest that the faster fluorescence quenching the slower dark reversibility. Pigment-induced differences were nevertheless more pronounced

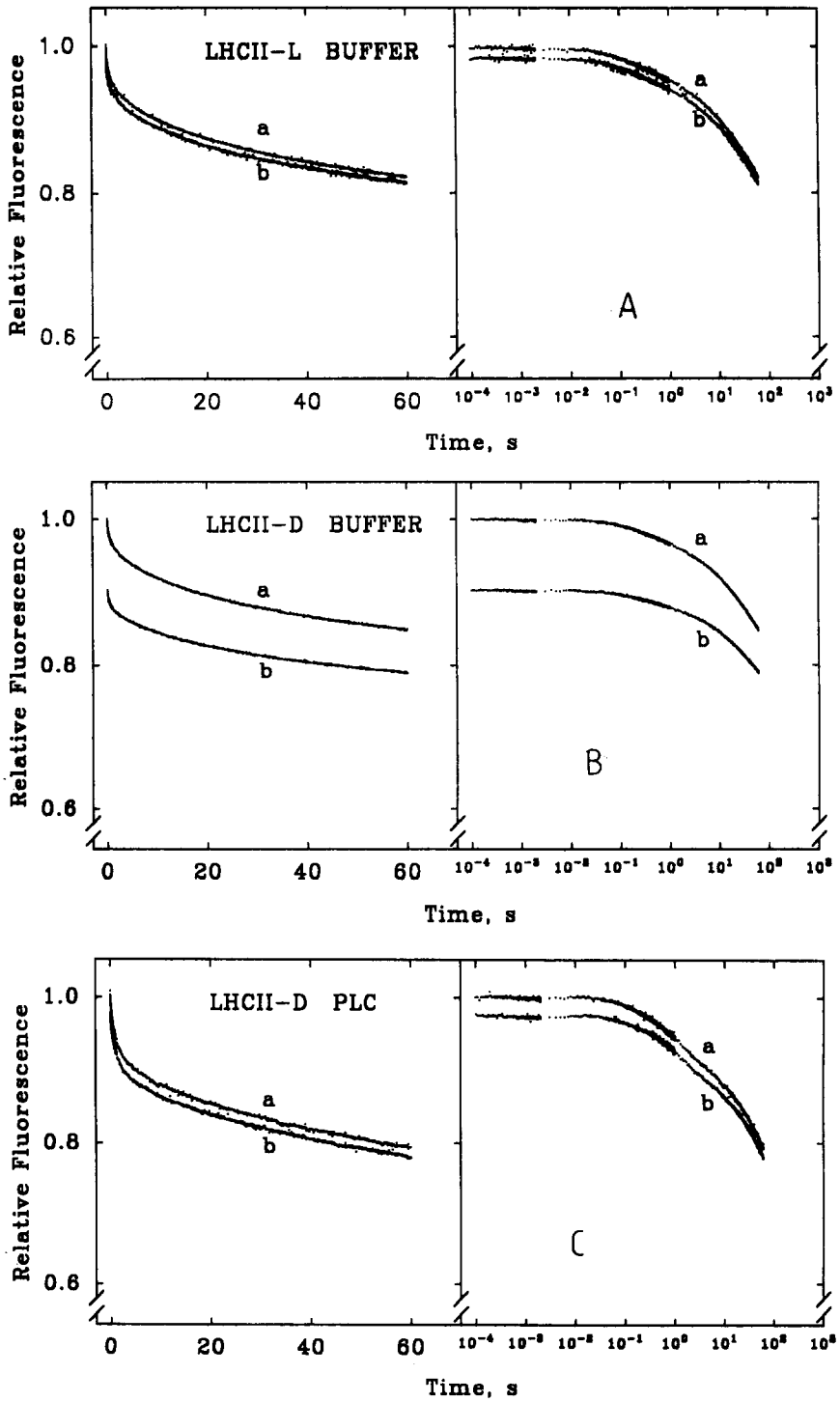

Fig. 2. Time-dependent chlorophyll *a* fluorescence emission induced by red light illumination of 220 Wm^{-2} (a) and second illumination of the same sample after 1 h in dark (b). Fluorescence was normalized to maximal emission during first illumination and presented in linear and logarithmic time scale. Logarithmic time scale enabled accurate determination of dark reversibility. (A) LHC II-L in buffer; (B) LHC II-D in buffer; (C) LHC II-D in protein–lipid-complex (PLC).

in dark reversibility than in the fluorescence quenching process. However, it should be noted that a faster fluorescence quenching of LHC II in liposomes (Fig. 3A) than in buffer (Fig. 2A) was followed by a much faster dark reversibility in the model membrane. Remembering that xanthophyll composition may be correlated with inhibition of reversibility of light-induced chlorophyll fluorescence quenching in LHC II (see results above), such an exception may be explained assuming that the xanthophyll pigment concentration in LHC II decreases during illumination in case the complex is located in a lipid membrane.

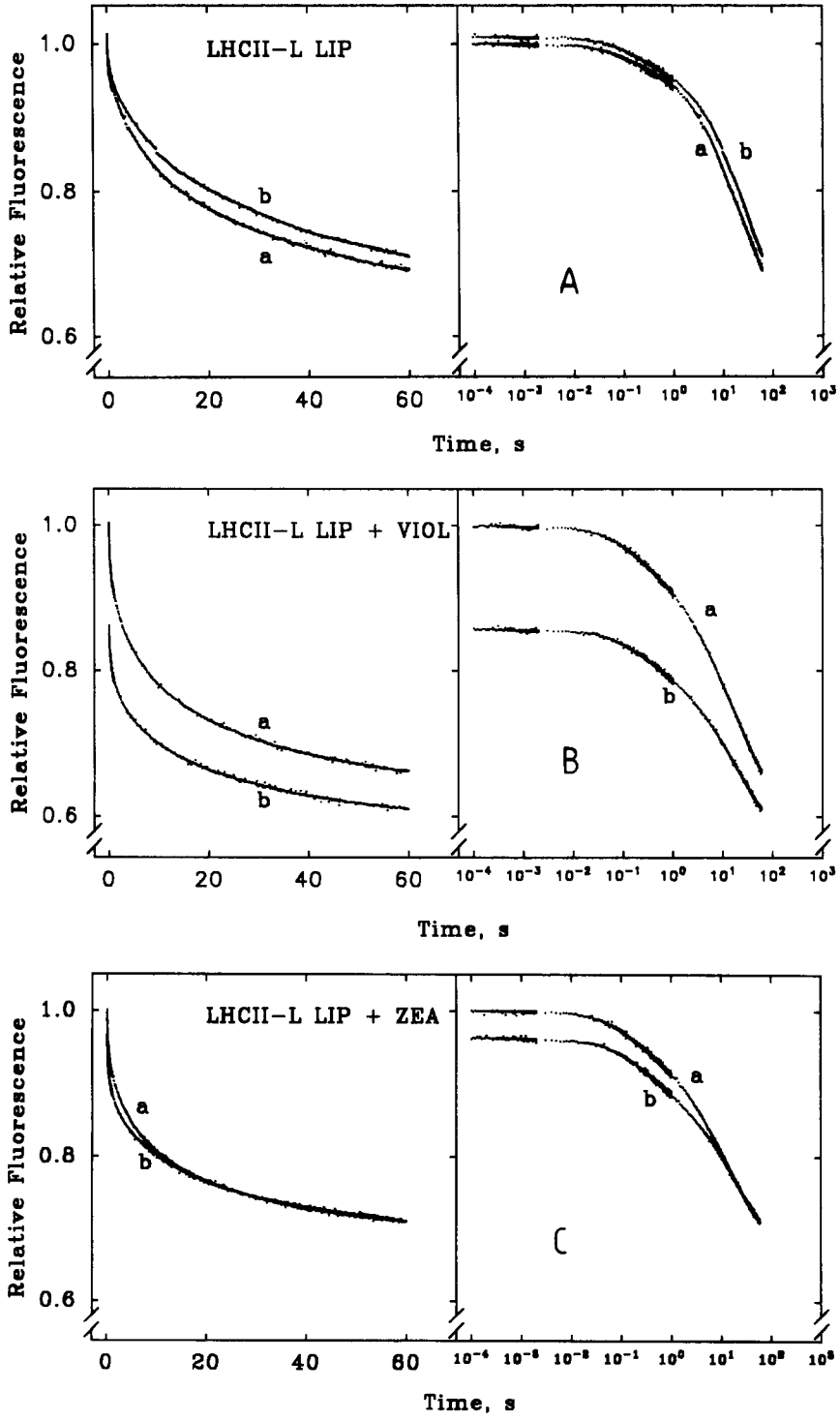

Fig. 3. Time-dependent chlorophyll *a* fluorescence of LHC II-L incorporated to asolectin liposomes. For details see Fig. 2 and Section 2. (A) Nonpigmented liposomes (please note that curve b is located above curve a in this case, in contrast to other figures); (B) liposomes contained 0.6 mol% violaxanthin; (C) liposomes contained 0.6 mol% zeaxanthin.

In order to test this hypothesis, fluorescence excitation spectra were recorded before and after illumination (Fig. 5). Relatively low light intensity was applied in order not to activate large-scale light-dependent chlorophyll fluorescence quenching linearly dependent on light intensity [10] which could overlap an expected xanthophyll effect. Probing light of the spectrofluorometer applied to record excitation spectra in the fast mode of the apparatus proved to have no effect on the shape of spectra (data not shown). As seen in Fig. 5, the difference chlorophyll excitation spectra (obtained in several experiments) resembled a typical xanthophyll absorption spectrum (with the 0–1 vibrational transition, the central maximum, at 450 nm). Additional maxima in the spectral region of 400 nm and a shoulder at 500 nm indicated that xanthophyll pigments, which lost their energy transfer activity during illumination, were partially in aggregated form (small aggregates) [23–25]. On the other hand a small maximum at 550 nm indicated for a presence of carotenoids in microcrystalline phase [26]. The excitation bands between 330 and 370 nm may suggest that carotenoid pigments adopted some cis conformation [27,28], at least just before energetic uncoupling from chlorophyll. The effect of light-induced energetic uncoupling of xanthophyll pigments from chlorophylls in LHC II was only found in complex incorporated to lipid membranes (no effect was observed in buffer and in PLC).

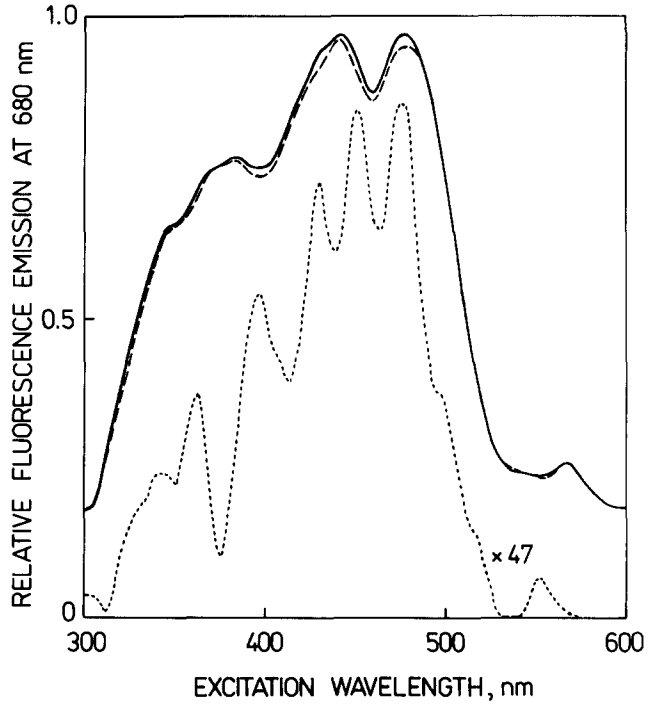

Fig. 5. Excitation spectra of chlorophyll *a* emission at 680 nm for LHC II-light in liposomes before (solid line) and after (dashed line) illumination for 60 s with light of 450 nm (10 Wm^{-2}). The difference spectrum (before illumination minus after illumination) was plotted as dotted line (47-fold amplification). Excitation and emission slits were set to 3 nm and 5 nm, respectively.

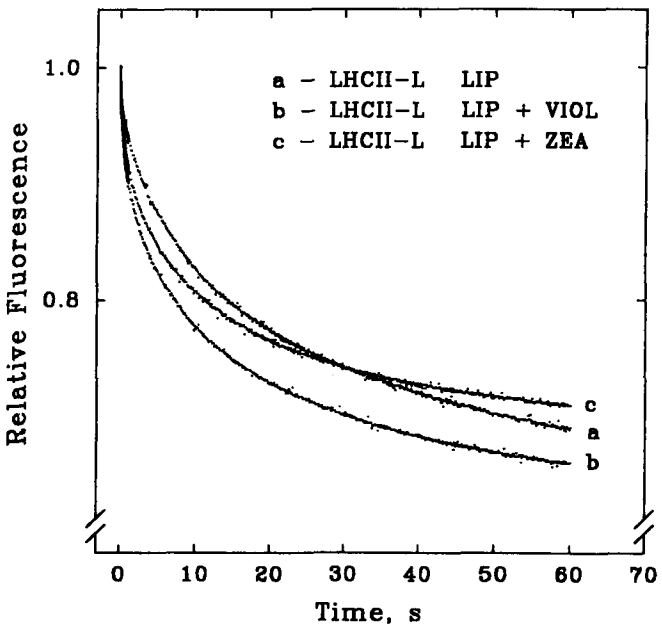

Fig. 4. Comparison of kinetics of chlorophyll *a* quenching during first illumination of LHC II-L incorporated into asolectin liposomes (LIP), (a) containing no exogenous pigments, (b) with 0.6 mol% exogenous violaxanthin (VIOL) and (c) with 0.6 mol% exogenous zeaxanthin (ZEA). Curves were extracted from Fig. 3A, B and C, respectively.

The effect was fully reversible after 5 min of darkness (data not shown).

4. Discussion

In this report we present results indicating the presence of xanthophyll pigments in LHC II and in particular that violaxanthin inhibits reversibility of light-driven chlorophyll *a* fluorescence quenching. All conclusions are based on LHC II behavior in model systems: LHC II-L and LHC II-D in buffer, LHC II-D as PLC and LHC II-L incorporated in violaxanthin- or zeaxanthin-pigmented liposomes.

Usually an inhibition of reversibility is combined with acceleration of fluorescence quenching. This effect may be understood, assuming that reversibility as a light-independent process is not only active in darkness but also during light-driven fluorescence quenching. The net effect in chlorophyll fluorescence decrease in illuminated LHC II is higher under conditions when the slow but constant back process is inhibited. An exceptional behavior was found instead for LHC II incorporated in lipid bilayer. In this system the other light-dependent mechanism was found to be active,

resulting in the energetic uncoupling of a certain xanthophyll pool from chlorophylls. It is highly probable that energetic uncoupling of xanthophylls is a result of physical desorption of pigment molecules from LHC II and their lateral diffusion within the membrane. Such a mechanism may not be active in a water environment because carotenoids are largely unpolar molecules and they would be preferentially adsorbed to lipophilic segments of LHC II (see Fig. 1). The effect was also not observed in PLC (data not shown), probably because xanthophyll pigments were mostly washed out (especially those not tightly bound which may detach in a lipid bilayer).

On the basis of the present data, the exact molecular mechanism of xanthophyll pigment-mediated inhibition of fluorescence quenching reversibility in LHC II may be speculative. Nevertheless the hydrophobic interaction of a rigid rod-like xanthophyll molecule with unpolar protein residues will stabilize a certain LHC II conformation (the complex would be less susceptible to conformational changes). Indeed several reports indicate that xanthophyll pigments are important structure-stabilizing factors in LHC II [29–31]. It was also shown that light-dependent conformational changes in LHC II proceed in the same time scale as light-driven chlorophyll fluorescence quenching [32] and that large-scale conformational changes in LHC II may be probably not responsible for quenching processes [10]. If fluorescence quenching is related to small conformational changes (partial unfolding?) of the pigment–protein complex [32], interaction with xanthophylls will preserve an unfolded structure and inhibit protein structure recovery. Such a mechanism would affect refolding process in a higher degree than the light-induced unfolding of LHC II since xanthophylls are detached (less tightly bound) in the illuminated complex (see Fig. 5). Possibly, protein unfolding is a process triggered or facilitated by xanthophylls detaching from LHC II. A preceding step would eventually be their photoisomerisation [27,28] as it could be concluded from the difference spectrum in Fig. 5 (cis-band). The light stress [33,16] or physiological light-induced energetic uncoupling of xanthophyll pigments [34] was reported to occur in the photosynthetic apparatus of higher plants. This process would increase the violaxanthin availability to de-epoxidation [22,23,34,35].

Several indications from our report may support that violaxanthin is the xanthophyll pigment being detached from LHC II and regulating light-induced fluorescence quenching:

(1) Violaxanthin concentration was the main difference between LHC II-L and LHC II-D.
(2) Violaxanthin inhibited fluorescence quenching reversibility stronger than zeaxanthin.
(3) Violaxanthin accelerated light-dependent fluorescence quenching stronger than zeaxanthin.
(4) Violaxanthin demonstrated lower adsorption affinity to LHC II than zeaxanthin.
(5) The shape of the fluorescence excitation difference spectrum of detached xanthophyll pigment (Fig. 5) with a central maximum at 450 nm is very similar to the absorption spectrum of violaxanthin incorporated to lipid bilayer with a main maximum at 449 nm [36]. Position of absorption maxima is dependent on the refractive index of environment of the pigment chromophore and a xanthophyll pigment having the possibility to be easily detached should be located at a protein–lipid interface.
(6) The position of 'aggregation' maxima of violaxanthin [23–25] are different from those of other xanthophylls and they are reasonably close to those which may be observed in the difference spectrum in Fig. 5.

An additional argument supporting the concept about light-induced detaching of violaxanthin from LHC II, is the very weak binding of this pigment to proteins so it appears to various degrees, dependent on experimental conditions, as free pigment fraction during thylakoid isolation [37].

As discussed previously [22,24,25,34,35], it is very probable that light-dependent violaxanthin detaching from LHC II and solubilization of this pigment within the lipid phase of thylakoid membrane is a process making violaxanthin available to enzymatic de-epoxidation. Whereas lowering of the luminal pH to a value of 5 (optimal condition for enzymatic de-epoxidation [38]) does not require strong light, large-scale zeaxanthin accumulation starts essentially at light intensities saturating photosynthesis [1–4] and violaxanthin availability to de-epoxidation seems to be the main limiting step of the xanthophyll cycle [2,35]. Violaxanthin availability may serve as an important signal transduction process to the xanthophyll cycle when the photosynthetic apparatus is overexcited. The status of LHC II under overexcitation conditions may be simply described in terms of a too high exciton density utilizable by the reaction centre. Under this conditions exciton quenching mechanism(s) is (are) decreasing the exciton density to protect against photodestruction. Obviously any light, irrespective of its intensity, is recognized as excess energy by the isolated LHC II since there is no way to transfer excitons out of the complex. In this case light drives the exciton quenching (Figs. 2, 3) as well as violaxanthin desorption (Fig. 5) from LHC II. At present it is not clear whether both light-dependent processes have the same origin and how they are related.

It is possible that violaxanthin desorption from LHC II facilitates protein unfolding ([29–31] which has an effect on exciton quenching. On the other hand zeaxanthin conversion to violaxanthin directly within LHC II [22] slows down reversibility of exciton quenching and increases the net rate of the quenching process

itself (this report). Such an effect may accelerate or amplify exciton quenching in LHC II and relate by this way an operative xanthophyll cycle. Such an interpretation is consistent with the concept of Horton's group that the xanthophyll cycle is not directly involved in the exciton quenching phenomena but may be active in a quenching amplification [5]. Our model of a cooperation of the xanthophyll cycle and light-driven exciton quenching in LHC II may explain why blockage of violaxanthin de-epoxidation results in a decreased extent of in vivo chlorophyll fluoresecence quenching [4]. Demmig-Adams also presented a linear correlation of fluorescence quenching and zeaxanthin level and concluded a direct physical involvement of the xanthophyll pigment zeaxanthin in chlorophyll singlet excited state quenching [4]. We now demonstrate that violaxanthin shows a stronger ability to inhibit reversibility of light-driven exciton quenching in LHC II and consequently accelerates the rate of the light-dependent process. The fact that zeaxanthin, the less polar xanthophyll pigment, has a higher adsorption affinity to the pigment–protein complex should become particularly important after illumination when the process of light-induced violaxanthin desorption from LHC II is active. The xanthophyll cycle may therefore regulate both the rate as well as the extent of quenching by violaxanthin de-epoxidation. It is difficult to propose an exact molecular mechanism leading to a light-driven chlorophyll excitation quenching in LHC II. At the present stage of research, one may speculate only that it is related to light-dependent conformational changes of a complex apoprotein. On the other hand, findings presented here indicate that this mechanism is strongly influenced by pigments of the xanthophyll cycle. In addition, the molecular mechanism of making violaxanthin available to de-epoxidation in the xanthophyll cycle may be proposed in conclusion of presented research as a process consisting in light-induced detachment of pigment molecules from antenna pigment–protein complex.

Acknowledgements

This work was financially supported by the Committee of Scientific Research of Poland under project KBN 6 P203 036 06 and by Grant no. 31-33678.92 of the Swiss National Science Foundation to R.J.S.

References

[1] Hager, A. (1980) in Pigments in Plants (Czygan, F.-C., ed.), pp. 57–79, Fischer, Stuttgart.
[2] Siefermann-Harms, D. (1977) in Lipids and Lipid Polymer in Higher Plants (Tevini, T., and Lichtenthaler, H.K., eds.), pp. 218–230, Springer, Berlin.
[3] Yamamoto, H.Y. (1979) Pure Appl. Chem. 51, 639–648.
[4] Demmig-Adams, B. (1990) Biochim. Biophys. Acta 1020, 1–24.
[5] Noctor, G., Rees, D., Young, A. and Horton, P. (1991) Biochim. Biophys. Acta 1057, 320–330.
[6] Gruszecki, W.I. and Strzalka, K. (1991) Biochim. Biophys. Acta 1060, 310–314.
[7] Krause, G.H. and Weiss, E. (1991) Annu. Rev. Plant Physiol. Plant Mol. Biol. 41, 313–349.
[8] Horton, P., Ruban, A.V., Rees, D., Pascal, A.A., Noctor, G. and Young, A.J. (1991) FEBS Lett. 292, 1–4.
[9] Ruban, A.V. and Horton, P. (1992) Biochim. Biophys. Acta 1102, 30–38.
[10] Jennings, R.C., Garlaschi, F.M. and Zucchelli, G. (1991) Photosynth. Res. 27, 57–64.
[11] Mullineaux, C.W., Pascal, A.A., Horton, P. and Holzwarth, A.R. (1993) Biochim. Biophys. Acta 1141, 23–28.
[12] Ruban, A.V., Young, A.J. and Horton, P. (1993) Plant Physiol. 102, 741–750.
[13] Rees, D., Young, A., Noctor, G., Britton, G. and Horton, P. (1989) FEBS Lett. 256, 85–90.
[14] Foyer, C.H., Dujardyn, M. and Lamoine, Y. (1989) Plant Physiol. Biochem. 27, 751–760.
[15] Pfündel, E. and Strasser, R.J. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), vol. II, pp. 727–737, Kluwer, Dordrecht.
[16] Havaux, M., Gruszecki, W.I., Dupont,I. and Leblanc, R.M. (1991) J. Photochem. Photobiol. B 8, 361–370.
[17] Jahns, P. and Krause, G.H. (1994) Planta 192, 176–182.
[18] Krupa, Z., Huner, N.P.A., Williams, J.P., Maissan, E. and James, D.R. (1987) Plant Physiol. 84, 19–24.
[19] Krupa, Z., Williams, J.P., Kahn, U.M. and Huner, N.P.A. (1992) Plant Physiol. 100, 931–938.
[20] Ayala, G., Nascimento, A., Gomez-Puyou, A. and Darszon, A. (1985) Biochim. Biophys. Acta 810, 115–122.
[21] Davies, B.H. (1976) in Chemistry and Biochemistry of Plant Pigments (Goodwin, T.W., ed.), pp. 38–165, Pergamon, London.
[22] Gruszecki, W.I. and Krupa, Z. (1993) Biochim. Biophys. Acta 1144, 97–101.
[23] Hager, A. (1970) Planta Berl. 91, 38–53.
[24] Gruszecki, W.I. (1990) Stud. Biophys. 139, 95–101.
[25] Gruszecki, W.I. (1991) J. Biol. Physics 18, 99–109.
[26] Wieckowski, S., Wloch, E. and Broniowska, B. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), pp. II.1.1–II.1.4, Martinus Nijhoff/Dr. W. Junk, Hague.
[27] Koyama, Y. (1991) J. Photochem. Photobiol. B 9, 265–280.
[28] Young, A. and Britton, G. (1990) in Stress Responses in Plants: Adaptation and Acclimation Mechanisms (Alscher, R.G. and Cumming, J.R., eds.), pp. 87–112, Willey-Liss, New-York.
[29] Plumley, F.G. and Schmidt, G.D. (1987) Proc. Natl. Acad. Sci. USA 84, 146–150.
[30] Paulsen, H., Römler, U. and Röddiger, W. (1990) Planta 181, 204–211.
[31] Paulsen, H., Finkenzeller, B. and Kuhlein, N. (1993) Eur. J. Biochem. 215, 809–816.
[32] Garab, G., Leegood, R.C., Walker, D.A., Sutherland, J.C. and Hind, G. (1988) Biochemistry 27, 2430–2434.
[33] Gruszecki, W.I., Veeranjaneyulu, K. and Leblanc, R.M. (1991) Biochem. Cell Biol. 69, 399–403.
[34] Gruszecki, W.I. and Krupa, Z. (1993) Z. Naturforsch. 48c, 46–51.
[35] Sielewiesiuk, J. and Gruszecki, W.I. (1991) Biophys. Chem. 41, 125–129.
[36] Gruszecki, W.I. and Sielewiesiuk, J. (1990) Biochim. Biophys. Acta 1023, 405–412.
[37] Siefermann-Harms, D. (1985) Biochim. Biophys. Acta 811, 325–355.
[38] Hager, A. and Holocher, K. (1994) Planta 192, 581–589.