Planta (1994) 192:176–182

Planta

Xanthophyll cycle and energy-dependent fluorescence quenching in leaves from pea plants grown under intermittent light

Peter Jahns, G. Heinrich Krause

Institut für Biochemie der Pflanzen, Heinrich-Heine-Universität Düsseldorf, Universitätsstrasse 1, D-40225 Düsseldorf, Germany

Received: 11 June 1993 / Accepted: 14 August 1993

Abstract. The possible role of zeaxanthin formation and antenna proteins in energy-dependent chlorophyll fluorescence quenching (qE) has been investigated. Intermittent-light-grown pea (*Pisum sativum* L.) plants that lack most of the chlorophyll a/b antenna proteins exhibited a significantly reduced qE upon illumination with respect to control plants. On the other hand, the violaxanthin content related to the number of reaction centers and to xanthophyll cycle activity, i.e. the conversion of violaxanthin into zeaxanthin, was found to be increased in the antenna-protein-depleted plants. Western blot analyses indicated that, with the exception of CP 26, the content of all chlorophyll a/b-binding proteins in these plants is reduced to less than 10% of control values. The results indicate that chlorophyll a/b-binding antenna proteins are involved in the energy-dependent fluorescence quenching but that only a part of qE can be attributed to quenching by chlorophyll a/b-binding proteins. It seems very unlikely that xanthophylls are exclusively responsible for the qE mechanism.

Key words: Chlorophyll a/b-binding protein – Energy-dependent fluorescence quenching – Intermittent light – Photosynthesis – *Pisum* – Xanthophyll cycle

Introduction

The fluorescence of chlorophyll (Chl) a can be used as a measure for the utilization of absorbed light energy in photosynthesis of higher plants (for a review, see Krause and Weis 1991). Room-temperature fluorescence originates predominantly from photosystem II (PSII). Quenching of the maximum Chl a fluorescence upon illumination has been ascribed to either photochemical activity (photochemical quenching, qP) or to energy transfer and thermal energy dissipation (non-photochemical quenching, qN). The latter process occurs predominantly under high illumination and plays an important role in protecting plants from damage due to non-utilized light energy. Three major mechanisms of non-photochemical fluorescence quenching have been described: (i) the pH-dependent or energy-dependent mechanism, (ii) changes in transfer of energy to PSII and PSI (state transitions) and (iii) photoinhibition. In fluorescence analyses, the contributions of these mechanisms to fluorescence quenching are expressed by the quenching coefficients qE, qT and qI, respectively.

The energy-dependent mechanism of fluorescence quenching has been investigated intensively during recent years. It is generally accepted that acidification of the thylakoid lumen triggers the qE mechanism. The subsequent processes, however, are a matter of controversy. One hypothesis relates qE to a donor-side inhibition of PSII at low pH and favours a reaction-center component as fluorescence quencher (Weis and Berry 1987; Schreiber and Neubauer 1990; Krieger et al. 1992). Two other hypotheses consider the involvement of antenna pigments in the qE mechanism. One line correlates the synthesis of zeaxanthin during lumen acidification via the xanthophyll cycle (see e.g. Siefermann-Harms 1977) to qE formation, and postulates zeaxanthin or antheraxanthin itself as the quenching component (Demmig-Adams 1990; Gilmore and Yamamoto 1993). Another model argues that acidification of the thylakoid lumen induces structural changes of the PSII antenna which generate quenching centers in the proteins of the light-harvesting complex of PSII (LHC II) (Horton et al. 1991; Ruban et al. 1992). There is experimental evidence that a zeaxanthin-dependent and a zeaxanthin-independent quenching component contribute to qE (e.g. Demmig-Adams et al. 1990). Gilmore and Yamamoto (1993) explained the

Abbreviations: CAB = chlorophyll a/b-binding; Chl = chlorophyll; F_V = variable fluorescence; IML = intermittent light; LHC = light harvesting complex; PFD = photon flux density: qP = photochemical quenching of chlorophyll fluoresence; qN = non-photochemical quenching; qE = energy-dependent quenching; qI = photoinhibitory quenching; qT = quenching by state transition

Correspondence to: P. Jahns; FAX: 49 (211) 311 3706

zeaxanthin-independent component of qE as quenching by antheraxanthin. Horton and coworkers suggested that zeaxanthin may function as an amplifier of qE formation, perhaps by activation of the LHC II aggregation (Noctor et al. 1991; Ruban et al. 1992). We have investigated the energy-dependent fluorescence quenching in pea plants grown under intermittent light (IML-plants). The IML-plants contain fully active photosynthetic reaction centers but are devoid of most of the Chl a/b-binding antenna proteins (CAB-proteins) (Argyroudi-Akoyunoglou 1979; Tzinas et al 1987; Jahns and Junge 1992). Thus this material allows in-vivo investigation of the qE mechanism in the absence of most of the CAB-proteins.

Materials and methods

Plant material. Pea (*Pisum sativum* L., cv. Kleine Rheinländerin, Aders, Düsseldorf, Germany) seedlings were grown either in a greenhouse (control plants) or under intermittent light (25 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 2 min light, 118 min dark; IML-plants). Intact leaves from 11-d-old IML-plants or 12- to 14-d-old control plants were used for all experiments.

Fluorescence measurements. Quenching of variable Chl a fluorescence after actinic illumination for 15 min (60–1100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was analyzed by using a PAM-fluorometer (Walz, Effeltrich, Germany). Leaves were kept in a tempered leaf-disc chamber (18°C) under a stream of moistened air. The photochemical and non-photochemical quenching components qP and qN, respectively, were discriminated by saturating pulses (5000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 1 s duration) applied to the leaves at different points of the fluorescence-induction signal (Bradbury and Baker 1984; Schreiber et al. 1986). The proportions of the non-photochemical quenching components qE, qT and qI were determined from the relaxation kinetics of the variable fluorescence (F_V) within 20 min dark adaption of the leaves (see also Quick and Stitt 1989; Walters and Horton 1991). Relaxation of F_V was tested by saturating pulses (1 s duration, 5000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) given every 2 min to the darkened leaves. For quantification of qE, qT and qI, the semi-logarithmic plot of F_V versus time was analyzed considering the relationship $(1 - qN) = (1 - qE) \times (1 - qT) \times (1 - qI)$.

Pigment analysis. For pigment analysis, whole leaves were frozen in liquid nitrogen and disrupted very carefully in 3 ml of acetone in a mortar for about 1 min under dim green light. About 250 mg of leaves from control plants and 500 mg of leaves from IML-plants were used for each experiment. The pigment extract was filtered through a 0.2-µm membrane filter (Schleicher and Schüll, Dassel, Germany) and usually applied immediately to the HPLC column. If necessary, the extracts were stored for up to 1 h at −20°C in the dark. No pigment degradation occurred during this time.

Pigments were separated in an HPLC system (Pharmacia, Freiburg, Germany) using a reversed-phase column (ET 250/8/4, Nucleosil 120–5 C_{18}; Macherey-Nagel, Düren, Germany) following a protocol from C. Wilhelm (Mainz; personal communication) based on the method described by Gilmore and Yamamoto 1991): Elution for 10 min with 74:6: 1 (by vol.) of acetonitrile: methanol: Tris-HCl (200 mM, pH 8.0) was followed by a linear 0–100% gradient of methanol: hexane (5:1; v/v) built up in 12 min. All pigments were recovered from the column within about 30 min at a flow rate of 1 $ml \cdot min^{-1}$. The eluted pigments were monitored at 440 nm. The peak area was calculated automatically by an integrator (LKB 2221; Pharmacia). Chlorophylls a and b were quantified photometrically for each sample according to Porra et al. (1989). Concentrations of the other pigments were estimated by using the conversion factors for peak area to nanomoles given by Gilmore and Yamamoto (1991).

Electrophoresis. The polypeptide compositions of thylakoid preparations from control plants and IML-plants were determined by denaturating SDS-PAGE. Following the procedure by Laemmli (1970), with the modifications by Engelbrecht et al. (1986), 5% acrylamide was used for the stacking gels and 15% acrylamide for the separating gels; 5.3 M urea was present in the separating gels.

Western blot analysis. For Western blot analysis, polypeptides were transferred from the gel to nitrocellulose (Schleicher and Schüll) using a semi-dry blot chamber (Biometra, Göttingen, Germany) for 20 min at $2 mA \cdot cm^{-2}$ in a carbonate buffer (10 mM $NaHCO_3$, 3 mM Na_2CO_3) containing 10% (v/v) methanol. Membranes were blocked by incubation for 1 h in 50 mM Tris-HCl (pH 7.4), 120 mM NaCl and 3% (w/v) bovine serum albumin (BSA) prior to incubation (over night) with the primary antibody. Goat anti-rabbit or anti-mouse IgG coupled to alkaline phosphatase (Sigma, Deisenhofen, Germany) was used as secondary antibody. Alkaline phosphatase was visualized by incubation with 100 $\mu g \cdot ml^{-1}$ nitroblue tetrazolium (Sigma) and 50 $\mu g \cdot ml^{-1}$ 5-bromo-4-chloro-3-indolylphosphate (Sigma) in 50 mM Na_2CO_3 (pH 9.5) and 4 mM $MgCl_2$ at room temperature for about 10 min.

Results

To investigate the role of CAB-proteins in the energy-dependent inactivation of PSII (qE mechanism), we com-

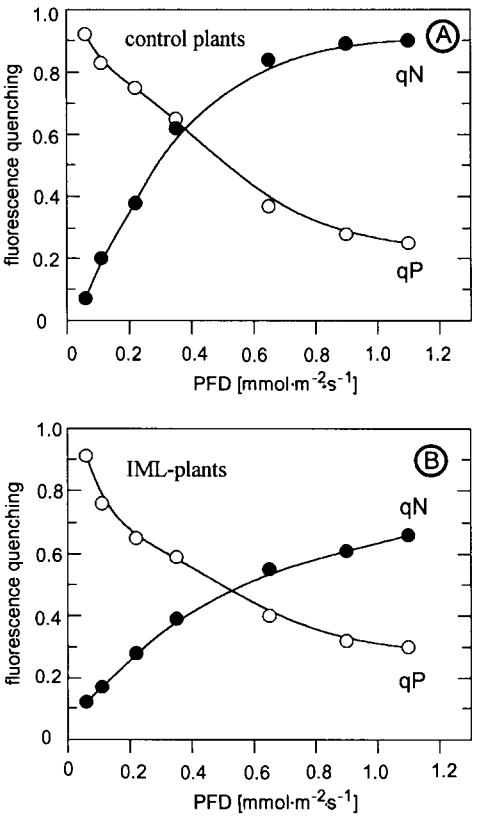

Fig. 1A, B. Quenching of variable Chl a fluorescence as function of the actinic light flux density (PFD). Leaves from pea plants were illuminated for 15 min. Photochemical (*qP*) and non-photochemical (*qN*) fluorescence-quenching coefficients were determined from the fluorescence changes induced by a saturating flash at the end of illumination. **A** Control plants; **B** intermittent-light-grown plants

Table 1. Relaxation kinetics of qN in the dark. Recovery half-times of qN were determined from semi-logarithmic plots of F_V versus time. The deviations of the values for the fast and medium phases represent the upper and lower limits determined in five different experiments at each PFD. For IML-plants we found only a biphasic relaxation of qN

	$t_{1/2}$ (fast)	$t_{1/2}$ (medium)	$t_{1/2}$ (slow)
Control plants	50–90 s	4–6 min	>20 min
IML-plants	120–180 s	–	>20 min

pared the quenching of the variable Chl a fluorescence in leaves of IML-plants and control plants. Leaves were illuminated for 15 min and fluorescence quenching was monitored by means of saturating light pulses.

Dark-adapted leaves of control plants had ratios of variable to maximum fluorescence (F_V/F_M) of 0.83 ± 0.01. In IML-plants this ratio was 0.69 ± 0.02. This lower F_V/F_M ratio in IML-plants is most likely caused by a higher spillover of excitation energy from PSII to PSI in IML-plants due to the absence of grana stacks. Figure 1 shows the extent of the photochemical (qP) and non-photochemical (qN) quenching as a function of the photon flux density (PFD). For both sets of plants, qP decreased in a comparable way from values of about 0.9 at 60 μmol · m^{-2} · s^{-1} to about 0.3 at 1100 μmol · m^{-2} · s^{-1}. The high photochemical quenching at low PFD indicates a similar photosynthetic utilization of absorbed light energy in IML-plants and controls. The same conclusion can be drawn when applying the expression given by Genty et al. (1989) for the estimation of the quantum yield of non-cyclic electron transport (data not shown).

However, qN was significantly reduced in IML-plants, indicating a less effective thermal energy dissipation in these plants. For discrimination between the different mechanisms of photochemical quenching (qE, qT, qI) we analyzed the relaxation kinetics of qN in the dark. For control plants, three different phases of recovery were resolved (see Table 1) with half-times of about 1 min, 5 min and more than 20 min. In agreement with other authors (Quick and Stitt 1989; Walters and Horton 1991) we attributed these phases to qE, qT and qI, respectively. For IML-plants, on the other hand, only two phases with half-times of 2–3 min and more than 20 min were resolved. Thus it appears that IML-plants show delayed relaxation kinetics of qE with respect to controls and that no fluorescence quenching occurs by the qT mechanism. Quenching by state transition is generally attributed to the transfer of energy from PSII to PSI by the migration of mobile LHC II proteins from the grana into the stroma region of the thylakoid membrane following phosphorylation (see Allen 1992). Since IML-thylakoids are unable to form grana stacks (due to the absence of LHC II proteins), the absence of qT in these plants can easily be explained.

The extent of each component of qN in both types of plants is shown in Fig. 2. Obviously, IML-plants exhibit a substantial qE, although it is significantly reduced in comparison to control plants. The reduction of qE in

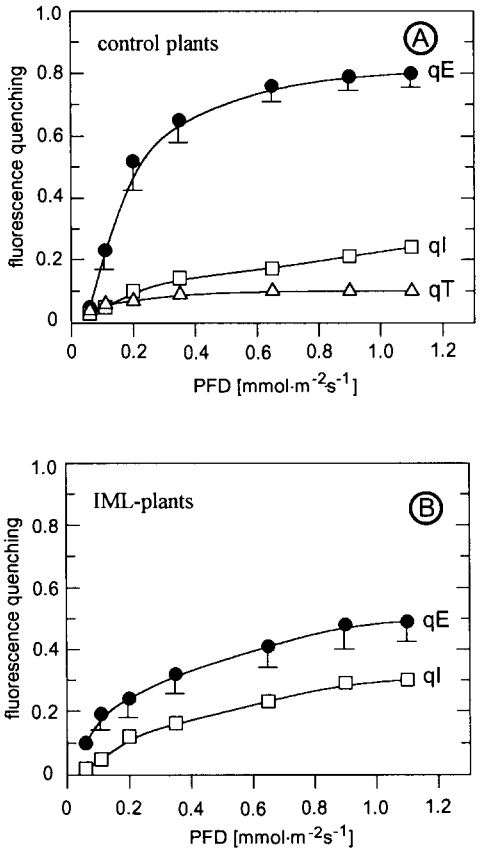

Fig. 2A, B. Components of non-photochemical fluorescence quenching. The different components of non-photochemical fluorescence quenching and the respective coefficients were derived from semi-logarithmic plots of the dark relaxation of F_V after 15 min illumination at different PFDs. Energy-dependent quenching (*qE*) was attributed to the fast phase, quenching by state transitions (*qT*) to the medium phase and photoinhibitory quenching (*qI*) to the slow phase of relaxation. The quenching coefficients (qE, qT, qI) were calculated from the amplitude of the respective phase considering the relationship (1–qN) = (1 –qE) × (1–qT) × (1–qI). **A** control plants; **B** intermittent-light-grown plants. No medium phase (and thus no qT) was found for IML-plants (see Table 1). Each value represents the mean of five different experiments; bars indicate ± SD of qE. Deviations of qI were in a similar range, that of qT generally smaller (both not shown)

IML-plants may be explained by the absence of most of the CAB-proteins, in agreement with the hypothesis by Horton et al. (1991). Alternatively, a reduced qE could result from a possible deficiency in zeaxanthin formation. It has been shown that zeaxanthin synthesis parallels the energy-dependent thermal deactivation of PSII (see *Introduction*). We analyzed the pigment content of leaves and the activity of the xanthophyll cycle. Table 2 summarizes the pigment content of dark-adapted IML and control plants. Pigment concentrations are given in relation to 1 mol total Chl content. For IML-plants the data are, in addition, normalized to the amount of reaction centers (right-hand column in Table I). The latter was calculated on the basis of recent flash-spectrophotometrical data (Jahns and Junge 1992) from which the PSII: PSI ratio in IML-plants was determined to be 5:2 instead of 1:1 in controls with respect to the same total chlorophyll con-

Table 2. Pigment contents of leaves from control and intermittent light (IML)-grown plants. Pigments were separated by HPLC. Pigment concentrations were calculated from the conversion factors of peak areas at 440 nm as given by Gilmore and Yamamoto (1991): 20.72 (neoxanthin and violaxanthin); 27.1 (antheraxanthin and lutein); 26.9 (zeaxanthin); 38.53 (Chl b); 34.94 (Chl a); 18.94 (β-carotene)

Pigments	Control plants	IML-plants	
	mmol pigments		per[a]
	1	1	0.286 mol Chl
Unknown[b]	0	60.0	17.1
Neoxanthin	52.8	71.4	20.4
Violaxanthin	76.6	602.0	172.0
Antheraxanthin	0.9	49.1	14.0
Lutein	207.1	996.3	284.6
Zeaxanthin	0	8.0	2.3
Chlorophyll b	277	26.0	7.4
Chlorophyll a	723	974.0	278.3
β-Carotene	87.4	238.3	68.1

[a] Data are normalized to 1 mol total Chl content. For IML-leaves the data were converted additionally to a total Chl content of 286 mmol considering the smaller antenna size of reaction centers in IML-plants

[b] Calculated from the relative peak area using the conversion factor of neoxanthin

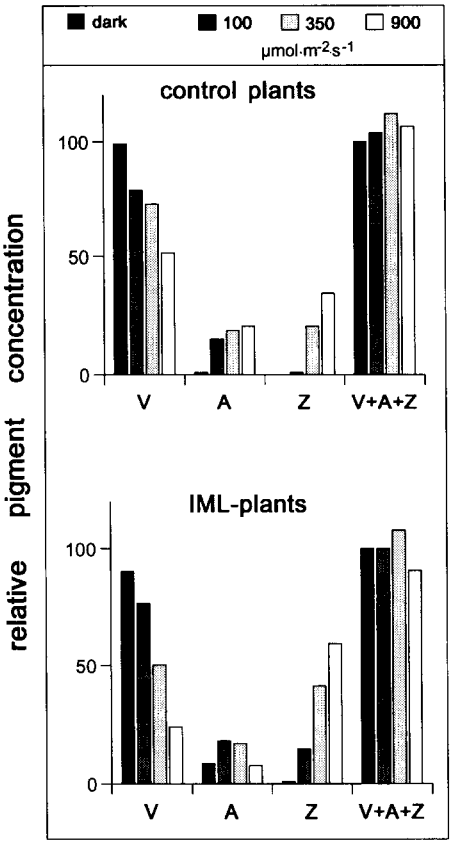

Fig. 3. Conversion of violaxanthin to zeaxanthin upon illumination of pea leaves. Control plants (*upper part*) and IML-plants (*lower part*) were kept in the dark for 16 h. The relative content of the xanthophylls violaxanthin (*V*), antheraxanthin (*A*) and zeaxanthin (*Z*) was determined by HPLC analysis. Values of dark-adapted plants are given in comparison to plants illuminated subsequently at different PFDs for 12 min (100, 350 and 900 μmol · m ^{2} · s^{-1}). The total content of the three xanthophylls (sum of $V+A+Z$) of each sample is given on the right in relation the respective dark controls

tent, leading to a 3.5-times higher amount of reaction centers in IML-thylakoids. This calculation (division by 3.5 of the respective values in the middle column) takes into account the smaller antenna size of PSII and PSI in IML-plants. Per reaction center, IML-plants contain about equal amounts of lutein and β-carotene as control plants, but appreciably higher amounts of violaxanthin and antheraxanthin, and reduced amounts of neoxanthin. Additionally, an unknown pigment was present in IML-plants. Comparison of the absorption at 430, 440 and 455 nm let us conclude that this pigment is most probably a chlorophyllide a. This assumption is supported by the low retention time, indicating a polar character of the pigment. This pigment was only present in darkened IML-plants (1–16 h) and disappeared upon illumination.

The synthesis of zeaxanthin upon illumination was determined under the same illumination conditions as described above for the quenching analyses. The relative amounts of violaxanthin, antheraxanthin and zeaxanthin in dark-adapted and illuminated (100, 350 and 900 μmol · m^{-2} · s^{-1}) leaves are illustrated in Fig. 3. The relative pigment concentrations were normalized to the total amount of the three pigments in the respective dark controls. It is obvious that the conversion from violaxanthin to zeaxanthin is even more effective in IML-thylakoids than in controls. Illumination with 900 μmol · m^{-2} · s^{-1} induces the conversion of about 75% of violoxanthin into zeaxanthin in IML-leaves, while only less than 50% of the violaxanthin can be converted in controls.

Thus the reduced qE is clearly not related to decreased zeaxanthin formation in IML-plants. The more efficient xanthophyll cycle in IML-plants rather corroborates the suggestion that the reduced amount of CAB-proteins is responsible for the decrease in qE. The absence of most of the CAB-proteins in IML-thylakoids has already been shown by SDS-PAGE (Jahns and Junge 1992). This is confirmed by the more sensitive Western blot analysis for the plant material used here. Polyclonal antibodies against the D1 reaction-center protein and the extrinsic 23-kDa protein of the water oxidase were used as PSII indicators. Monoclonal antibodies against the major LHC II proteins and CP 29 served as indicators for CAB-proteins. The amount of sample loaded on the gel was adjusted to yield comparable staining of the D1 and 23-kDa proteins for controls and IML-thylakoids at the different dilution steps (see Fig. 4). By this procedure we ensured that comparable amounts of PSII reaction centres were present in IML and control thylakoids.

For IML-thylakoids, samples equivalent to 300 ng Chl contained comparable amounts of PSII reaction centers as obtained with 1.2 μg Chl for controls. This result confirms the earlier estimation of a higher PSII content in IML-leaves on the basis of Chl concentration. The relative content of CAB-proteins, on the other hand, was drastically diminished in IML-thylakoids. This holds

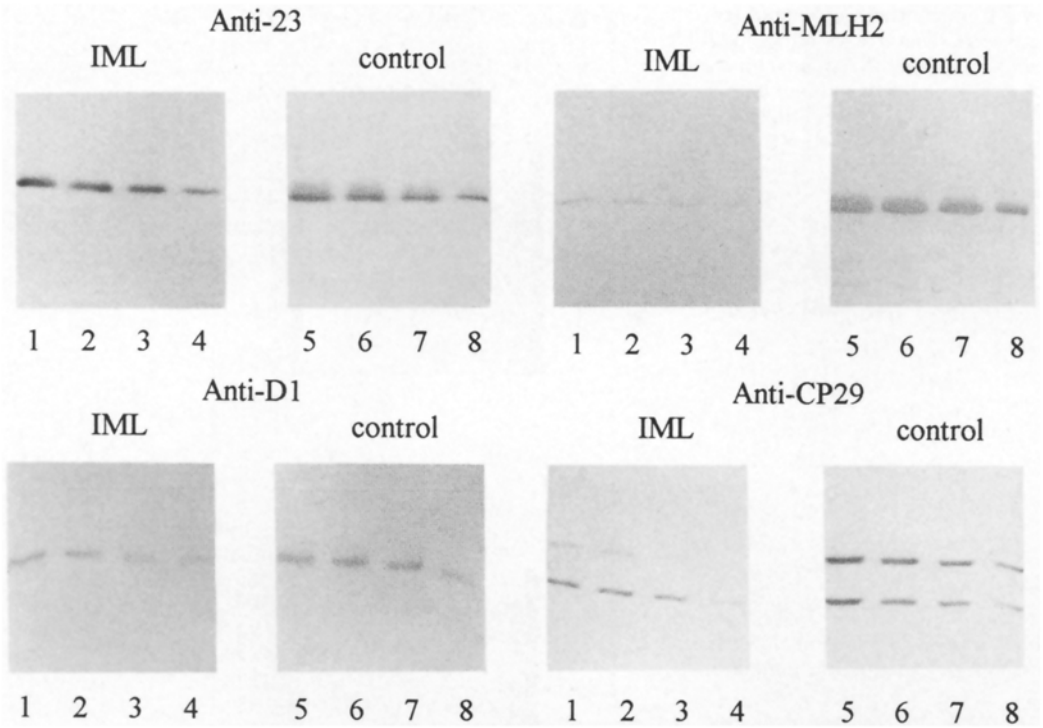

Fig. 4. Quantification of PSII proteins in thylakoids from IML and control pea leaves. Four different dilutions of IML-thylakoids (*lanes* 1–4) and control thylakoids (*lanes* 5–8) were blotted onto nitrocellulose and assayed with four different antibodies as indicated above each series. Following antibodies have been used: Polyclonal antibodies raised against the extrinsic 23 kDa protein of the PSII water oxidase (*Anti*-23) and the D1 reaction-center protein of PSII (*Anti-D1*); monoclonal antibodies raised against the major light-harvesting protein of PSII (*Anti-MLH2*) and CP 29 (*Anti-CP29*). For IML-thylakoids, samples equivalent to 300, 200, 100 and 25 ng Chl were applied on lanes 1–4, respectively; for control thylakoids samples equivalent to 1.2, 0.8, 0.4 and 0.1 µg were applied on lanes 5–8, respectively. Staining of the Anti-23 and Anti-D1 antibodies was used as indicator for the PSII reaction centers present in each sample. From the reduction of the staining of the Anti-MLH2 and Anti-CP29 antibodies we estimated the relative amounts of the respective antenna proteins present per PSII reaction center

true at least for the major LHC proteins: only traces of these could be detected by the antibodies raised against the LHC II proteins.

Monoclonal antibodies against CP 29, however, indicate a higher amount of this inner CAB-protein in IML-thylakoids in comparison to the amount of LHC II. Moreover, two proteins cross-react with these antibodies in both samples. As pointed out by Falbel and Staehelin (1992), the Berg CP 29 antibody may cross-react also with CP 26. Thus it seems likely that the upper bands in Fig. 4 represent CP 29 and the lower ones CP 26. Both proteins are present in IML-thylakoids to a greater extent than the major LHC proteins. The relative CP 26 content can be estimated to be about 90–100% of that of controls, the CP 29 content to be about 10% of that of controls.

Discussion

The fluorescence analyses clearly show that in illuminated leaves of CAB-protein-lacking IML-plants a substantial energy-dependent fluorescence quenching occurs, although it is significantly reduced in comparison to controls. Under the same illumination conditions that induce qE we found an enhanced zeaxanthin synthesis. Thus the loss of qE in IML-plants must be related to changes which are independent of zeaxanthin formation. Since IML-thylakoids seem to contain unchanged PSII reaction centers, the reduction of qE most probably results from the reduced amount of CAB-proteins. The major component of LHC II proteins (often referred to as the LHC IIb component) is almost completely absent in this material. Also the amount of inner CAB-protein CP 29 apparently is reduced to less than 10% of that of controls, while only CP 26 is present in nearly unchanged stoichiometry. Similar results for the CAB-protein content in IML-plants of maize have been found by Marquardt and Bassi (1993), although the maize IML-thylakoids clearly contained more CAB-proteins than found here with pea. The differences may be caused by a higher chlorophyll b content of the maize IML-plants in comparison to IML-pea, which is obvious from the relative low Chl a/b ratio in IML-maize (Marquardt and Bassi 1993). Thus their material which exhibits intermediate amounts of CAB-proteins reprents a higher stage of greening than IML-pea. Interestingly, Marquardt and Bassi (1993) found that in these more developed IML-plants CP 24 was reduced to the same amount as the major LHC II, while CP 29 and CP 26 were less reduced, but both to a similar extent. Taking both sets of data together, one can speculate that CP 26 represents the innermost CAB-protein of PSII, followed by CP 29, which is more reduced in IML-plants of pea but not of

maize. CP 24, which has not been tested in this study, would then represent the most peripheral of the 'inner CAB-proteins'.

The substantial qE observed in IML-plants shows that the mechanism of energy-dependent quenching cannot be explained exclusively by structural changes of the PSII antenna as proposed by Horton et al. (1991). Thus a second mechanism must be postulated. So far, it is uncertain to what extent zeaxanthin formation may be involved. The high xanthophyll-cycle efficiency in IML-plants does not corroborate a direct correlation of qE with zeaxanthin and antheraxanthin formation. Our results are therefore in contrast to the recently proposed function of zeaxanthin/antheraxanthin in thermal energy dissipation by Gilmore and Yamamoto (1993). Although the relative antheraxanthin content decreases in IML-plants at high PFDs (Fig. 3) this does not hold true for the absolute antheraxanthin content. Additionally, the sum of antheraxanthin and zeaxanthin in IML-plants exceeds that of controls at least by a factor of two at any PFD.

Very recently, Bassi et al. (1993) have investigated the pigment-binding properties of PSII proteins. They showed that about 80% of the violaxanthin in thylakoid membranes is bound to the inner CAB-proteins (CP 24, CP 26 and CP 29). In our IML-plants we found twice the amount of violaxanthin per reaction center but a reduction of the inner CAB-proteins. Thus it looks unlikely that all violaxanthin in pea IML-plants is bound to the inner CAB-proteins. Either other proteins have to be involved in violaxanthin binding or there are substantial amounts of free xanthophylls in the thylakoid membrane of IML-plants. We have one piece of experimental evidence for the latter case. In IML-plants not only the amount of violaxanthin per se is increased but also the amount of violaxanthin that can be converted into zeaxanthin (Fig. 3). On the other hand, no increase in qE is correlated with this increase in violaxanthin (and violaxanthin conversion), possibly due to the lack of CAB-proteins which are required for the interaction with zeaxanthin. If one calculates roughly the amount of non-convertible violaxanthin in controls and IML-plants, similar values in both types can be estimated. We speculate that the additional (and completely convertible) violaxanthin in IML-plants may not be bound to distinct proteins and therefore cannot contribute to qE. Thus there would exist two different fractions of violaxanthin in IML-plants, one unconnected and zeaxanthin-convertible, and one protein-associated fraction. The latter, however, seems to be only partly convertible into zeaxanthin (under our illumination conditions) and is present in controls and IML-plants in similar amounts. Assuming that only this protein-bound violaxanthin/zeaxanthin can contribute to qE, it seems possible that the qE observed in IML-plants depends on the action of zeaxanthin.

In conclusion, our studies are only partly in agreement with the hypothesis by Horton and co-workers that LHC II proteins are involved in energy-dependent fluorescence quenching. Although the reduction of qE in IML-plants is most probably due to the absence of LHC proteins, it is obvious that the remaining qE cannot be explained by this mechanism. Therefore, we have to postulate at least two different mechanisms to explain the energy-dependent quenching. Our results imply that most of the violaxanthin that is converted into zeaxanthin in IML-plants does not contribute to qE and, rather, is not connected to distinct proteins of the thylakoid membrane. Thus either a protein-connected fraction of violaxanthin/zeaxanthin is responsible for the qE in IML-plants or the quenching is related to processes occurring in the reaction centre of PSII itself, as proposed by several authors (see e.g. Krieger et al. 1992).

References

Allen, J. F. (1992) Protein phosphorylation in regulation of photosynthesis. Biochim. Biophys. Acta **1098**, 275–335

Argyroudi-Akoyunoglou, J. H., Akoyunoglou, G. (1979) The chlorophyll-protein complexes of the thylakoid in greening plastids of *Phaseolus vulgaris*. FEBS Lett. **104**, 78–84

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

Bradbury, M., Baker, N. R. (1984) A quantitative determination of photochemical and non-photochemical quenching during the slow phase of the chlorophyll fluorescence induction curve of bean leaves. Biochim. Biophys. Acta **765**, 275–281

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

Demmig-Adams, B., Adams III, W. W., Heber, U., Neimanis, S., Winter, K., Krüger, A., Czygan, F.-Z., Bilger, W., Björkman, O. (1990) Inhibition of zeaxanthin formation and of rapid changes in radiationless energy dissipation by dithiothreitol in spinach leaves and chloroplasts. Plant Physiol. **92**, 293–301

Engelbrecht, S., Lill, H., Junge, W. (1986) The proton channel, CF_o, in thylakoid membranes – Only a low proportion of CF_1-lacking CF_o is active with a high unit conductance (169 fS). Eur. J. Biochem. **160**, 635–643

Falbel, T.G., Staehelin, L.A. (1992) Species-related differences in the electrophoretic behavior of CP 29 and CP 26: An immunochemical analysis. Photosynth. Res. **34**, 249–262

Genty, B., Briantais, J.-M., Baker, N.R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta **990**, 87–92

Gilmore, A. M., Yamamoto, H. Y. (1991) Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C_{18} high-performance liquid chromatographic column. J. Chromatogr. **543**, 137–145

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

Horton, P., Ruban, A. V., Rees, D., Pascal, A. A., Noctor, G., Young, A. J. (1991) Control of light-harvesting function of chloroplast membranes by aggregation of the LHC II chlorophyll-protein complex. FEBS Lett. **292**, 1–4

Jahns, P., Junge, W. (1992) Thylakoids from pea seedlings grown under intermittent light: biochemical and flash-spectrophotometric properties. Biochemistry **31**, 7390–7397

Krause, G. H., Weis, E. (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. **42**, 313–349

Krieger, A., Moya, I., Weis, E. (1992) Energy-dependent quenching of chlorophyll a fluorescence: effect of pH on stationary fluorescence and picosecond-relaxation kinetics in thylakoid membranes and photosystem II preparations. Biochim. Biophys. Acta **1102**, 167–176

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

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

Noctor, G., Rees, D., Young, A., Horton, P. (1991) The relationship between zeaxanthin, energy-dependent quenching of chlorophyll fluorescence, and trans-thylakoid pH gradient in isolated chloroplasts. Biochim. Biophys. Acta **1057**, 320–330

Porra, R. J., Thompson, W., Kriedemann, P. E. (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim. Biophys. Acta **975**, 384–394

Quick, W. P., Stitt, M. (1989) An examination of factors contributing to non-photochemical quenching of chlorophyll fluorescence in barley leaves. Biochim. Biophys. Acta **977**, 287–296

Ruban, A. V., Rees, D., Pascal, A. A., Horton, P. (1992) Mechanism of Δ pH-dependent dissipation of photosynthetic membranes. II. The relationship between LHCII aggregation in vitro and qE in isolated thylakoids. Biochim. Biophys. Acta **1102**, 39–44

Schreiber, U., Neubauer, C. (1990) O_2-dependent electron flow, membrane organization and the mechanism of non-photochemical quenching of chlorophyll fluorescence. Photosynth. Res. **25**, 279–293

Schreiber, U., Schliwa, U., Bilger, W. (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth. Res. **10**, 51–62

Siefermann-Harms, D. (1977) The xanthophyll cycle in higher plants. In: Lipids and lipid polymers in higher plants, pp. 218–230, Tevini, M., Lichtenthaler, H. K., eds. Springer, Berlin Heidelberg New York

Tzinas, G., Argyroudi-Akoyunoglou, J.H., Akoyunoglou, G. (1987) The effect of the dark interval in intermittent light on thylakoid development: photosynthetic unit formation and light harvesting protein accumulation. Photosynth. Res. **14**, 241–258

Walters, R. G., Horton, P. (1991) Resolution of components of non-photochemical chlorophyll fluorescence quenching in barley leaves. Photosynth. Res. **27**, 121–133

Weis, E., Berry, J. A. (1987) Quantum efficiency of photosystem II in relation to 'energy'-dependent quenching of chlorophyll fluorescence. Biochim. Biophys. Acta **894**, 198–208