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Research article

LHCII organization and thylakoid lipids affect the sensitivity of the photosynthetic apparatus to high-light treatment

Kolyo G. Dankov [a], Anelia G. Dobrikova [a], Bettina Ughy [b], Balázs Bogos [b], Zoltan Gombos [b], Emilia L. Apostolova [a,*]

[a] Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria
[b] Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, Hungary

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ABSTRACT

Pulse-amplitude-modulated (PAM) chlorophyll fluorescence and photosynthetic oxygen evolution were used to investigate the role of the different amount and organization of light-harvesting complexes of photosystem II (LHCII) in four pea species on the susceptibility of the photosynthetic apparatus to high-light treatment. In this work we analyzed the thylakoid membrane lipid composition of the studied pea plants. A relationship between the structural organization of LHCII proteins, the amount of the main lipid classes and the sensitivity of the photosynthetic apparatus to high-light treatment was found. The results reveal that the photosynthetic apparatus, enriched in oligomeric forms of LHCII concomitant with decreased amount of anionic lipids and increased content of the monogalactosyldiacylglycerol (MGDG), is less sensitive to high light. Our data also suggest that the degree of LHCII oligomerization, as well as the lipid composition do not influence the degree of recovery of the PSII photochemistry after excess light exposure.

1. Introduction

Photosynthesis is a light-driven reaction, but exposure of plants to strong light intensities causes a decrease of photosynthetic efficiency, a phenomenon referred as photoinhibition [1,2]. The susceptibility of plants to photoinhibition depends on plant species and growth light condition. It is well established that acclimation, which is a basic feature of the photosynthetic apparatus of higher plants to adapt to the external environmental conditions, is accompanied with distinct changes in gene expression followed by changes of the organization of the protein complexes, lipids and the function of the thylakoid membranes [3].

The primary site of photodamage is the multiprotein complex of photosystem II (PSII) [1,2]. It is supposed that the extent of the high-light inhibition depends on the balance between the rate of damage and repair of PSII [2,4]. Strong visible light has two effects: a direct effect on photodamage, and an inhibitory effect on repair of PSII via the production of reactive oxygen species [2]. Vass and Cser [5] proposed a model of photoinhibition by visible light in which charge recombination processes are involved not only in damaging, but also in protecting PSII via the modulation of singlet oxygen formation [5]. It is considered that the earlier detectable step of the light-induced photoinhibition is the release of the Mn ion to the thylakoid lumen, which is followed by oxidative damage to the PSII reaction center [6]. Recent investigations developed a new scheme for the molecular mechanisms of photoinhibition, which propose two steps of the photodamage. The first step is the light-dependent destruction in the Mn cluster of the oxygen-evolving complex (OEC), and the second step is the inactivation of the reaction center of PSII [2,7]. It has been shown that phosphorylation of the PSII core protein facilitates the repair of the photodamaged PSII at strong light [8].

Plants have developed several adaptive regulatory mechanisms to optimize the utilization of light energy and to protect themselves against over-excitation-related damage. The dynamic properties of the proteins and pigments of the chlorophyll *a*/*b* light-harvesting complex of photosystem II (LHCII) allow excess energy to be dissipated as heat [9]. During the long-term acclimation of plants, the LHCII content in the photosynthetic membranes often changes, which affects the ultrastructure of the grana [10].

The role of light-harvesting antenna size of PSII in susceptibility of the photosynthetic apparatus towards high-light treatment is still

* Corresponding author at: Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria. Tel.: +359 2 979 26 21; fax: +359 2 971 24 93.
E-mail address: emya@obzor.bio21.bas.bg (E.L. Apostolova).

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controversial. Some investigators suggested that photoinhibition depends on the size of PSII antenna [11 and refs. therein], while others [12] claimed that it is independent of LHCII size. In our recent study we have shown that the effect of UV-A radiation on the photosynthetic apparatus depends not only on the antenna size of PSII, but also on the degree of LHCII oligomerization [13].

On the other hand, it is well known that the specific interactions between lipids and membrane proteins are involved in the activation of protein function and protein packing of the macrocomplexes in the thylakoid membranes [14–16]. Monogalactosyldiacylglycerol (MGDG) is the major lipid constituent of thylakoid membranes and has been proposed to be included in various important photosynthetic processes [17,18]. Several lines of evidence show that thylakoid lipid desaturation and composition influence photoinhibition [19–24]. Earlier observations demonstrated that polyunsaturated fatty acids of membrane lipids are important in the resistance of the photosynthetic apparatus to low-temperature photoinhibition [15]. The role of the digalactosyldiacylglycerol (DGDG) [25], as well as of the unsaturated fatty acids of phosphatidylglycerol (PG) [23,26], in recovery of the PSII protein complex was revealed. In spite of numerous studies, the overall role of lipids in the protection mechanisms of the photosynthetic apparatus against photoinhibition has not been completely elucidated.

In this study, we used previously described pea plants, which have different ratio of oligomeric to monomeric forms of LHCII (LHCIIo/LHCIIm). The ratio increases in the following order: mutant *Chlorotica XV/1422* (2.45), Auralia wild type (2.85), mutant *Costata 2/133* (3.34) and Borec wild type (4.87) [27–29]. Earlier studies also revealed that the amount of LHCII proteins in Auralia wt and its mutant *Chlorotica XV/1422* is smaller than in Borec wt and the mutant *Costata 2/133* [28,29]. These differences were accompanied with alterations in the pigment composition, as well as in the photochemical and physicochemical characteristics of thylakoid membranes [27–29].

In order to get better insight into the role of the degree of LHCII oligomerization on sensitivity of photosynthetic apparatus to high-light treatment, we studied the effect of excess light on the parameters of the PAM chlorophyll fluorescence and the photosynthetic oxygen evolution of studied pea plants. In present study we show that the sensitivity of the photosynthetic apparatus to high-light treatment depends on the structural organization of LHCII and the lipid composition.

2. Materials and methods

2.1. Plant material and thylakoid membrane isolation

The plants from *Pisum sativum* L. cv. Borec and Auralia and their mutants (*Costata 2/133* and *Chlorotica XV/1422*) were grown under controlled conditions with 16 h light/8 h dark period. The mutants that we used in this study are well-defined and stable. In each new series of studies of these mutants we checked their properties and they did not reveal any differences in their physicochemical characteristics and functions.

The thylakoid membranes were isolated from non-treated and high-light irradiated leaves as described in [30] and suspended in a medium containing: 40 mM HEPES (pH 7.6), 10 mM NaCl, 5 mM $MgCl_2$ and 400 mM sucrose.

2.2. High-light treatment

Leaves were cut from plants and the petioles were immersed in water or in solution of 10 mM lincomycin (Sigma) and incubated in dim light for 2 h before high-light treatment. The leaves were illuminated up to 3 h at room temperature (20–25 °C) with white high-intensity light (1300 $\mu mol\, m^{-2}\, s^{-1}$). For recovery from photoinhibitory treatment the leaves were transferred to a dim light at room temperature.

2.3. Lipid analysis

The analysis of lipids of thylakoid membranes from pea types was carried out according to the method of Sato and Murata [31]. The total lipid and the various lipid classes, which were fractionated on precoated thin-layer chromatography (TLC) plates (Merck 5721) developed with $CHC1_3/CH_3OH/28\%\ NH_4OH$ (65/35/5, v/v/v), were subjected to methanolysis with 5% HCl in methanol at 85 °C for 2 h. The resulting methyl esters were analyzed with a Hewlett Packard (Palo Alto, California, USA) HP6890 gas chromatograph equipped with a hydrogen flame-ionization detector. Fatty acid methyl esters were separated on a 30 m × 0.25 mm i.d. SP-2330 capillary column (Supelco, Bellefonte, Pennsylvania, USA). Temperatures of the column and the flame-ionization detector were 180 and 260 °C, respectively. The relative amounts of fatty acid methyl esters were determined by comparison of areas under the peaks on the chromatogram to the area of internal fatty acid standard, pentadecanoic acid (15:0). Fifty μg of pentadecanoic acid was added to each band of the TLC plate.

2.4. Pulse-amplitude-modulated chlorophyll fluorescence

Modulated chlorophyll fluorescence was measured on leaf discs by a PAM fluorometer (H. Walz, Effeltrich, Germany, model PAM 101-103). The leaves were dark adapted for 15 min. The initial fluorescence level (F_0) was measured at instrument frequency of 1.6 kHz and measuring beam set at 0.120 $\mu mol\, m^{-2}\, s^{-1}$ PFD. For evaluation of maximal fluorescence level (F_m), saturating flashes of 3000 $\mu mol\, m^{-2}\, s^{-1}$ PFD with duration of 0.8 s were provided by Schott lamp KL 1500 (Schott Glaswerke, Mainz, Germany). The saturating flash gives F_m in dark-adapted state and F_m' in light-adapted state. The interval between two consecutive flashes was 60 s. The actinic light illumination (250 $\mu mol\, m^{-2}\, s^{-1}$ PFD) was provided by second Schott lamp KL 1500 for the induction of photosynthesis. The maximum quantum yield of primary photochemistry in dark-adapted state was calculated, $(F_m - F_0)/F_m = F_v/F_m$ [32], which is proportional to de-excitation rate constant of photochemical (k_p) and non-photochemical processes (k_n) of PSII deactivation, $F_v/F_m = k_p/(k_n + k_P)$ [33]. The non-photochemical quenching, $NPQ = (F_m - F_m')/F_m'$, was calculated as in [34].

2.5. Oxygen evolution measurements

Oxygen flash yields and initial oxygen burst of isolated thylakoid membranes were measured by a home-built polarographic oxygen rate electrode described in [35]. Thylakoid membranes were suspended in a medium containing: 40 mM HEPES (pH 7.6), 10 mM NaCl, 5 mM $MgCl_2$ and 400 mM sucrose. The chlorophyll concentration was 150 $\mu g\, ml^{-1}$. The measurements were performed as in [29]. The induction curves after oxygen burst exhibit biphasic exponential decay. The deconvolution of the oxygen burst decay was performed as in [13]. Initial S_0 and S_1 state distribution, misses and double hits were determined by the fitting of the theoretically calculated yields according to the model of Kok et al. [36] with the experimentally obtained oxygen flash yields using the least square deviations procedure.

2.6. Statistical analysis

The results are mean values from 3 to 5 independent experiments. The statistical differences among the means were determined using