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Photoinhibition of photosystem I in a pea mutant with altered LHCII organization

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ABSTRACT

Comparative analysis of *in vivo* chlorophyll fluorescence imaging revealed that photosystem II (PSII) photochemical efficiency (F_v/F_m) of leaves of the *Costata 2/133* pea mutant with altered pigment composition and decreased level of oligomerization of the light harvesting chlorophyll a/b-protein complexes (LHCII) of PSII (Dobrikova et al., 2000; Ivanov et al., 2005) did not differ from that of WT. In contrast, photosystem I (PSI) activity of the *Costata 2/133* mutant measured by the far-red (FR) light inducible P700 ($P700^+$) signal exhibited 39% lower steady state level of $P700^+$, a 2.2-fold higher intersystem electron pool size (e^-/P700) and higher rate of $P700^+$ re-reduction, which indicate an increased capacity for PSI cyclic electron transfer (CET) in the *Costata 2/133* mutant than WT. The mutant also exhibited a limited capacity for state transitions. The lower level of oxidizable P700 ($P700^+$) is consistent with a lower amount of PSI related chlorophyll protein complexes and lower abundance of the PsaA/PsaB heterodimer, PsaD and Lhca1 polypeptides in *Costata 2/133* mutant. Exposure of WT and the *Costata 2/133* mutant to high light stress resulted in a comparable photoinhibition of PSII measured *in vivo*, although the decrease of F_v/F_m was modestly higher in the mutant plants. However, under the same photoinhibitory conditions PSI photochemistry ($P700^+$) measured as $\Delta A_{820-860}$ was inhibited to a greater extent (50%) in the *Costata 2/133* mutant than in the WT (22%). This was accompanied by a 50% faster re-reduction rate of $P700^+$ in the dark indicating a higher capacity for CET around PSI in high light treated mutant leaves. The role of chloroplast thylakoid organization on the stability of the PSI complex and its susceptibility to high light stress is discussed.

1. Introduction

The exposure of photosynthetic organisms to various environmental stresses such as low and high temperatures, excess light and water and nutrient stress may cause an imbalance between the capacity for harvesting light energy and the capacity to dissipate this energy through metabolic activity, resulting in excess PSII excitation pressure. Excess excitation pressure, measured as the relative redox state of Q_A, the first stable quinine electron acceptor of photosystem II (PSII) reaction centers, reflects the overall reduction state of the photosynthetic electron transport chain [3–5]. The imbalance between the reducing equivalents produced in excess that exceeds the capacity of the metabolic sinks to utilize the electrons generated from the absorbed energy may be caused by either exposure to an irradiance that exceed the light harvesting capacity or by any environmental constrains that may decrease the capacity of the metabolic pathways downstream of photochemistry (C, N, and S assimilation) to utilize photosynthetically generated reductants [4–6]. This imbalance, which is a prerequisite for any stress

Abbreviations: AG, afterglow thermoluminescence band; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenolindophenol; F_v/F_m, maximum photochemical efficiency of PSII in the dark adapted state; Fr, capacity for state transitions; LHCII *a*/*b*, light-harvesting chlorophyll a/b-protein complex of PSII; MV, methyl viologen; P700, reaction center chlorophyll of PSI; PQ, plastoquinone; PSI, photosystem I; PSII, photosystem II; qN, non-photochemical quenching; qP, photochemical quenching parameter; SDS, sodium dodecyl sulfate; TL, thermoluminescence; T_M, thermoluminescence peak temperature; Tricine, N-(tris(hydroxymethyl)methyl)glycine.

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can potentially result in generation of reactive oxygen species (ROS) such as $^1O_2$ and O_2^-, leading to photoinhibition and photooxidative damage of photosystem II (PSII) [7–13]. Apart from PSII, various environmental stress conditions can also cause photoinhibitory damage on photosystem I (PSI) [14–22].

This potential for photoinhibition makes it necessary for the plant to develop mechanisms for photoprotection of the photosynthetic apparatus. The major photoprotective mechanism playing a key role for de-excitation of excess light energy in green plants and algae is considered to be the ΔpH- and zeaxanthin (Zx)-dependent non-photochemical quenching (NPQ) occurring in the pigment bed of LHCII proteins [23–26], although alternative/supplementary photoprotective mechanisms for effective thermal deactivation of excess light energy have also been proposed [13,24,27–29].

In addition to its essential role in development of NPQ, light-harvesting Chl *a*/*b*-binding protein complex of PSII (LHCII) is the major component of the chloroplast thylakoid membranes, which mediates their macrostructural arrangement (granal stacking), and is believed to regulate the excitation energy transfer between photosystem I (PSI) and photosystem II (PSII) *via* redox-dependent reversible phosphorylation of its major component [13,29,30–34]. LHCII is also known to be involved in adaptation of plants to the light environment [35] and is dynamically regulated by short-term or long-term changes in the environmental growth conditions such as temperature, irradiance and nutrient availability [6,36].

Electron crystallography at 3.4 Å resolution of LHCII crystals from pea revealed that LHCII exists in the trimeric form, which is believed to predominate in the thylakoids *in vivo* [37,38]. The importance of high ordered oligomeric structural organization of LHCII for the dynamics and functioning of the photosynthetic membranes has been well established [39–43]. Indeed, light induced LHCII trimer to monomer transitions as well as LHCII trimer–trimer interactions in higher plants have been suggested to have significant impact on light harvesting/quenching capacity of thylakoid membranes *in vivo* [42,43]. It has been suggested that the xanthophylls cycle pigments loosely bound to the periphery of LHCII, are important element in stabilizing the structure of LHCII trimer aggregates [25,44,45]. More importantly, it has been demonstrated that trimeric organization of LHCII is better adapted for efficient light harvesting, exhibit enhanced protein stability and possess the optimal capacity for non-radiative energy dissipation [46,47]. However, the traditional view of the organization of PSII and PSI as separate entities has been challenged recently. New evidence indicates that PSII and PSI are energetically connected because they are embedded in a common lake of LHCII in *Arabidopsis thaliana* [13,29,48,49].

In view of these versatile and important roles of LHCII trimers in functioning of the photosynthetic apparatus, the impact of structural organization of the LHCII complexes was assessed in a *Costata 2/133* pea mutant with altered pigment content and decreased level of LHCII oligomerization [1,2,50]. The response of the *Costata* mutant to high light stress at low temperature was compared to wild type plants by *in vivo* measurements of PSII and PSI photochemical performance and pigment and polypeptide composition of thylakoid membranes. We demonstrate that while exposure of the *Costata 2/133* mutant to excess light caused stronger inhibition of both PSII and PSI photochemistry compared to wild type plants, PSI photochemistry is more severely affected. The higher susceptibility to photoinhibition of PSI correlates with lower abundance of PSI-related proteins in the mutant.

2. Materials and methods

2.1. Plant material

Wild type (WT) (*Pisum sativum* L. cv. Borek) and *Costata 2/133* mutant with altered pigment content and decreased level of LHCII oligomerization [1,2,50] pea plants were germinated from seeds in coarse vermiculite with 16 h light/dark period in controlled environment growth chambers (Conviron, Winnipeg, MB, Canada). Fluorescent tubes (Cool White, 160 W, F72T12/CW/VHO, Sylvannia, Drummondville, QC, Canada) provided PAR which was adjusted to 250 μmol photons m^{-2} s^{-1} PPFD. Day/night temperatures were 20°C/16 °C. Relative humidity was 50%. Fully expanded leaves harvested 2–3 h after the beginning of the light cycle were used in all experiments.

2.2. Non-denaturating SDS–PAGE

Isolation of thylakoid membranes for non-denaturating SDS–PAGE was performed as described previously [1]. Chloroplast membranes for electrophoretic separation of Chl–protein complexes were prepared according to [51]. Samples were resuspended in a deoxycholic acid (DOC):SDS:Chl ratio of 20:10:1 in a 0.3 M Tris–HCl (pH 8.0) solubilization buffer containing 13% (w/v) glycerol. Separation of the chlorophyll–protein complexes by non-denaturing SDS–PAGE was performed on an 8% (w/v) polyacrylamide resolving gel containing 150 mM Tris–HCl (pH 6.35) buffer and a 4% (w/v) stacking gel containing 40 mM Tris–HCl (pH 6.14) buffer. Samples were loaded with an equal amount of protein (20 μg per line). Protein concentration was determined using Pierce BCA Protein Assay Kit-Reducing Agent Compatible (Thermo Scientific, USA). The excised lanes were scanned at 671 nm on a Beckman DU 640 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA, USA).

2.3. SDS–PAGE and immunoblotting

Thylakoid membranes for SDS-PAGE WT type and *Costata 2/133* mutant of pea were isolated as described earlier [52]. Benzamidine and aminocaproic acid were present in the homogenization buffer at concentrations of 2 mM. Samples containing equal amounts of protein were separated on a 15% (w/v) linear polyacrylamide gel and electrophoretically transferred to a nitrocellulose membrane (0.2 μm pore size, Bio-Rad) at 5 °C for 2 h at 25 mA. Immunoblot analysis was performed as in [1] and the thylakoid proteins were detected with specific antibodies at the following dilutions: PsbA (D1), 1:5000; Lhcb2, 1:5000, Lhcb3, 1:5000; PsaA/B, 1:500; PsaD, 1:1000; Lhca1, 1:2000. All antibodies, except the antibody against PsaA/B heterodimer, were obtained from AgriSera AB (Vanas, Sweden). PsaA/B antibody was acquired as described in [51]. After incubation with anti-rabbit horseradish peroxidase-conjugated secondary antisera (Sigma–Aldrich, St. Louis, 1:20,000 dilution), the antibody complexes were visualized by incubation of the blots in ECL™ chemiluminescent detection reagents (Amersham Biosciences, GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA) and developed on Cronex 4 X-ray film (Kodak). Immunoblots were performed on samples from at least three independent replicate experiments. Densitometric scanning and analysis of X-ray films from each replicate immunoblot was performed with a Hewlett Packard ScanJet 4200C desktop scanner and ImageJ 1.41o densitometry software (Wayne Rosband, National Institute of Health, USA, http://rsb.info.nih.gov.ij).

2.4. Modulated chlorophyll fluorescence

Chlorophyll *a* fluorescence of a dark adapted (30 min) leaves of WT and *Costata 2/133* pea mutant plants was measured under ambient CO_2 conditions using a PAM 101 chlorophyll fluorescence measuring system (Heinz Walz GmbH, Effeltrich, Germany) as described in [18,53]. Alternatively, a modulated imaging fluorometer (IMAGING-PAM, Heinz Walz GmbH, Effeltrich, Germany) was used for capturing the chlorophyll fluorescence images and