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Research Article

The PsbS protein plays important roles in photosystem II supercomplex remodeling under elevated light conditions☆

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ABSTRACT

Leaves from three different *Arabidopsis* lines with different expression levels of PsbS protein showed different levels of non-photochemical quenching. The PsbS deficient plant *npq4* showed remarkable reduction of electron transport rate, while the other two lines with a moderate amount (wild type) or an overexpression of PsbS (*L17*) presented unchanged electron transport rates under the same range of high light intensities. Biochemical investigation revealed that the plant with the highest PsbS content (*L17*) sustained the highest level of stable PSII–LHCII supercomplex structure, and displayed the smallest fluorescence quenching in the thylakoid membranes, the most efficient linear electron transport and the smallest cyclic electron transport. Based on these observations, it is proposed that the remodeling of PSII–LHCII supercomplexes affected by PsbS plays important roles in regulating the energy balance in thylakoid membrane and in ensuring the sophisticated coordination between energy excitation and dissipation.

Introduction

Solar light is the ultimate source for photosynthesis. However, too much light brings about over-excitation that leads to photodamage of the photosynthetic apparatus. During 3.5 billion years of evolution, photosynthesis has developed sophisticated thylakoid membranes that manage to harvest and convert enough energy for carboxylation, and to avoid harmful over-excitation to protect the photosynthetic apparatus at the same time (Kanervo et al., 2005; Takahashi and Badger, 2011). The two important chlorophyll (Chl) protein supercomplexes for harnessing solar energy are photosystem (PS) II and PSI. The two PSs are both multi-component supercomplexes containing about 20 subunits, each with a reaction center (RC) and the adhering light harvesting complexes (LHC). One of the significant features of the thylakoid membrane ensuring a highly effective usage of the excitation energy is the lateral segregation of grana and stroma organization in higher plants, which separates "the slow process of PSII" from "the quick process of PSI" (Trissl and Wilhelm, 1993). PSII, combining more than half of the photosynthetic pigments, is located in the pigment–protein densely occupied grana region where the most important processes for harnessing solar energy occur and therefore, it is also the main target of over-excitation attack (Barber, 2002; Nixon et al., 2010). It is proposed that the assembly of the Chl *b* containing light harvesting protein in PSII is the basis for grana stacking, which is always accompanied by the PsbS assembly (Wilk et al., 2013). This indicates clearly that the development of a system for enhanced quantum efficiency of photosynthesis is synchronized by the development of a capacity for harmless dissipation of excess excitation energy (Chow et al., 2000).

Among all the mechanisms adapting to excess light conditions, the most important one is the non-photochemical quenching (NPQ) that dissipates the over-excitation into heat within a time range from minutes to hours (Horton et al., 2005; Goss and Lepetit, 2015). In photosynthesis, two different types of NPQ mechanisms have been characterized, one being LHCSR-dependent and the other PsbS-dependent (Niyogi and Truong, 2013). The LHCSR-dependent

Abbreviations: AL, actinic light; BN-PAGE, Blue native polyacrylamide gel electrophoresis; CEF, cyclic electron flow; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_0, the minimum fluorescence intensity in the dark-adapted state; F_m, the maximum fluorescence intensity in the dark-adapted state; F'_m, the maximum chlorophyll fluorescence intensity of leaves exposed to actinic light; FR, far red; F_t, the steady state fluorescence intensity during illumination; F_v, variable fluorescence; F_v/F_m, maximal photochemical efficiency of PSII; HL, high light; LHC, light harvesting complexes; ML, measuring light; NPQ, non-photochemical quenching; PAM, pulse amplitude modulation fluorometer; PS, photosystem; PSI, photosystem I; PSII, photosystem II; Φ_{PSII}, quantum yield of PSII; rETR, relative electron transport rate; SP, saturating pulse; β-DM, dodecyl-β-D-maltoside; RC, reaction center; SDS, sodium dodecyl sulfate; WT, wild type.

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NPQ is active in eukaryotic photosynthetic algae, while the PsbS-dependent NPQ is a product of land colonization (Gerotto and Morosinotto, 2013). It has been found that the moss *Physcomitrella patens*, diverging from vascular plants after the land colonization, contains both LHCSR and PsbS actively responding to excess light conditions (Gerotto et al., 2011). Later on, PsbS has been found in species as early as the late Streptophyte algae, *Ulva prolifera* and *Ulva Linza* (Gerotto and Morosinotto, 2013; Mou et al., 2013; Zhang et al., 2013), which evolved the same mechanisms as higher plants responding to high light intensity. These findings suggest that both PsbS-dependent and LHCSR-dependent NPQ may co-exist during the photosynthesis evolution. For more details see Büchel (2015) in this issue. More research proposed that PsbS was the second protein that appeared during the evolution of the Lhcb protein family, directly after the emergence of CP29 (Koziol et al., 2007). Analyzing the trace of photosynthesis evolution, it is clear to see that the appearance of PsbS, accompanying earlier or later the appearance of all the Chl *b* binding proteins, the membrane embedded peripheral antenna of PSII, and the formation of PSII–LHCII supercomplexes, is related to or facilitates the appearance of grana organization. Incomplete grana formation is found in primitive green algae which may have played a special role in the transition from the aquatic environment to landlife (Quaas et al., 2015). PSII–LHCII supercomplexes, which contains homodimeric PSII core with three minor antenna and different amounts of major antenna, provide the basis for forming the semicrystalline arrays and grana stacking (Dekker and Boekema, 2005) that facilitates the excitation energy transfer among different PSII complexes (Chow et al., 2005). It is reasonable to propose that, unlike LHCSR that is a pigment-binding protein, PsbS is not likely to bind any Chl, which may facilitate the adjustment of energy utilization in the grana membrane characteristically packed densely with pigments (Bonente et al., 2008). This proposal can be supported by several evidences. PsbS has been found to be very tightly combined to PSII supercomplexes (Nield et al., 2000; Thidholm et al., 2002; Haniewicz et al., 2013), and plays important roles in regulating the interaction between LHCII and PSII and the amount of semi-crystalline structure in the grana membrane (Kiss et al., 2008; Kereïche et al., 2010; Goral et al., 2012). The existence of PsbS in thylakoid membranes leads to an increase in the fluidity of the membrane that facilitates the reorganization of PSII macro-structure, which in turn, assists the protein migration under strong light conditions (Kirchhoff et al., 2011; Goral et al., 2012; Kirchhoff, 2013). Besides, PsbS has been demonstrated to be very important in regulating the dissociation of a LHCII assembly composed of CP29, CP24 and LHCII, the prerequisite for inducing NPQ under strong light conditions (Betterle et al., 2009).

Besides its effect on grana membrane reorganization and membrane fluidity, the protonation of PsbS induced by increased *trans*-thylakoid membrane pH gradient under excess light conditions plays important roles in the xanthophyll cycle, which converts violathantin to zeathantin (Li et al., 2002b), which in turn induces several quenching centers for LHCII, or changes in the energy transport in the thylakoid membrane (Li et al., 2002b, 2004). To summarize, PsbS plays important roles in triggering overall NPQ responses to high light conditions, dissipating over-excitation harmlessly to heat.

Although PsbS is found to be very important in triggering NPQ under elevated light conditions and in regulating the semi-crystalline structure of grana membrane, its significance in PSII supercomplex remodeling is still not demonstrated. In the present study, three different *Arabidopsis* lines, namely a wild type (WT), and two mutant lines, one lacking the PsbS expression, and the other with an overexpression of PsbS, were employed to study the characteristics of PsbS's function responding to excess light conditions. The responses in photosynthetic performances of different *Arabidopsis* lines upon starting of excess light treatment were investigated. PsbS's role in regulating the grana stacking and its evolutionary significance were discussed.

Methods

Plant materials and growth conditions

The *Arabidopsis thaliana* (ecotype Columbia) T-DNA insertion line *npq4* (SALK_095156) were obtained from the Arabidopsis Biological Resource Center (Ohio State University, Columbus, OH). Seeds for *L17* were a kind gift of Prof. Krishna K. Niyogi. Seeds of WT, *npq4* and *L17* were imbibed in the dark at 4 °C for 2 days to ensure synchronized germination and then transferred to growth chamber (100 μmol photons m^{-2} s^{-1}, 12 h photoperiod at 22 °C, 70% relative humidity). Mature rosette leaves from 5-week-old plants were used for experiment. During high irradiance treatments, detached leaves, floating with the adaxial side on water, were maintained at 1200 μmol photons m^{-2} s^{-1} with light passed through a heat filter to avoid overheating the floating leaves. The temperature was maintained at 22 °C in a temperature-controlled chamber.

Pigment analysis

Pigments were extracted from leaf discs of known area, either dark-adapted or light-treated (1200 μmol photons m^{-2} s^{-1}, 30 min), at 4 °C. Samples treated were frozen in liquid nitrogen, and then leaves were ground and pigments were extracted with 80% acetone. The homogenates were centrifuged (10 min at 15,000 × *g*, 4 °C), and then the supernatant was taken. The pigments were quantified as described in Porra et al. (1989).

Thylakoid membrane preparation

Preparation of thylakoid membrane proteins was performed as described previously (Zhang et al., 1999). The leaves were homogenized in an ice-cold isolation buffer containing 400 mM sucrose, 50 mM HEPES-KOH (pH 7.8), 10 mM NaCl, and 2 mM $MgCl_2$. The homogenate was then filtrated through four layers of cheesecloth. The filtrate was centrifuged at 5000 × *g* for 10 min. The pellets were washed twice with isolation buffer and finally resuspended in the same buffer.

Electrophoresis analysis and immunoblot analysis

Blue native polyacrylamide gel electrophoresis (BN-PAGE) was performed according to Peng et al. (2006). The thylakoid membranes were washed twice, and then solubilized at a final Chl concentration of 0.5 mg ml^{-1} with 1% dodecyl-β-D-maltoside (β-DM) at 4 °C for 5 min. After centrifugation (12,000 × *g* for 10 min at 4 °C), the supernatant was used for electrophoresis carried out with a 5–12% polyacryamide gradient gel at 4 °C.

The amounts of PsbS protein and the phosphorylation of CP43 were analyzed with protein blot analysis. The protein components of the thylakoid membrane were separated in a 15% sodium dodecyl sulfate (SDS)-PAGE containing 6 M urea (Laemmli, 1970) and protein blot analysis was performed according to standard techniques by probing with specific primary antibodies (against PsbS or anti-phosphothreonine, purchased from Agrisera or Cell Signaling respectively). DyLight™800-labeled secondary antibody (Kirkegaard and Perry Laboratories, Inc., USA) was used for infrared visualization of protein bands. Quantification of protein was done with the Odyssey Infrared Imaging System (Li-Cor Biosciences, Lincoln, NE).