Functional Analysis of PsbP-Like Protein 1 (PPL1) in Arabidopsis

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Abstract: Higher plants have a number of PsbP homologs (PsbP-like proteins: PPLs, PsbP-domain proteins: PPDs) in addition to the authentic PsbP in the oxygen-evolving complex of photosystem II (PSII). Among the PsbP homologs, the PPL1 protein is most homologous to a cyanobacterial PsbP (cyanoP), and we previously reported that PPL1 is required for the efficient repair of photo-damaged PSII under high light conditions [Ishihara *et al*. (2007) Plant Physiol. 145: 668-679]. However, functional role of PPL1 in the PSII repair cycle has not been clarified yet. In this study, we further investigated molecular function of PPL1 by characterizing the phenotypes of the PPL1 knockdown plants (*ppl1i*) in which PPL1 expressions were differently suppressed. Although growth of the *ppl1i* mutants under low intensity light was comparable with that of wild type plants, PSII activity of the *ppl1i* mutants was more sensitive to high intensity light and the extent of photoinhibition was correlated with the levels of the knocked-down *PPL1*. The possible functional role of PPL1 in PSII repair is discussed.

Keywords: Photosystem II; Photoinhibition; PsbP homolog; PSII repair cycle; Thylakoid lumen

Introduction

It is known that the composition of the extrinsic subunits of photosystem II (PSII) in the lumenal side of thylakoids are significantly different among the photo-oxygenic organisms (Seidler, 1996): higher plants and green algae have a set of three extrinsic proteins [PsbO (33 kD), PsbP (23 kD), and PsbQ (17 kD)], whereas cyanobacteria have a different set of proteins [PsbO, PsbU (12 kD), and PsbV (cytcrome *c*550)] (Enami *et al*., 2008). In addition, recent genomic and proteomic studies have demonstrated that prokaryotic cyanobacteria have a homolog of PsbP (cyanoP) and PsbQ (cyanoQ). Furthermore, it turns out that higher plants have a number of PsbP and PsbQ homologs (PsbP-like proteins, PPL; PsbP-domain proteins, PPD; PsbQ-like protein, PQL) in thylakoid lumen (Roose *et al*., 2007). Significant progresses have been made to understand the functions of PsbP and PsbQ homologs in higher plants (Ifuku *et al*., 2008, 2010). However, further research is still needed to elucidate their molecular function fully.

Among the PsbP homologs, PPL1 is most homologous to cyanoP. We previously reported that PPL1 seems to express under stress conditions (Ishihara *et al*., 2007). In fact, PSII activity in an Arabidopsis mutant lacking PPL1 (*ppl1*) was more sensitive to high-intensity light than wild type, and the recovery of photoinhibited PSII activity was delayed in *ppl1* mutants. Therefore, we conclude that PPL1 is required for efficient repair of photodamaged PSII. However, the protein-protein interaction between PPL1 and PSII was not detected, so that functional role of PPL1 in the PSII repair cycle has not been clarified yet.

In this study, we characterized phenotypes of the RNAi mutants (*ppl1i*) in which PPL1 expressions are differently suppressed. Preliminary results of biochemical studies about PPL1 are also introduced.

Materials and Methods

Growth conditions

Arabidopsis wild-type (ecotype Columbia-0, Col-0),

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and the *ppl1i* plants were grown in soil under growth chamber conditions (10/14 h light-dark photoperiod at 50 µmol photons m^{-2} s^{-1}, 21 °C).

Production of the ppl1i transgenic line

The following PCR primers were used to amplify the 408 bp fragment of *PPL1* cDNA: 5'-CACCTGCT CCTTGGATCTCATTGC-3', 5'-AACCGTGATGGTA CCCAGAG-3'. The PCR products were cloned into pENTR/D-TOPO vector and then transferred into pHellsgate8 vector by a LR recombination reaction (Gateway, Invitrogen). The plasmids pHellsgate8-PPL1 was used to transform Col-0 via *Agrobacterium tumefaciens* strain GV3101 by floral dip method. Seeds were collected and the transformants were selected on the medium containing Murashige and Skoog salt mix, 50 µg ml^{-1} kanamycin, and 0.8% agar.

SDS-PAGE and Immunoblotting

Total proteins extracted from 5-week-old leaves were solubilized and separated on 12.5% SDS-polyacrylamide gels including 6 mol urea. Separated proteins were transferred to a PVDF membrane using a semidry blotting system (Bio-Rad). Detection was performed with ECL-plus reagent (GE Healthcare).

RT-PCR Analysis

Total RNA was extracted from 3-week-old leaves and cDNA was reverse transcribed using Super Script III reverse transcriptase (Invitorogen). The *PPL1* cDNA was then amplified using following primers: *PPL1* (5′-CACCATGGCTTCTCTGAAGCTTTCAC-3′ and 5′-TCAAACAGTGATCTTGAAGGAATCT-3′). The products were visualized by ethidium bromide staining after agarose gel electrophoresis.

Photoinhibition Assay

Detached leaves from 5 weeks-old plants were placed adaxial side up on the filter papers steeped with water and illuminated at 650 µmol photons m^{-2} s^{-1}. Maximum efficiency of PSII (F_v/F_m) was monitored during exposure to an irradiance of high-intensity light. The measurements were done after 10 min dark-adaptation with Mini-PAM chlorophyll fluorometer (Walz, Germany).

Result and Discussion

Our previous report showed that PSII activity in an Arabidopsis mutant lacking PPL1 (*ppl1*) was more sensitive to high-intensity light than wild type. However, this *ppl1* mutant (Salk_014843) has a T-DNA insertion in the intron of the *PPL1* gene and residual *PPL1* expression was expected (Ishihara *et al.*, 2007). Therefore, we tried to establish the RNAi transgenic lines (*ppl1i*) in which *PPL1* expressions are severely suppressed. In addition, we expected to analyze whether *PPL1* knockdown affects PSII activity in a dose-dependent manner.

When three independent transgenic lines (*ppl1i-1*, *ppl1i-2*, *ppl1i-3*) were analyzed, all transgenic lines showed the severe suppression of the expressions of the *PPL1* gene (At3g55330) in both transcript (Fig. 1B) and protein (Fig. 1C) levels. Among the *ppl1i* transgenic lines, the *ppl1-2* plants showed the lowest accumulation of PPL1 protein (Fig. 1C). Growth of *ppl1i* transgenic plants under 50 µmol photons m^{-2} s^{-1} (moderate light condition) was comparable with that of wild type, which is consistent with the previous observation using the *ppl1* mutant (Fig. 1D).

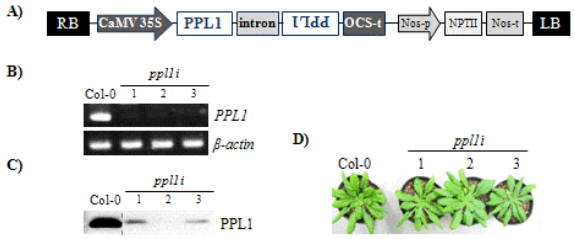

Fig. 1 Production of the *ppl1i* transgenic line A) RNAi construct. A 408bp fragment of the *PPL1* gene was amplified by RT-PCR and cloned into pHELLSGATE8 vector. B) The mRNA expression level of *PPL1* was determined by RT-PCR. *β-actin* was analyzed as control. C) Immunodetection of PPL1. Total protein (30 µg) was used for the SDS-PAGE / Immuno-blot analysis D) Visible phenotypes of Arabidopsis wild-type (Col-0) and the *ppl1i* plants grown in soil for 5 weeks under moderate light condition (~50 µmol photons m^{-2} s^{-1}).

To examine the performance of PSII in wild type and *ppl1i* leaves, detached leaves were irradiated with high-intensity light, and the changes in the F_v/F_m value were monitored by a PAM fluorometer. Wild type leaves showed little decline in F_v/F_m even under high-intensity light condition (650 µmol photons m^{-2} s^{-1}). On the other hand, the F_v/F_m value in the *ppl1i* leaves was decreased to 60%–70% of the initial value within 360 minutes of illumination. Among the *ppl1i* transgenic lines, *ppl1i-2*, in which the expression level of PPL1 protein was most severely reduced, showed

greatest reduction in F_v/F_m (Fig. 2). This suggests that the *PPL1* knockdown affects PSII activity in dose-dependent manner. In the presence of chloramphenicol that inhibits plastid protein synthesis, F_v/F_m decreased similarly in both wild type and the *ppl1i* leaves to ~40% within 360 minutes of illumination (data not shown). These data are fully consistent with our previous observation (Ishihara *et al*., 2007), and demonstrate that *PPL1* knockdown slows the rate of PSII repair but does not accelerate the rate of the PSII photodamage.

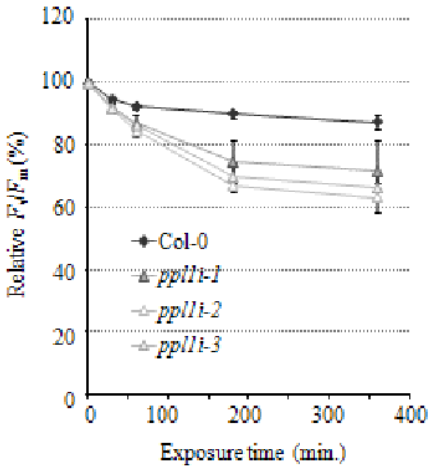

Fig. 2 Time course of photoinhibition under high-intensity light condition Detached leaves were illuminated at 650 µmol photons m^{-2} s^{-1}. Maximum efficiency of PSII (F_v/F_m) was measured during exposure to an irradiance of high-intensity light after 10 minutes of dark-adaptation. Values were averages ±SD (n = 3).

Recently, we observed that PPL1 was mainly localized in the stroma-exposed (unstacked) thylakoid regions where the PSII repair is thought to be conducted (Mulo *et al*., 2008) (data not shown). This is compatible with the results showing that PPL1 is involved in PSII repair. Furthermore, PPL1 was fractionated in relatively high-density fractions in a sucrose density gradient ultracentrifuge analysis (data not shown), suggesting that PPL1 should interact with some protein complexes. Because PPL1 knockdown affected PSII repair in dose-dependent manner (Fig. 2), it is possible that PPL1 may interact with and stabilize the PSII assemblies during the repair of photo-damaged PSII in stroma-exposed thylakoid regions. Further biochemical analyses to identify proteins or protein complexes interacting with PPL1 will clarify functional roles of PPL1 in the PSII repair cycle.

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