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Insights into the function of PsbR protein in *Arabidopsis thaliana*

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Abstract

The functional state of the Photosystem (PS) II complex in Arabidopsis *psbR* T-DNA insertion mutant was studied. The ΔPsbR thylakoids showed about 34% less oxygen evolution than WT, which correlates with the amounts of PSII estimated from Y_D^{ox} radical EPR signal. The increased time constant of the slow phase of flash fluorescence (FF)-relaxation and upshift in the peak position of the main TL-bands, both in the presence and in the absence of DCMU, confirmed that the $S_2Q_A^-$ and $S_2Q_B^-$ charge recombinations were stabilized in ΔPsbR thylakoids. Furthermore, the higher amount of dark oxidized Cyt-b559 and the increased proportion of fluorescence, which did not decay during the 100s time span of the measurement thus indicating higher amount of $Y_D^+Q_A^-$ recombination, pointed to the donor side modifications in ΔPsbR. EPR measurements revealed that S_1-to-S_2-transition and S_2-state multiline signal were not affected by mutation. The fast phase of the FF-relaxation in the absence of DCMU was significantly slowed down with concomitant decrease in the relative amplitude of this phase, indicating a modification in Q_A to Q_B electron transfer in ΔPsbR thylakoids. It is concluded that the lack of the PsbR protein modifies both the donor and the acceptor side of the PSII complex.

Keywords: Arabidopsis; Photosynthesis; Photosystem II; PsbR; Thylakoid membrane

1. Introduction

Photosystem (PS) II is a multisubunit pigment protein complex embedded in the thylakoid membrane. In oxygen-evolving photosynthetic organisms the oxidation of water occurs at a tetramanganese-cluster, known as a water oxidizing complex (WOC). The WOC is bound to the reaction center of the PSII complex [1].

The mechanism of water oxidation is still unknown. Although many models have been suggested, the site of water binding and oxidation has not been unequivocally identified. There are a number of studies in the literature concerning the structure and assembly of the WOC, particularly the position and function of extrinsic WOC proteins [2]. In higher plants three extrinsic proteins (PsbO, PsbP, PsbQ) with the molecular masses of 33, 23 and 17 kDa are bound to the lumenal surface of the PSII complex. Modeling of WOC and its association with the reaction center of the PSII complex has demonstrated that the position of the PsbO protein is the same as in cyanobacteria, whereas PsbP and PsbQ are located in different positions as compared to the cyanobacterial PsbU and PsbV [3]. The PsbO protein is the most strongly bound extrinsic protein. Some experimental evidence suggests that the PsbP protein cannot bind efficiently to the PSII complex in the absence of PsbO and that the binding of PsbQ requires the presence of PsbP [4]. PsbP not only interacts with PsbO but also with the lumenal surface of the PSII core in the vicinity of CP43. There is some evidence that PsbP can bind weakly to the lumenal surface of the PSII complex even in the absence of PsbO [5].

Abbreviations: Chl, chlorophyll; DCMU, 3-(3′,4′-dichlorphenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; DMBQ, 2,6-dimethyl-*p*-benzoquinone; DPC, 2,2′-diphenylcarbonic dihydrazide; EPR, electron paramagnetic resonance; F_v, variable fluorescence yield; PSII, Photosystem II; Q_A and Q_B, primary and secondary quinone acceptors in PSII; Y_Z and $Y_Z^\bullet$, tyrosine 161 of the PSII D1 polypeptide and its radical; Y_D and $Y_D^\bullet$, tyrosine 161 of the PSII D2 polypeptide and its radical; TL, thermoluminescence

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While the structures of the extrinsic proteins have become solved, their function still remains obscure [6,7]. PsbO pretein is known as a Mn-stabilizing protein. In addition to the Mn ions, the ionic cofactors, especially Ca^{2+} and Cl^{-} are essential for the activity of WOC, although their specific functions still remain unknown [2]. The two extrinsic proteins—PsbP and PsbQ have been proposed to facilitate the binding of Ca^{2+} and Cl^{-} cofactors, to the WOC [8,9]. There is evidence that Ca^{2+} is required for the structure of the Mn cluster of the WOC [10] and modulates electron transfer between Y_z and $P680^{+}$ [11,12]. It has also been reported that Ca^{2+} influences the redox potential of Cyt-b559 [13] and Q_A [14], as well as the oxidation of Q_A^{-} [11,12]. In spite of the fact that the role of Ca^{2+} in water oxidation by PSII has been extensively studied over the years [2], a clear understanding of the involvement of Ca^{2+} ions in the function of the PSII complex is still missing. The importance of Ca^{2+} in oxygen evolution was realized when characterizing preparations, where the PsbP and PsbQ extrinsic proteins had been removed by 2 M NaCl treatment. After this treatment the electron transfer from the Mn cluster to $Y_z^{\bullet +}$ was inhibited, however addition of Ca^{2+} at millimolar concentration restored oxygen evolution to a large extent [10]. Later it was shown that the loss of oxygen evolving activity is due to the loss of Ca^{2+} rather than the PsbP and PsbQ proteins. These extrinsic proteins are thought to shield WOC and modulate the affinity of the Ca^{2+} binding site. Cl^{-} ions have been proposed to regulate the redox properties of WOC, which are readily interconvertible by addition or removal of Cl^{-}. Also the involvement of Cl^{-} in the process of PSII photoactivation has been suggested [15].

Recently PsbP has been suggested to play an important role in providing Mn during the process of photoactivation [16]. This protein also has structural features, which suggest that it might be a GTPase activating protein [6].

Besides PsbO, PsbP and PsbQ yet another PSII protein, PsbR, is thought to exert important structural stability to the WOC and is probably also required for proper function of the PSII complex [17,18].

In order to investigate the role of the PsbR protein in higher plant PSII, we have here functionally characterized the Arabidopsis T-DNA insertion mutant *psbR*.

2. Materials and methods

2.1. Plant material and isolation of thylakoid membranes

Arabidopsis thaliana L. ecotype Columbia (Col-0) plants were grown at 23 °C under 60-80 μE m^{-2} s^{-1} light intensity in 8-h light/16-dark photoperiod (for more details see [18]). The *psbR* T-DNA insertion line (SALK_114469) was purchased from the Salk Institute. PCR analysis of the plants confirmed that mutant was homozygous [18]. Fully-grown rosettes were used for the experiments. Leaves were homogenized in ice-cold buffer containing 40 mM HEPES/NaOH pH 7.6, 5 mM $MgCl_2$, 10 mM NaCl, 2 mM EDTA, 0.4 M sucrose, and 2 g/L BSA, 2 g/L NaAsc. The homogenate was filtered through Miracloth and centrifuged for 4 min at 6000×*g*. The resulting pellet was washed with 10 mM HEPES/NaOH pH 7.5, 5 mM $MgCl_2$, 10 mM NaCl and finally re-suspended in 40 mM Hepes/NaOH pH 7.0, 5 mM $MgCl_2$, 15 mM NaCl, and 0.4 M sucrose and stored at −80 °C until use. Unstacked thylakoids were isolated by the same procedure, however, $MgCl_2$ and NaCl were excluded from all buffers. PSII-enriched membranes were isolated as described by Berthold et al. [19].

Chlorophyll was extracted in 80% (v/v) buffered acetone and quantified as described [20].

2.2. Measurements of electron transport activity

Light-saturated steady-state rates of oxygen evolution from thylakoids were measured at 20 °C with Clark-type oxygen electrode (Hansatech, King's Lynn, UK) using 1 mM DMBQ as an artificial electron acceptor. Electron transfer from H_2O to DCIP and from H_2O+DPC to DCIP was measured as DCIP photoreduction at 560 nm in Shimadzu UV3000 spectrophotometer equipped with side illumination as described in [21].

2.3. Fluorescence measurements

Flash induced increase and subsequent relaxation of the chlorophyll fluorescence yield (FF-relaxation) were measured by a double-modulation fluorometer (Photon System Instruments, Brno, Czech Republic) in the 150 μs–100 s time range. Samples at 10 μg Chl mL^{-1} concentration were dark adapted for 5 min before fluorescence detection. Multicomponent deconvolution of the relaxation curves was performed by using a fitting function with two exponential and one hyperbolic component as shown earlier [22]. The nonlinear correlation between the fluorescence yield and the redox state of Q_A was corrected by using the Joliot model [23] with a value of 0.5 for the energy-transfer parameter between PSII units. For in vivo measurements DCMU (50 μM) was vacuum infiltrated into leaf discs. After 10 min dark adaptation FF-relaxation was measured as described above.

2.4. Thermoluminescence

Thermoluminescence (TL) was measured with home-build apparatus as described in [24]. Samples were excited with single turnover (2 μs, 1 Hz) Stroboslave Xenon flashes at −10 °C in the presence or absence 40 μM DCMU. TL curves were recorded from −40 to +80 °C at a heating rate 40 °C min^{-1}.

2.5. EPR spectroscopy

X-band low-temperature EPR spectra were recorded at 9.43 GHz microwave frequency with a Bruker ELEXSYS E500 spectrometer equipped with an Oxford-900 cryostat and ITC-503 temperature controller (Oxford Instruments Ltd, UK). Samples were pre-illuminated at room light for 1 min to fully oxidize Y_D and incubated in the dark for 5 min before freezing. The S_2-state multiline EPR signal was induced by illumination at 200 K for 6 min as described in [25]. Oxidation level of Cyt-b559 in the dark adapted samples was estimated from the amplitude of the EPR-signal in g_z-region ($g \sim 3.0$). Full oxidation of Cyt-b559 in PSII centers was achieved by illumination at 77 K for 6 min as in [25]. Analysis of EPR spectra was performed using Bruker Xepr 2.1 software.

3. Results

We have functionally characterized the T-DNA insertion mutant line SALK_114469, having an insert located in the third exon of the *psbR* gene (At1g79040). ΔPsbR mutant plants showed no specific phenotype under normal growth conditions when compared to WT plants (for more details see [18]).

3.1. Reduced amount and less activity of the oxygen evolving PSII complex in ΔPsbR mutant

Steady-state oxygen evolution measurements, the FF-relaxation, TL and EPR spectroscopy were applied to clarify the functional state of the PSII complex in an Arabidopsis *psbR* T-DNA insertion mutant. The ΔPsbR mutant thylakoids