Biochimica et Biophysica Acta 1807 (2011) 205–215

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Electron paramagnetic resonance study of the electron transfer reactions in photosystem II membrane preparations from *Arabidopsis thaliana*

Guiying Chen [a], Yagut Allahverdiyeva [b], Eva-Mari Aro [b], Stenbjörn Styring [a], Fikret Mamedov [a,*]

[a] Department of Photochemistry and Molecular Science, Ångström Laboratory, Box 523, Uppsala University, SE-751 20 Uppsala, Sweden
[b] Department of Biochemistry and Food Chemistry, Plant Physiology and Molecular Biology, University of Turku, FI-20014 Turku, Finland

ARTICLE INFO

Article history:
Received 1 August 2010
Received in revised form 6 October 2010
Accepted 8 October 2010
Available online 15 October 2010

Keywords:
Photosystem II
Electron paramagnetic resonance
Arabidopsis thaliana

ABSTRACT

Arabidopsis thaliana is widely used as a model organism in plant biology as its genome has been sequenced and transformation is known to be efficient. A large number of mutant lines and genomic resources are available for *Arabidopsis*. All this makes *Arabidopsis* a useful tool for studies of photosynthetic reactions in higher plants. In this study, photosystem II (PSII) enriched membranes were successfully isolated from thylakoids of *Arabidopsis* plants and for the first time the electron transfer cofactors in PSII were systematically studied using electron paramagnetic resonance (EPR) spectroscopy. EPR signals from both of the donor and acceptor sides of PSII, as well as from auxiliary electron donors were recorded. From the acceptor side of PSII, EPR signals from $Q_A^- Fe^{2+}$ and $Phe^- Q_A^- Fe^{2+}$ as well as from the free Phe^- radical were observed. The multiline EPR signals from the S_0- and S_2-states of $CaMn_4O_x$-cluster in the water oxidation complex were characterized. Moreover, split EPR signals, the interaction signals from Y_Z• and $CaMn_4O_x$-cluster in the S_0-, S_1-, S_2-, and the S_3-state were induced by illumination of the PSII membranes at 5 K and characterized. In addition, EPR signals from auxiliary donors Y_D•, Chl^+ and cytochrome b_{559} were observed. In total, we were able to detect about 20 different EPR signals covering all electron transfer components in PSII. Use of this spectroscopic platform opens a possibility to study PSII reactions in the library of mutants available in *Arabidopsis*.
© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Higher plants are widely spread over terrestrial ecosystems and exhibit a high degree of diversity, which enables them to grow under very different environmental conditions. One of the reasons for such plasticity is that plants possess the most efficient and dynamic photosynthetic apparatus. Their photosynthetic machinery is situated in chloroplasts and the light harvesting and energy transduction systems are embedded into highly organized membrane structures.

Arabidopsis thaliana (hereafter *Arabidopsis*) is a small flowering plant with a modest genome size which has been sequenced in the year 2000 [1]. Since then *Arabidopsis* has been widely used as a model organism in plant biology for the following reasons: (i) it has a rapid life cycle (about 6 weeks from germination to mature seed); (ii) extensive genetic and physical maps of all 5 chromosomes of *Arabidopsis* are available; (iii) efficient transformation methods have been developed and (iv) a large number of mutant lines and genomic resources are available from Stock Centers [2–4]. All this makes *Arabidopsis* an important object also for studies of the primary photosynthetic reactions in higher plants.

In recent years, the use *Arabidopsis* mutants for detailed analysis of the photosynthetic apparatus in the thylakoid membrane has increased. This approach has been pivotal for studies focusing on regulation of light harvesting [5–7], linear and cyclic electron flow [8–12], the structure and function of photosystem I (PSI)[1], Cyt b_6f and NDH-complexes [8,10–14], oxidative, temperature stress and photoinhibition [15–18], chloroplast development and biogenesis of the photosynthetic complexes [19,20], the redox signaling in chloroplasts [19], etc.

This also holds for studies of structure and function of PSII. PSII is a large multiprotein–pigment complex which in its active form in higher plants is mostly found in the stacked granal membranes of chloroplasts [21–23]. It initiates the photosynthetic electron flow by using light energy to extract electrons from water and to reduce the pool of the plastoquinone molecules [24,25]. About ten redox active cofactors, mostly bound to the D1/D2 protein heterodimer participate in this reaction. The $CaMn_4O_x$-cluster and the redox active tyrosine

Abbreviations: Car, carotenoid; Chl, chlorophyll; Cyt b_{559}, cytochrome b_{559}; DCIP, 2,6-dichlorophenolindophenol; DPC, 2,2′-diphenylcarbonic dihydrazide; EPR, electron paramagnetic resonance; HP and LP, high and low potential forms of oxidized Cyt b_{559}; MA, modulation amplitude; NIR, near infrared; RT, room temperature; P680, primary electron donor chlorophylls in PSII; PpBQ, phenyl-*p*-benzoquinone; PSII, photosystem II; Phe, pheophytin, acceptor of PSII; Q_A and Q_B, primary and secondary quinine acceptors of PSII; S states, intermediates in the cyclic turnover of the WOC; WOC, water oxidation complex; WT, wild type; Y_D and Y_D•, tyrosine 161 of the D2 polypeptide of PSII and its radical; Y_Z and Y_Z•, tyrosine 161 of the D1 polypeptide of PSII and its radical

* Corresponding author. Tel.: +46 18 471 6581; fax: +46 471 6844.
E-mail address: fikret.mamedov@fotomol.uu.se (F. Mamedov).

0005-2728/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2010.10.010

residue, Y_Z, constitute the catalytic site — WOC, where the water oxidation takes place [26–30]. P_{680} is the primary electron donor in PSII and is composed of a tetramer of Chls. After excitation from antenna Chls, P_{680} transfers an electron to the acceptor side of PSII. P_{680}^{+} is strongly oxidizing and extracts electrons from the WOC which circulates through the five intermediate states, denoted $S_0 \rightarrow S_4$ [26,27,30,31]. After excitation, electrons from P_{680} first reduce the Phe and subsequently Q_A and Q_B, the primary and secondary quinone acceptors in PSII. After accepting two electrons and two protons, Q_B leaves PSII in the plastoquinol form.

The many redox components in PSII can be studied by a diversity of spectroscopic methods. One of the few techniques that give access to nearly all of the redox components, including the WOC in all S states, is EPR spectroscopy. With EPR spectroscopy both the structure and the function of the redox center can be studied, often in molecular details. In PSII research EPR has been applied also to studies of the effects of many site-directed mutants on for example the $CaMn_4O_x$-cluster. Most EPR work applied to mutants has been performed in cyanobacteria and algae but there also valuable EPR studies in naturally occurring mutants in plants. This does not hold for *Arabidopsis* and there are very few EPR studies in this plant despite its huge genetic importance.

The wild type and mutants in PSII subunits of *Arabidopsis* have been widely used in studies of the protein composition of PSII [32–38], electron transfer reactions and the mechanism of water oxidation [33,35,38]. However, these experiments were mostly performed in intact leaves or isolated thylakoid membranes. In this study, we have used highly active PSII enriched membranes isolated from thylakoids of *Arabidopsis* plants [38] to perform a systematic characterization with EPR spectroscopy of the electron transfer cofactors in PSII and intermediates in the water oxidation process. Our study describes nearly 20 different EPR signals representing all redox components in PSII. Thus, presented results provide the first strong spectroscopic platform for PSII studies in *Arabidopsis* and extend the molecular studies of PSII to another higher plant species.

2. Materials and methods

2.1. Plant material and isolation of PSII-enriched membranes

Arabidopsis plants (ecotype Columbia) were grown on soil under standard growth chamber conditions (23 °C, 120 μmol photons $m^{-2} s^{-1}$, a light/dark cycle 8/16 h) for 7 weeks. PSII-enriched membranes (BBY-type) were isolated from mature plant leaves according to the procedure of Berthold et al. [39] with some modifications. Leaves were ground in ice-cold buffer containing 20 mM Tricine/NaOH (pH 8.4), 0.45 M sorbitol, 10 mM Na-EDTA, 5 mM NaCl, 5 mM $MgCl_2$ and freshly added 0.2% BSA and 0.2% Na-ascorbate. The homogenate was filtered through Miracloth and centrifuged at 4200×*g* for 10 min at 4 °C. The pellet was washed with 20 mM Tricine/NaOH (pH 7.6), 0.33 M sorbitol, 5 mM $MgCl_2$ and re-suspended in a buffer containing 20 mM Tricine/NaOH (pH 7.6) and 5 mM $MgCl_2$. After centrifugation at 4200×*g*, the pellet was re-suspended in 20 mM MES/NaOH (pH 6.3), 5 mM $MgCl_2$ and 15 mM NaCl. After the Chl concentration was adjusted to 2.67 mg/ml, 1/3 volume of 20% Triton X-100 was added slowly to the sample suspension and stirred for 30 min on ice in darkness. The sample was then centrifuged at 9300×*g* for 3 min and the supernatant again at 42 000×*g* for 30 min. The pellet was re-suspended in the same buffer without Triton and again centrifuged at 42 000×*g* for 30 min. Finally, the pellet was suspended in buffer containing 20 mM Mes/NaOH (pH 6.3), 0.4 M sorbitol, 15 mM NaCl, 10 mM $CaCl_2$ and 5 mM $MgCl_2$. The Chl concentration was measured according to Porra et al. [40].

2.2. Analysis of the protein composition and general characterization

Polypeptides were separated with SDS-PAGE (15% polyacrilamide, 6 M urea) [41]. After electrophoresis polypeptides were stained with Coomassie Blue or electroblotted to a polyvinylidenefluoride membrane and immunodetected with specific antibodies. Oxygen evolution and variable fluorescence were measured as in [34]. The number of active PSII centers was determined by measuring DCIP reduction in the absence and presence exogenous electron donor DPC as described in [21]. The PSI/PSII ratio was determined by EPR measurements at room temperature as in [42].

2.2.1. EPR samples preparation

PSII enriched membranes were diluted to 2 mg Chl/ml and filled into calibrated EPR tubes. For quantification of Y_D•, the sample was exposed to room light for 3 min to fully oxidize Y_D and thereafter dark incubated for 15 min at room temperature before freezing or application of the pre-flash protocol [43]. All spectra obtained at this point are considered as EPR spectra of dark-adapted samples.

2.2.2. Synchronization of the WOC in the S_1-state

PSII in the samples with fully oxidized Y_D were synchronized to contain an absolute majority of the dark stable S_1-state by the application of two saturating pre-flashes from a Nd:YAG laser from Spectra Physics, Newport, USA (532 nm, 450 mJ, 6 ns, 1.25 Hz) followed by dark adaptation for 20 min at room temperature [43–46].

2.2.3. Flash-induced turnover of the WOC

To study EPR signals from PSII in the different S states, the synchronized samples were advanced to the other S states by giving a corresponding number of saturating laser flashes [44]. Before the flashes, P*p*BQ was added as an external electron acceptor to a final concentration of 0.5 mM (from a stock solution in DMSO or methanol, final solvent concentration 3% v/v) in darkness at room temperature. 30 s after the addition of P*p*BQ, the samples were transferred to an ethanol bath at 1 °C and allowed to equilibrate for 1 min. After the equilibration, the samples were transferred to the flash cell and were immediately given one, two or three turnover flashes (532 nm, 450 mJ, 6 ns, 1.25 Hz). After flashes, the EPR samples were immediately frozen in an ethanol-dry ice bath (198 K) within 1–2 s and flushed with argon gas before being transferred to liquid nitrogen for EPR measurements.

2.2.4. Induction of EPR signals from the acceptor side of PSII

To chemically induce the $Q_A^- Fe^{2+}$ interaction signal, EPR samples were incubated for 15 min after the addition of 50 mM Na-formate followed by addition with 50 mM Na-dithionite and a second incubation for 10 min in the darkness at room temperature [47–50]. To induce the split Phe^- signal (the $Phe^- Q_A^- Fe^{2+}$ interaction signal) the formate and dithionite treated samples were illuminated at 198 K for 10 min [48,51]. The Phe^- radical signal was photo-accumulated in the formate and dithionite treated samples by illumination at room temperature for 10 min [47,52].

To photo-induce the spin-polarized triplet $^3P_{680}$ EPR signal, PSII samples were incubated at anaerobic conditions with 50 mM Na-dithionite and 30 μM benzyl viologen at room temperature for 1 h [53]. The signal was generated by direct illumination into the EPR cavity.

2.2.5. Illumination conditions

The S_2-state multiline EPR signal was in some cases also induced by illumination for 6 min at 198 K in an ethanol-dry ice bath. Complete oxidation of Cyt b_{559} was achieved by illumination for 6 min at 77 K. White light from a halogen lamp (800 W) filtered with a 5 cm thick $CuSO_4$ solution was used in both cases.

Download English Version:

https://daneshyari.com/en/article/1942636

Download Persian Version:

https://daneshyari.com/article/1942636

Daneshyari.com