Biochimica et Biophysica Acta 1787 (2009) 1492–1498

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Investigation of the low-affinity oxidation site for exogenous electron donors in the Mn-depleted photosystem II complexes

V.N. Kurashov [c], E.R. Lovyagina [a], D.Yu. Shkolnikov [b], M.K. Solntsev [b], M.D. Mamedov [c], B.K. Semin [a,*]

[a] Department of Biophysics, Faculty of Biology, Moscow State University, Moscow, Russia
[b] Department of Biophysics, Faculty of Physics, Moscow State University, Moscow, Russia
[c] A.N.Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia

ARTICLE INFO

Article history:
Received 14 April 2009
Received in revised form 24 June 2009
Accepted 9 July 2009
Available online 17 July 2009

Keywords:
Chlorophyll fluorescence
Iron
Manganese
Oxygen-evolving complex
Photosystem II
Photovoltage
Thermoluminescence

ABSTRACT

In the manganese-depleted photosystem II (PSII[−Mn]) preparations, oxidation of exogenous electron donors is carried out through the high-affinity (HA) and the low-affinity (LA) sites. This paper investigates the LA oxidation site in the PSII(−Mn) preparations where the HA, Mn-binding site was blocked with ferric cations [[11] B.K. Semin, M.L. Ghirardi, M. Seibert, Blocking of electron donation by Mn(II) to $Y_Z^{\bullet}$ following incubation of Mn-depleted photosystem II membranes with Fe(II) in the light, Biochemistry 41 (2002) 5854–5864.]. In blocked (PSII[−Mn,+Fe]) preparations electron donation by Mn(II) cations to $Y_Z^{\bullet}$ was not detected at Mn(II) concentration 10 μM (corresponds to K_m for Mn(II) oxidation at the HA site), but detected at Mn concentration 100 μM (corresponds to K_m for the LA site) by fluorescence measurements. Comparison of pH-dependencies of electron donation by Mn(II) through the HA and the LA sites revealed the similar pK_a equal to 6.8. Comparison of K_m for diphenylcarbazide (DPC) oxidation at the LA site and K_d for A_T thermoluminescence band suppression by DPC in PSII(−Mn,+Fe) samples suggests that there is relationship between the LA site and A_T band formation. The role of D1-His190 as an oxidant of exogenous electron donors at the LA site is discussed. In contrast to electrogenic electron transfer from Mn(II) at the HA site to $Y_Z^{\bullet}$, photovoltage due to Mn(II) oxidation in iron-blocked PSII(−Mn) core particles was not detected.

1. Introduction

Photosystem II (PSII) of cyanobacteria, algae and higher plants is a multisubunit integral thylakoid membrane protein complex that catalyses the light-driven oxidation of water and reduction of plastoquinone. The oxidation of water occurs in catalytic site of oxygen-evolving complex (OEC) composing of four Mn cations, one Ca^{2+} cation, and one or more chloride anion(s) [1]. The catalytic site is localized on the luminal side of the PSII reaction center and access of components in the medium to the site is limited by three extrinsic proteins: PsbO (the 33 kDa protein), PsbP (the 23 kDa protein, in cyanobacteria PsbU (cytochrome *c*-550)), PsbQ (the 17 kDa protein, in cyanobacteria PsbV (12 kDa)), shield the Mn_4/Ca cluster [2]. Therefore the catalytic site of the intact PSII is capable of oxidizing only the molecules of water delivered to the site by special channels from the lumen [3]. Extraction of Mn from the PSII OEC also results in the removal of Ca^{2+} and extrinsic proteins and as a consequence, the redox-active tyrosine, Y_Z of the D1 polypeptide, becomes accessible to the exogenous electron donors including Mn(II).

By studying the light-induced oxidation of exogenous Mn(II) cations, DPC and I^- anions by Mn-depleted PSII membranes, Blubaugh and Cheniae [4] concluded that there are two sites of oxidation with different rate constants. One of the sites is a high-affinity (HA) site through which $Y_Z^{\bullet}$ is reduced [4–7]. A carboxylic residue (D1-D170), which is a ligand of Mn cation in the tetranuclear manganese cluster of native PS II, participates in the coordination of manganese cation at the HA site [2,8]. Thus, the HA site for the binding and oxidation of Mn (II) is either native site for one of the four manganese cations or substantially overlapped with it. Mn(II) cation oxidation by the redox-active $Y_Z^{\bullet}$ through the HA site is regarded as the first step in the photoligation/photoactivation of the manganese cluster [4]. It was initially proposed that the Y_D radical is the oxidant at the LA site [4]. However, the lack of Y_D radical reduction by Mn(II) [5] or DPC [9] in PSII(−Mn) samples indicates another nature of an oxidant at this site. Note that since the main electron transport path through the HA site dominate, investigation of the LA oxidation site is complicated.

Abbreviations: Chl, chlorophyll; DCPIP, 2,6-dichlorophenolindophenol; DEPC, diethylpyrocarbonate; DPC, 1,5-diphenylcarbazide; HA, high-affinity electron donation site to $Y_Z^{\bullet}$ by exogenous donors; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; K_m, Michaelis–Menten constant for an enzymatic reaction; K_d, dissociation constant of the enzyme–ligand complex; LA, low-affinity electron donation site by exogenous donors; MES, 2-(*N*-morpholino)-ethanesulfonic acid; OEC, oxygen-evolving complex; PSII, photosystem II; PSII(−Mn), Mn-depleted PSII; PSII(−Mn,+Fe), Fe-blocked, Mn-depleted PSII; Tris, tris(hydroxymethyl)aminomethane; Y_Z, redox-active tyrosine (D1-Tyr161), the first electron donor to P_{680}^+ in PSII; Y_D, redox-active tyrosine (D2-Tyr160), an alternative electron donor to P_{680}^+ in PSII; $\Delta\Psi$, transmembrane electric potential difference; τ, characteristic time constant

* Corresponding author. Tel.: +7 495 939 3315; fax: +7 495 939 1115.
E-mail address: semin@biophys.msu.ru (B.K. Semin).

0005-2728/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2009.07.002

We have previously shown that Fe(II) and Mn(II) cations bind to the HA, Mn-binding site with a high and comparable efficiencies in PSII(−Mn) membranes [10]. Under photoactivating conditions (weak room light for 0.5–3 min, depending on the iron concentration and light), oxidation of Fe(II) is accompanied by the irreversible binding of Fe(III) to the HA, Mn-binding site [10–13]. This "Fe block" prevents further oxidation of exogenous Mn(II), Fe(II) and DPC by $Y_Z^{\bullet}$ [11]. On the other hand, oxidation of donors through the LA site still occurs in Fe-blocked membranes [12]. Such selective blocking of the HA oxidation site by ferric cations allows the direct study of the oxidation process through the LA site using the instrumental methods. In the present work the given methodological approach was applied to investigate the nature of the LA oxidation site in PSII(−Mn,+Fe) preparations.

2. Materials and methods

2.1. PSII preparations

PSII-enriched membrane fragments (BBY-type) were prepared from market spinach according to the procedure of Ghanotakis and Babcock [14]. The functional and spectral characteristics of these preparations matched the previously reported ones [15]. The O_2-evolving activity of the PSII membranes, measured polarographically, ranged from 400 to 500 μmol O_2 mg Chl^{-1} h^{-1} when 0.2 mM 2,6-dichloro-*p*-benzoquinone was used as an artificial electron acceptor. The preparations were stored in liquid nitrogen in a buffer A (15 mM NaCl, 400 mM sucrose and 50 mM MES/NaOH, buffer, pH 6.5). Chlorophyll concentrations were determined in 80% acetone, according to the method of Arnon [16]. To extract Mn from PSII, the preparations were treated with 0.8 M Tris/HCl buffer (pH 8.5) for 15 min at room temperature in the weak light. Treated membranes were washed twice with buffer A. The residual oxygen-evolving activity of Mn-depleted PSII particles was about 15 μmol O_2 mg Chl^{-1} h^{-1}.

Oxygen-evolving PSII core particles were prepared from market spinach according to the method of Enami et al. [17] with minor modifications. To prepare core PSII samples depleted of Mn(II) and the extrinsic protein(s), thawed particles (0.5 mg Chl/ml) were treated with 0.9 M Tris/HCl buffer, pH 9.0 for 30 min, washed twice with 25 mM HEPES (pH 7.5) buffer containing 300 mM sucrose and 20 mM NaCl [18] and employed immediately or after storage at −80 °C. PSII core complexes were reconstituted into liposomes prepared from soybean phospholipids (L-α-Lecithin, type II-S, Sigma) by sonication as described in [19]. The lipid to protein ratio was about 50 (w/w).

2.2. Blocking of the HA Mn-binding site

Blocking of the high-affinity Mn-binding site was performed as described in [20]. PSII(−Mn) membranes (25 μg of Chl/ml) were incubated in buffer A (pH 6.5) with 15 μM Fe(II) for 3 min under continuous illumination (irradiance was about 15 $\mu E/m^2 s$) and constant stirring. Only freshly prepared solutions of $FeSO_4$ were used. Following the incubation, the preparations were centrifuged for 7 min at 16000 ×*g* and resuspended in buffer A.

2.3. Variable fluorescence

The photoinduced changes of chlorophyll fluorescence yield (ΔF) related to photoreduction of primary plastoquinone acceptor Q_A, were measured in a tightly closed 1-cm cuvette at 20 °C using a dual-modulation kinetic fluorimeter (Photon Systems Instruments, FL3000, Czech Republic).

2.4. Fluorescence induction kinetics

Fluorescence induction kinetics were measured using a portable Plant Efficiency Analyser (Hansatech Instruments, Ltd, UK). LED sources of excitation light (λ_{max} = 650 nm; spectral range, 580–710 nm) were employed in the fluorimeter. The time resolution of fluorescence detection were 10 μs (within the initial 2 ms); 1 ms (within the time interval 2 ms–1 s); and 100 ms (at a time interval >1 s). Fluorescence induction kinetics were measured under continuous actinic light (1200 $\mu E/m^2 \cdot s$). The fluorescence signal at 50 μs after application of actinic light was defined as F_0, because the fluorescence yield at that time did not depend on the exciting light intensity [21]. A logarithmic time scale was used in the plot as it is commonly employed for the presentation of fluorescence induction kinetics.

The effects of pH on fluorescence induction kinetics in PSII(−Mn) and PSII(−Mn,+Fe) membranes were examined using MES buffer (50 mM MES/NaOH, 15 mM NaCl, and 400 mM sucrose) in the pH range of 5.5–7.0 and HEPES buffer (50 mM HEPES/NaOH, 15 mM NaCl, and 400 mM sucrose) in the pH range of 7.5–8.0. No differences in the apparent fluorescence induction kinetics of the samples in MES or HEPES buffers poised at the same pH (pH 7.0) were observed.

2.5. Thermoluminescence

The intensity of the thermoluminescence bands in PSII preparations was measured using the luminometer developed at the Department of Biophysics, Faculty of Physics, Lomonosov Moscow State University [22]. The procedure of measurement included the following stages: a 100 μl suspension of PSII particles in buffer A (200 μg Chl per ml) was applied to a filter paper support, incubated in the dark for 1 min at 0 °C, illuminated with a KGM-30-300 incandescent lamp (light intensity, 15 W/m^2) at −30 °C for 3 min (the temperature and light exposure time were optimal for detecting the A_T band of thermoluminescence), and rapidly cooled to −60 °C. Thermoluminescence was recorded upon sample heating at a rate of 30°/min over the temperature range from −60 to +80 °C.

2.6. Time-resolved electrometrical technique

Photovoltage measurements in PSII core-containing proteoliposomes were performed as described in [23]. The technique includes an arrangement of proteoliposomes on the surface of a colodion (nitrocellulose) phospholipid impregnated film separating two partitions of the measuring cell, and recording $\Delta\Psi$ with the light protected Ag/AgCl macroelectrodes. The measuring instrument time constant was 200 ns. Pulsed illumination was provided by a Nd-YAG laser (Quantel, wavelength 532 nm with a pulse energy of 40 mJ and a halfwidth of 15 ns). The kinetic traces were resolved into individual exponentials using GraphView from PLUK software developed on the basis of PLUK language [24].

3. Results

The DPC inhibition assay has shown that binding of the Mn(II) cation to the HA, Mn-binding site in PSII(−Mn) preparations results in a decrease in the rate of electron donors oxidation from 40 to 70% [25–28]. The remaining activity is determined by oxidation of the Mn (II) or DPC at the LA site. Inhibition is due to binding of the oxidized Mn(III) cations to the HA site [4,27]. In addition, bound Mn(III) cation substantially decreases the efficiency of Mn(II) oxidation at the HA site: K_m increases from ≤1 μM to 10 μM [4,27]. However, Mn (III) is weakly bound and since it has a high redox potential, is easily reduced and leaves the binding site [27]. This feature(s) complicates the use of manganese cations as a component blocking the HA site in order to study the LA donating site.