Biochimica et Biophysica Acta 1827 (2013) 438–445

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

# Electronic structure of $S_2$ state of the oxygen-evolving complex of photosystem II studied by PELDOR

Mizue Asada [a], Hiroki Nagashima [a], Faisal Hammad Mekky Koua [b], Jian-Ren Shen [b], Asako Kawamori [c], Hiroyuki Mino [a,*]

[a] Division of Material Science (Physics), Graduate school of Science, Nagoya University, Furo-cho, Chikusa, Nagoya, 464-8602, Japan
[b] Graduate School of Natural Science and Technology/Faculty of Science; Okayama University, Okayama 700-8530, Japan
[c] AGAPE-Kabutoyama Institute of Medicine, Nishinomiya, Hyogo 662-0001, Japan

ARTICLE INFO

*Article history:*
Received 25 August 2012
Received in revised form 25 December 2012
Accepted 27 December 2012
Available online 8 January 2013

*Keywords:*
Photosystem II
Oxygen-evolving complex
Mn cluster
EPR
PELDOR

ABSTRACT

Photosynthetic water splitting is catalyzed by a $Mn_4CaO_5$ cluster in photosystem II, whose structure was recently determined at a resolution of 1.9 Å [Umena, Y. et al. 2011, *Nature*, 473:55–60]. To determine the electronic structure of the $Mn_4CaO_5$ cluster, pulsed electron–electron double resonance (PELDOR) measurements were performed for the tyrosine residue $Y_D^{\bullet}$ and $S_2$ state signals with non-oriented and oriented photosystem II (PS II) samples. Based on these measurements, the spin density distributions were calculated by comparing with the experimental results. The best fitting parameters were obtained with a model in which Mn1 has a large positive projection, Mn3 has a small positive projection, and Mn2 and Mn4 have negative projections (the numbering of Mn*i* ($i=1$–4) is based on the crystal structure at a 1.9 Å resolution), which yielded spin projections of 1.97, −1.20, 1.19 and −0.96 for Mn1–4 ions. The results show that the Mn1 ion, which is coordinated by H332, D342 and E189, has a valence of Mn(III) in the $S_2$ state. The sign of the exchange interactions $J_{13}$ is positive, and the other signs are negative.



## 1. Introduction

Photosynthetic $O_2$ evolution is one of the most essential reactions for life on earth, because it produces $O_2$, protons and electrons from water. Protons and electrons are utilized to synthesize energy-rich compounds that are required for the synthesis of carbohydrates from carbon dioxide, whereas the $O_2$ maintains the oxygenic atmosphere that we enjoy today. The reaction is catalyzed by an oxygen-evolving complex (OEC) bound to a trans-membrane, multi-subunit protein complex designated photosystem (PS) II, located in the thylakoid membranes of cyanobacteria, algae, and green plants [1–3].

In addition to other protein subunits, PS II contains two reaction center proteins, D1/D2, whose structures are related by a pseudo $C_2$ symmetry. Light energy is absorbed by the reaction center chlorophylls P680 bound to the D1/D2 proteins, inducing charge separation and subsequent electron transfer reactions. The unpaired electron generated is subsequently transferred to bound plastoquinone molecules, $Q_A/Q_B$, in the stromal side of the membrane via pheophytin on the D1 protein. The paired hole on $P680^+$ is transferred to OEC in the lumenal side of PS II via a redox-active tyrosine residue, D1-Tyr161, which is designated $Y_Z$. Another tyrosine residue, D2-Tyr160, is located on the D2 protein in a position symmetric to that of $Y_Z$, giving rise to the stable tyrosine radical $Y_D^{\bullet}$.

In the water oxidation reaction, two water molecules are oxidized to yield an oxygen molecule through a cycle of five distinct redox states of OEC, which are designated as $S_n$ ($n=0$–4). The $S_1$ state is the most stable state in the dark, and each $S_n$ state advances to $S_{n+1}$ by a single photon reaction in PS II. Upon successive photoreactions, the OEC advances to the highest oxidation state $S_4$ which is spontaneously converted to the lowest oxidation state $S_0$, concomitant with the release of an oxygen molecule [1–3]. The structure of OEC has been extensively studied with various techniques including visible, UV, and X-ray spectroscopic measurements, EPR and X-ray crystallography. In particular, previous X-ray crystallographic analysis has revealed the 3D structure of PS II at resolutions of 2.9–3.8 Å, which provided significant information on the overall structure of PS II as well as the locations of various subunits and cofactors including OEC containing 4 Mn atoms and 1 Ca atom [4–6]. However, the detailed structure of the $Mn_4Ca$ cluster including the location of each metal ion and the oxo-bridges that connect them has not been resolved. Extended X-ray absorption fine structure (EXAFS) and polarized EXAFS

*Abbreviations:* EPR, electron paramagnetic resonance; PELDOR, pulsed electron–electron double resonance; ESE, electron spin echo; ESEEM, electron spin-echo envelope modulation; ENDOR, electron-nuclear double resonance; CW, continuous wave; MW, microwave; Mes, 2-Morpholinoethasulfonic acid; PS II, photosystem II; OEC, oxygen-evolving complex; $Y_D$, Tyr161 for higher plants (Tyr160 for cyanobacteria) of the D2 subunit in PS II; $Y_Z$, Tyr161 of the D1 subunit in PS II; ZFS, zero-field splitting

* Corresponding author. Tel./fax: +81 52 789 2882.
*E-mail address:* mino@bio.phys.nagoya-u.ac.jp (H. Mino).

0005-2728/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbabio.2012.12.011

have yielded various models for the structure of the Mn-cluster, and some of which have reached similar structures as that revealed by the crystallographic studies [7–12]. EXAFS studies have also provided information regarding the distances and orientations of Mn–Mn and Mn–Ca pairs [8,13–16]. In spite of these studies, ambiguities regarding the exact structure of the OEC still remained. This situation was changed dramatically by the success of recent structural analysis of PSII at a resolution of 1.9 Å, which revealed not only the locations of the individual metal ions but also the presence of the oxo-bridges connecting the metal ions, giving rise to a chemical formula of $Mn_4CaO_5$ for the OEC [17]. All of the amino acid residues ligating the metal cluster were identified, showing that each of the Mn ions is 6-coordinated and the Ca ion is 7-coordinated. The $Mn_4CaO_5$ cluster is organized in a distorted chair form with 3 Mn atoms (labeled Mn1–Mn3) and 1 Ca atom connected by 4 oxygen atoms to form a distorted cubic structure, and the 4th Mn (Mn4) and 5th oxygen (O4) connected outside of the cubane. The high-resolution structure also revealed that there are four water molecules ligated to the cluster, among which, two are ligated to Ca and the other two are ligated to Mn4. The amino acid residues ligated to Mn ions were D1-H332, D1-D342 and D1-E189 for Mn1, D1-D342, D1-A344 and CP43-E354 for Mn2, D1-E333 and CP43-E354 for Mn3 and D1-D170 and D1-E333 for Mn4 (Fig. 1).

During the S-state transition, the $Mn_4CaO_5$-cluster generates an EPR multiline signal in the $S_2$ state, which is the signpost for OEC in EPR studies. The multiline signal is centered at $g=2$ with an expansion over approximately 60 mT and is characterized by 19–21 hyperfine lines with a spacing of 8.5–9 mT between each pair of adjacent lines [18]. This signal has been ascribed to an overall $\boldsymbol{S_T}=1/2$ ground state arising from magnetically coupled 4 Mn ions. It has been proposed that the Mn cluster is a multinuclear complex in the $S_2$ state that includes a Mn(III)–Mn(IV) pair (reviewed in references [1–3]). Another multiline signal was found to arise in the $S_0$ state. This multiline signal is composed of at least 26 peaks that correspond to an $\boldsymbol{S}=1/2$ ground state in the presence of methanol [19,20]. $^{55}$Mn-pulsed ENDOR results have revealed that the Mn cluster is a multinuclear complex in the $S_2$ state [19,20]. Kulik et al. suggested an oxidation state of 4Mn (III, IV, IV, IV) for the $S_2$ state based upon $^{55}$Mn-pulsed ENDOR for the $S_0$ state [21]. $^{55}$Mn-pulsed ENDOR has addressed the 4 sets of hyperfine constants (HFC) for the 4 Mn ions, where the largest set of HFC were assigned to the Mn(III) ion. In addition the HFC and spin projections of the Mn ions have been calculated. Because a unique solution of 6 exchange interactions $J$ among the 4 Mn ions has not been experimentally determined, various models have been proposed. Peloquin et al. proposed a model with Mn ions coupled antiferro-magnetically [22]. Kulik et al. proposed a model with three Mn ions coupled antiferromagnetically in a triangular form, and one Mn ion isolated and coupled weakly to the three Mn ions antiferro-magnetically (Y-shape structure) [23]. Cox et al. proposed that the 4 Mn ions are connected with 6 exchange couplings based on a structure proposed by Siegbahn, which was obtained from theoretical studies using medium resolution X-ray structures and EXAFS data [24–27]. ESEEM studies suggested that the spin projection of the Mn ion coordinated to $^{14}$N is Mn(III), where His332 has been proposed as a good ligand candidate based on a comparison with the results of H332E mutant studies [28,29].

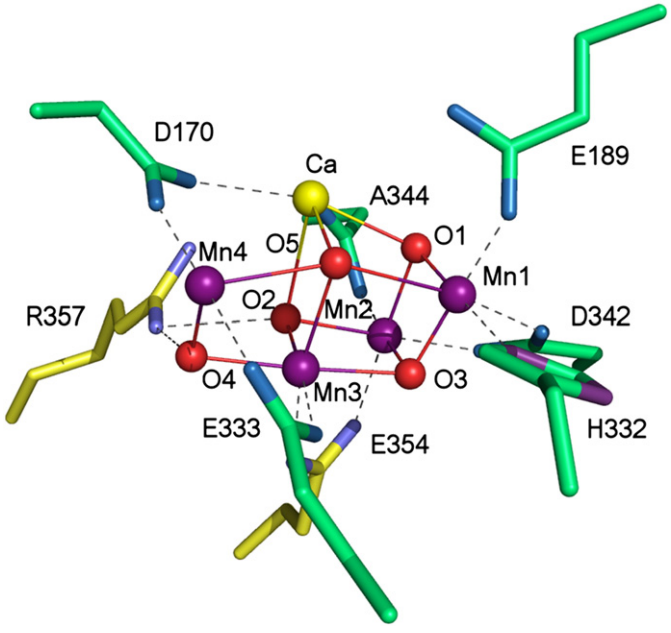


**Fig. 1.** The structure of the oxygen-evolving complex and its coordinating environment based on X-ray crystal spectroscopy (PDB: 3ARC).

Pulsed electron–electron double resonance (PELDOR) measurement is a well-established technique to determine the distances between electron spins [30]. PELDOR has been previously employed to measure the distance between $Y_D^{\bullet}$ and the $S_2$ Mn cluster [31]. The point dipole approximation showed that the distance between $Y_D^{\bullet}$ and the Mn cluster was 27 Å. However, the recent 1.9 Å PS II structure shows that the distances between the center of the aromatic ring of $Y_D^{\bullet}$ and each Mn ion are approximately $30\pm2$ Å. The inconsistency between the results may correspond to delocalization of the electron spins. In this work, we performed the PELDOR measurement and calculated the spin projections of each Mn ion in the $S_2$ state. Based on these results, the electronic structure of the $Mn_4CaO_5$-cluster in the $S_2$ state was determined.

## 2. Materials and methods

### 2.1. Sample preparation

Oxygen-evolving PS II core complexes were isolated from a thermophilic cyanobacterium *Thermosynechococcus vulcanus* as described previously [32,33]. The samples were suspended in a buffer containing 20 mM Mes (pH 6.0), 20 mM NaCl and 3 mM $CaCl_2$. Spinach PS II membranes were prepared as described in [34,35]. The samples were suspended in a buffer containing 400 mM sucrose, 20 mM NaCl and 20 mM Mes/NaOH (pH 6.5) and packed into EPR tubes. For the preparation of the oriented membrane samples, spinach PSII was dried on an OHP sheet under a humid nitrogen gas flow for 15 h at 4 °C. The sheets were cut into $2.5\times25$ mm$^2$ pieces and 6 pieces were collected, placed in an EPR tube, and frozen by rapidly placing the tube into liquid nitrogen (within 1 s). The bottom of the EPR tube contained glycerol to improve the heat conductivity in the EPR tubes. For the measurement of the $S_1$ state, the PS II samples were dark-adapted for 2–3 h after pre-illumination. The $S_2$ state of the samples was formed by illumination with white light for 5 min at 200 K.

### 2.2. EPR measurements

CW EPR measurements were performed by using a Bruker ESP-300E ESR spectrometer with a gas flow temperature control system (CF935, Oxford Instruments, Oxford, GB). ESE spectra were recorded on a Bruker pulsed EPR spectrometer ESP-380E by using an Oxford Instruments liquid helium cryostat. The pulsed ESE field swept spectra were measured by using a $\pi/2$–$\tau$–$\pi$ sequence with a time interval $\tau$ of 200 ns between the microwave (mw) pulses. A three-pulse PELDOR sequence was employed for the PELDOR measurements. The $\pi/2$–$\tau$–$\pi$ sequence with a time interval $\tau$ of 1000 ns between the mw pulses from the ESP380 source was used for observation. An mw synthesizer (HP83751A, Hewlett-Packard) was used as the mw source of 24 ns pumping pulse [31,36].