Magnetic structure of manganese cluster in photosystem II investigated by electron paramagnetic resonance

Hiroki Nagashima¹,², Mizue Asada¹,³ and Hiroyuki Mino¹

¹Division of Material Science (Physics), Graduate School of Science, Nagoya University, Nagoya, Aichi 464-8602, Japan
²Present address: Laser Molecular Photoscience Laboratory, Molecular Photoscience Research Center, Kobe University, Kobe, Hyogo 657-8501, Japan
³Present address: Department of Materials Molecular Science, Institute for Molecular Science, Okazaki, Aichi 444-8585, Japan

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The electronic structure of manganese (Mn) cluster in photosystem II was investigated by electron paramagnetic resonance (EPR) spectroscopy. In order to determine the spin density distribution in magnetically coupled Mn in the S² state Mn cluster, pulsed electron-electron double resonance (PELDOR) measurement was performed. The local environment of the Mn cluster was investigated by electron-nuclear double resonance (ENDOR). Using spin projections determined by PELDOR, ENDOR signals were assigned to the water molecules ligated to the Mn cluster. The location of a high-affinity Mn²⁺ site in apo-photosystem II, which is the initial site of photoactivation of the Mn cluster, was determined by PELDOR.

Key words: photosystem II, manganese cluster, EPR, PELDOR, ENDOR

Photosynthesis is a series of the energy-conversion reactions from light energy to chemical energy. Photosynthetic oxygen evolution is one of the greatest inventions given by the creature on earth. The oxygen evolution mechanism has been a great mystery for human beings since the establishment of modern science. Oxygen evolution occurs in a manganese (Mn) cluster in photosystem II (PS II) protein complex. In 2011, Umena et al. revealed the X-ray crystal fine structure of PS II with 1.9 Å [1]. This work provided a significant clue to elucidate the chemical reactions. Although great advancements have been achieved, the mechanism of oxygen evolution is still under debate. Photosystem II, with complicated structures and reactions, is attractive as it can be used to explore many scientific methods, such as X-ray free-electron laser (XFEL), large-scale quantum calculations, Fourier transform infrared spectroscopy (FTIR), and electron paramagnetic resonance (EPR). In this mini review, we introduce our recent work on magnetic structure of Mn cluster using EPR.

The main functional unit of PS II consists of two proteins, D1 and D2, with a pseudo C₂ symmetry (Fig. 1A) (see reviews [2,3]). In the initial reaction, photon is collected at the reaction center chlorophyll pair P680. The unpaired electron, generated by charge separation in P680, is subsequently transferred to a plastoquinone molecule, Qₐ, through pheophytin molecule on the D1 protein, and transferred to Cytochrome b/f complex via plastoquinone molecule, Qₐ.
The hole generated in P680* is transferred to the oxygen-evolving complex in the luminal side of PS II via a redox-active tyrosine residue, D1-Tyr161, named as YZ. There is another redox-active tyrosine residue, D2-Tyr160, called YD, which is located at a symmetrical position to YZ on the D1/D2 complex. YD forms a stable neutral radical state, and therefore, is a signpost in EPR studies.

X-ray crystal structure analysis reveals the chemical formula of Mn₄CaO₅ for the oxygen evolving complex. In addition, ten amino acid residues, which ligate the metal cluster, are identified [1]. The structure of Mn₄CaO₅ cluster is called as a “distorted chair” structure (Fig. 1B). Three Mn ions, labeled as Mn1–Mn3, and one Calcium ion, are connected with four oxygen atoms, and the 4th manganese ion (Mn4) and 5th oxygen ion (O4) form the part of “backrest.” The crystal structure revealed that there are four water molecules ligated to the cluster, labeled as W1–W4.

In the sequential reactions, two water molecules are oxidized to produce an oxygen molecule through a cyclic process of five distinct redox states, denoted as Sₙ states (n = 0–4) (Fig. 2). The S₀ state is the lowest redox state, and the S₄ state is the most stable state in the dark. Each Sₙ state advances to Sₙ₊₁ by a single photon reaction in PS II. After advancement to the highest oxidation state S₄, the state is spontaneously converted to the lowest oxidation state S₀ with the release of an oxygen molecule [2,3].

1. Determination of spin projections on the Mn cluster using the PELDOR technique

EPR is a powerful tool to investigate the electronic structure of a molecule. EPR signals in the Mn cluster in the S₀–S₃ states can be detected. Generally, it is difficult to detect the EPR signal in an integer spin system. Therefore, it is difficult to detect the EPR signals in the S₁ and S₃ states. Especially, the S₂ state multiline signal has been primarily studied as it is the main signal for the EPR studies. The S₂ state multiline signal is centered at g = 2 with an expansion over approximately 160 mT and is characterized by 19–21 hyperfine lines with a spacing of 8.5–9 mT between each pair of adjacent lines [4]. This signal has been ascribed to a spin state S₂ = 1/2 coupled magnetically with four Mn ions.

Generally, the spin Hamiltonian of the Mn cluster is written as follows:

\[
H = \sum_i [B \cdot g_i \cdot S_i + I_i \cdot A_i \cdot S_i + S_i \cdot D_i \cdot S_i] - \sum_j J_{ij} S_j \cdot S_i, \tag{1}
\]

where Sᵢ and Iᵢ are the operators of electron spin and nuclear spin of the i-th Mn ion, respectively, Dᵢ is the zero-field splitting (ZFS) tensor for the i-th Mn ion, gᵢ is the g-tensor, and Aᵢ is the effective hyperfine tensor of the i-th ion. Jᵢ is the exchange interaction between the i-th and j-th ions.

The determination of unique solutions of the six exchange interactions J among the Mn ions was experimentally difficult. Therefore, various models have been proposed. The effective hyperfine constants Aᵢ,iso in the S₂ state have been obtained by X- and Q-band ENDOR measurements, and the intrinsic hyperfine constants aᵢ,iso were estimated to be –165
The spin density distribution on each Mn atom was evaluated. The simulated results show that the best fitting parameters were 1.97, –1.19, 1.17, and –0.95 for Mn
and the Mn atoms (j = 1–4) Mn atom. \( \theta_d \) is the angle between the external magnetic field \( \mathbf{H} \) and the distance vector \( \mathbf{R}_{ij} \). The parameters \( g_i \) and \( g_j \) are the g-factors for the two electron spins, which were assumed to be 2.00 for \( Y_{D} \) and the S2 multilinear signal, and \( \beta \) is the Bohr magnetron.

In the oriented PS II sample, PELDOR intensity is given by:

\[
I(t') = \int \int X(t') G(\theta - \theta_o) \sin \theta d\theta d\phi ,
\]

with

\[
G(\theta - \theta_o) = \exp \left[ -\frac{1}{2} \left( \frac{\theta - \theta_o}{\Delta} \right)^2 \right] .
\]

where \( \theta \) is the angle between \( \mathbf{n} \) and external field \( \mathbf{H}_0 \), and \( \Delta \) is the distribution angle of the mosaic spread. \( G(\theta - \theta_o) \) is the mosaic spread function which is assumed to be Gaussian. The z-axis was set to \( \mathbf{n} \), along with the pseudo-symmetric C2 axis of PS II. The coordinates of \( Y_{D} \) and the Mn atoms were obtained from a 1.9 Å resolution X-ray structure (PDB: 3ARC). The spin density distribution on \( Y_{D} \) was taken from density functional theory (DFT) calculation [8]. Based on these parameters, the spin density distribution on each Mn ion was evaluated. The simulated results show that the best fitting parameters were 1.97, –1.19, 1.17, and –0.95 for Mn
and the Mn atoms (i = 1–4), respectively.

The obtained spin density on Mn1 is larger than the other Mn ions, indicating that the local spin at Mn1 is large. The valences of Mn in the S2 state are believed to be 1 for Mn(III) and 3 for Mn(IV). Therefore, the virtual valence of Mn1 is derived as Mn(III). This is the first result where the location of Mn(III) in the S2 state is experimentally obtained from the crystal structure. Figure 4 shows the relative signs of the exchange interaction \( J \) revealed by the PELDOR results, which connects the X-ray crystal structure with the magnetic structure.

**Figure 4** Spin projections on each manganese ion and the signs of exchange interaction \( J \) in the Mn cluster [15].

\[
D = \sum_{i,j} \rho_i \rho_j \frac{g_i g_j \beta}{R_{ij}^3} (1 - 3\cos^2\Theta) ,
\]

where \( \rho_i \) is the spin density at the \( i \)-th (\( i = 1–7 \)) carbon/oxygen atom of the \( Y_{D} \) radical and \( \rho_j \) is the spin projection at the \( j \)-th (\( j = 1–4 \)) Mn atom. \( R_{ij} \) is the length between the \( i \)-th (\( i = 1–7 \)) carbon/oxygen atom of the \( Y_{D} \) radical and the \( j \)-th (\( j = 1–4 \)) Mn atom. \( \Theta \) is the angle between the external magnetic field \( \mathbf{H} \) and the distance vector \( \mathbf{R}_{ij} \). The parameters \( g_i \) and \( g_j \) are the g-factors for the two electron spins, which were assumed to be 2.00 for \( Y_{D} \) and the S2 multilinear signal, and \( \beta \) is the Bohr magnetron.
2. Microenvironment of the Mn cluster using the ENDOR technique

It is essential to clarify the role of the surroundings of Mn,CaO in order to understand the oxygen evolving mechanism. Especially, the locations of the hydrogen atoms, which are essential to clarify the function of the Mn cluster because the hydrogen atoms are directly related to the water oxidation reaction and influence the DFT calculations. However, X-ray crystal analysis is difficult to resolve the protons.

Electron nuclear double resonance (ENDOR) is a powerful method to detect nuclear transitions surrounded in unpaired electrons. Figure 5A shows the proton matrix ENDOR of the S_2 state Mn cluster. Several pairs of signals, centered at about 15 MHz, are detected. Pair separation is determined by electron-nuclear magnetic dipole interaction and is proportional to 1/r^3, where r is the distance between the Mn cluster and proton. For example, the maximum peak separation at 4 MHz is estimated to be 2.7 Å, using point-dipole approximation. However, the electron spin is actually distributed on the Mn cluster. Using the spin density distribution obtained by the PELDOR experiment and the crystal structure, we have assigned the ENDOR signals to the hydrogen atoms and W1–W4 water protons, connected to the Mn cluster. The H/D exchange ratios of W1 and W3 are high, showing the possibility of existence of the substrate water [9]. On the other hand, the exchange ratio of W2 is very low.

Some biochemical treatments are effective to understand the chemistry of the Mn cluster. Ca^2+ depletion causes the loss of ENDOR signals assigned to the proton of W4 (Fig. 5B) [10], which connects Ca^2+ and Z directly. The role of Na^+ involves the maintenance of the hydrogen-bond network near the Ca^2+ site and the electron-transfer pathway to the Mn cluster.

It has been widely known that an alcohol molecule influences the magnetic structure of the S_2 state Mn cluster. Methanol has been proposed to bind to one or more Mn ions directly. Figure 6A shows the ENDOR spectra arising from a methanol molecule with (a) CH_3OH, (b) CD_3OH, and subtraction of (a) and (b). Two pairs of ENDOR signals, A, and A', are obtained for the methyl group of methanol in the subtraction spectrum. The obtained hyperfine constants show that the obtained distance between the methanol and the Mn ions is long, and therefore, methanol is not directly ligated to the Mn cluster. Figure 6B shows the issosurface plots of hyperfine couplings of the protons and carbon atoms. The blue and green surfaces show the possible locations for the methyl group of the methanol. The possible location for the methanol molecule is limited to the regions close to Mn1 (area I) and Mn4 (area II). The width of area I (located on Glu189) is approximately 4.0 Å perpendicular to the arc and 10 Å along the arc. The width of area II is approximately 3.5 Å. Glu189 and His332 amino acids near area I and Asp170 near area II are directly connected to the Mn cluster. Insertion of methanol near these amino acids might result in modification of the structure of the Mn cluster. Recent DFT calculations suggest that the S_2 state has two different isomers, which are called “open cubane” and “closed cubane” states [9,11]. The S_2 multiline signal is attributed to the S_2 = 1/2 open cubane state in which O5 is connected to Mn4, Mn3, and Ca; Mn1 is in the five coordinated Mn(III) state. The g = 4 signal is attributed to the S = 5/2 closed cubane state, where O5 is coordinated to Mn1, and the closed cubane structure is composed of Mn1–3 and Ca. If methanol is located in area I, close to Mn1, the structural modification via the amino acids surrounding Mn1 might cause modification of the hyperfine anisotropy of Mn1 [12]. The interaction with the hydrogen-bonding network via W4 and Z located nearby would lower the reaction rate [10]. On the other hand, modification of the spin structure is not necessary to be located close to Mn1 in a coupled spin system. Disruption of the hydrogen-bond network surrounding the Mn cluster would also cause the modification. Area II, near Mn4, would disrupt the whole hydrogen-bond structure and the equilibrium would be modified. In addition, Ser169 is in close proximity to Asn87, which is the only amino acid around the Mn cluster that differs between higher plants and cyanobacteria.

Figure 5 (A) ENDOR spectra of the S_2 state multiline in (black) untreated and (red) Ca^2+-depleted PS II and (B) locations of the corresponded protons [10,16].
cluster and a possible high Mn\textsuperscript{2+} affinity site indicated in gray. The results show that the high Mn\textsuperscript{2+} affinity site is located near Mn\textsubscript{4} in the structure of the native Mn cluster. Mn\textsuperscript{2+} would be fixed with axial ligands, Asp\textsubscript{170} and Glu\textsubscript{333}, in the D1 polypeptide. The position of Mn\textsubscript{1–3} in the native structure is difficult to bind axially to an amino acid, which is consistent with the PELDOR results. The structure connecting Asp\textsubscript{170} and Glu\textsubscript{333} with Mn\textsuperscript{2+} determines the orientation of the other C=O in the carboxylate of Asp\textsubscript{170} and Glu\textsubscript{333}, which is the same as that in the structure of the native Mn cluster. Therefore, the structure would form a mold of the Mn cluster.

### 4. Summary

In this mini review, we have presented recent EPR studies on magnetic structure of Mn cluster. EPR results showed the magnetic structure of the Mn cluster, which complemented the molecular structure. The development of a highly-resolved...
X-ray crystal analysis advanced the understanding of the oxygen evolution mechanism. Recently, the structure of the higher oxidation state S$_2$ state obtained using XFEL was reported [14]. With advancement in the X-ray crystal structure analysis, EPR would play an important role in future.

Conflicts of Interest

H.N., M.A. and H.M. declare that they have no conflict of interest.

Author Contribution

H.N. and M.A. performed EPR measurements. H.N., M.A. and H.M have analyzed the experimental results.

Reference

[1] Umena, Y., Kawakami, K., Shen, J. R. & Kamiya, N. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. Nature 473, 55–60 (2011).
[2] Satoh, K. Protein-pigments and the photosystem II reaction center: a glimpse into the history of research and reminiscences. Photosynth. Res. 98, 33–42 (2008).
[3] Shen, J. R. The Structure of Photosystem II and the Mechanism of Water Oxidation in Photosynthesis. Annu. Rev. Plant Biol. 66, 23–48 (2015).
[4] Dismukes, G.C. & Siderer, Y. Intermediates of a Polynuclear Manganese Center Involved in Photosynthetic Oxidation of Water. Proc. Natl. Acad. Sci. USA 78, 274–278 (1981).
[5] Peloquin, J. M., Campbell, K. A., Randall, D. W., Evanich, M. A., Pecoraro, V. L., Armstrong, W. H., et al. $^{55}$Mn ENDOR of the S$_2$ state multiline EPR signal of photosystem II: Implications on the structure of the tetranuclear Mn cluster. J. Am. Chem. Soc. 122, 10926–10942 (2000).
[6] Cox, N., Rapatskiy, L., Su, J. H., Pantazis, D. A., Sugihara, M., Kulik, L., et al. Effect of Ca$^{2+}$/Sr$^{2+}$ Substitution on the Electronic Structure of the Oxygen-Evolving Complex of Photosystem II: A Combined Multifrequency EPR, $^{55}$Mn- ENDOR, and DFT Study of the S$_2$ State. J. Am. Chem. Soc. 133, 3635–3648 (2011).
[7] Tsvelev, Y. D. & Grishin, Y. A. Techniques for EPR spectroscopy of pulsed electron double resonance (PELDOR): A review. Instrum. Exp. Tech. (USSR) 52, 615–636 (2009).
[8] Nagai, H., Fukushima, Y., Okajima, K., Ikeuchi, M. & Mino, H. Formation of Interacting Spins on Flavosemiquinone and Tyrosine Radical in Photooxidation of a Blue Light Sensor BLUF Protein TePixD. Biochemistry 47, 12574–12582 (2008).
[9] Yamada, H., Mino, H. & Itoh, S. Protons bound to the Mn cluster in photosystem II oxygen evolving complex detected by proton matrix ENDOR. Biochim. Biophys. Acta 1767, 197–203 (2007).
[10] Nagashima, H., Nakajima, Y., Shen, J. R. & Mino, H. Proton Matrix ENDOR Studies on Ca$^{2+}$-depleted and Sr$^{2+}$-substituted Manganese Cluster in Photosystem II. J. Biol. Chem. 290, 28166–28174 (2015).
[11] Pantazis, D. A., Ames, W., Cox, N., Lubitz, W. & Neese, F. Two Interconvertible Structures that Explain the Spectroscopic Properties of the Oxygen-Evolving Complex of Photosystem II in the S$_2$ State. Angew. Chem. Int. Ed. 51, 9935–9940 (2012).
[12] Su, J. H., Cox, N., Ames, W., Pantazis, D. A., Rapatskiy, L., Lohmiller, T., et al. The electronic structures of the S$_2$ states of the oxygen-evolving complexes of photosystem II in plants and cyanobacteria in the presence and absence of methanol. Biochim. Biophys. Acta 1807, 829–840 (2011).
[13] Ono, T. & Mino, H. Unique binding site for Mn$^{2+}$ ion responsible for reducing an oxidized Y$_Z$ tyrosine in manganese-depleted photosystem II membranes. Biochemistry 38, 8778–8785 (1999).
[14] Suga, M., Akita, F., Sugahara, M., Kubo, M., Nakajima, Y., Nakane, T., et al. Light-induced structural changes and the site of O=O bond formation in PSII catalyzed by XFEL. Nature 543, 131–135 (2017).
[15] Asada, M., Nagashima, H., Koua, F. H. M., Shen, J. R., Kawamori, A. & Mino, H. Electronic structure of S$_2$ state of the oxygen-evolving complex of photosystem II studied by PELDOR. Biochim. Biophys. Acta 1827, 438–445 (2013).
[16] Nagashima, H. & Mino, H. Highly resolved proton matrix ENDOR of oriented photosystem II membranes in the S$_2$ state. Biochim. Biophys. Acta 1827, 1165–1173 (2013).
[17] Nagashima, H. & Mino, H. Location of Methanol on the S$_2$ State Mn Cluster in Photosystem II Studied by Proton Matrix Electron Nuclear Double Resonance. J. Phys. Chem. Lett. 8, 621–625 (2017).
[18] Asada, M. & Mino, H. Location of the High-Affinity Mn$^{2+}$ Site in Photosystem II Detected by PELDOR. J. Phys. Chem. B 119, 10139–10144 (2015).

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