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Configurtion of electron transfer cofactors in photosystem II studied by pulsed EPR

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The major electron transfer cofactors in photosystem II have been studied by pulsed EPR, pulsed electron electron double resonance (PELDOR) and laser excited spin polarized electron spin echo envelope modulation (ESEEM) methods, in non-oriented and oriented photosystem II membranes. Distances between radical pairs were determined from the observed dipole interaction constants to be 27.3 Å for P680-QA, 30 Å, etc. with the error within 1 Å. Angles between the distance vector and membrane normal was determined by orientation dependence of oriented membranes with the accuracy of 5°. The results were compared with the recent structural data by X-ray analysis.

Key words: Photosystem II, Relative positions, Electron transfer, ESEEM, PELDOR, Radical pair, Dipole interaction.

INTRODUCTION

Recently, the crystal structure of photosynthetic reaction centers of photosystem II of cyanobacterium was analyzed by Zouni et al.[1] with the resolution of 3.8 Å. Pulsed EPR has given structural information by detecting dipolar interactions between the pair of electron transfer cofactors, $P680^{+}Q_A^{-}$ induced by pulsed laser irradiation [2] and others. The determined distances 27.3 Å was a little shorter than that fir $P860^{+}Q_A^{-}$ 28.3 Å and a little longer than that for $P700^{+}A_1^{-}$ 25.7 Å. These differences might have affected kinetics of charge recombination between the cofactors. To elucidate the mechanism of electron transfer, the detail structural information is essential. EPR provide the correct distance between spin centers with the accuracy within one Å. In this report the methods of observation and the material handling to obtain specific radical pairs are described. The results are summarized in Table 1and the values will be discussed in comparisonwith the recent ststructural data [1].

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MATERIALS AND METHODS

Oxygen evolving PS II particles

Oxygen evolving PS II membranes (about 400 μmol O_2/mg Chl/h) were prepared from market spinach by the method of Kuwabara & Murata or BBY and suspended in a MES buffer (at pH 6.5) with Chlorophyll concentration 3 to 15 mg depending on the experiment with 50 % glycerol as a cryoprotectant added if necessary. The PS II membranes were stored in 77 K until use.

Oriented membranes PS II membranes were painted on mylar sheets and were dried under 90 % humidity. The distribution of the orientation may be from 15 to 20° as defined by root mean square deviation. The sample

sheets were cut into the strips of 3 × 20 mm^2, and a bundle of piled five or six sheets was inserted into a quartz tube with the inner diameter of 4 mm.

Tris-treatment PS II membranes are suspended in Tris (tris(hydroxylmethyl) aminomethane) buffer at pH 8.7 and incubated under room light with gentle stir for 30 min at 4 °C. This treatment eliminates all manganese with three extrinsic proteins on the donor side, in which $Y_Z^{\bullet}$ radical becomes visible by cw EPR because quick donation of electron is inhibited.

Reduction by Hydroxylamine The S_1-state WOC was reduced with 80 μM NH_2OH to the S_0-state to observe PELDOR of $Mn_4(S_0)$-$Y_D^{\bullet}$ pair.

Site-directed mutagenesis A mutant of *chlamydomonas reinhardtii* lacking Y_D 160 will be investigated to know the distance from $Y_Z^{\bullet}$ radical without interference from $Y_D^{\bullet}$ signal.

Illumination and trapping To induce charge separation illumination of PS II sample with appropriate intensity and wave length is essential. A 500 W tungsten halogen lamp is used for continuous illumination, while the second harmonics of pulse Nd-YAG laser with 532 nm wave length is used for pulse irradiation on time resolved experiment. Illumination of the oxygen evolving PS II sample at 200 K produces the S_2-state of WOC at high yield. On the other hand, all S-states except for S_4 can be produced by flash illumination by laser or Xenon pulse light.

Below 243 K, electron transfer from Q_A to Q_B is inhibited. Trapping below 200 K is necessary to produce radicals such as $Y_Z^{\bullet}$ and Q_A immediately after illumination above 253 K or to stabilize generally a charge separated state, D^+A^-

Pulsed Electron-Electron Double Resonance (PELDOR)

Let us consider a pair of interacting spins A and B. The ESE signal of the spin A is observed at ω_A using a pulse sequence, the first $\pi/2$ and third π pulses separated by the time interval τ. The spin B produces an extra dipolar field on the spin A in addition to the applied magnetic field H_0 as given by

$$\varepsilon(r,\theta) = \gamma_A \Delta H = \mu_B/r^3(3\cos^2\theta - 1)m_S \qquad (1)$$

where m_S is the projection of the spin B on the applied field direction. When the second pulse at the frequency ω_B is applied to the spin B at the time τ' to turn the B spins, the sudden change of the extra field given by Eq. (1) produces a periodic change in the echo height of $V(2\tau)$ depending on τ' as given by

$$V(\tau') \propto -p[1-\cos(\Delta\omega\tau')], \quad \text{with } \Delta\omega = \varepsilon(r,\theta) \qquad (2)$$

For randomly oriented system $V(\tau')$ ahould bw cwefws over the orientation θ.

$$V(\tau') \propto \int <\cos(\Delta\omega\tau')>_\theta \propto \cos(\Delta\omega\tau')\sin\theta\, d\theta \qquad (3)$$

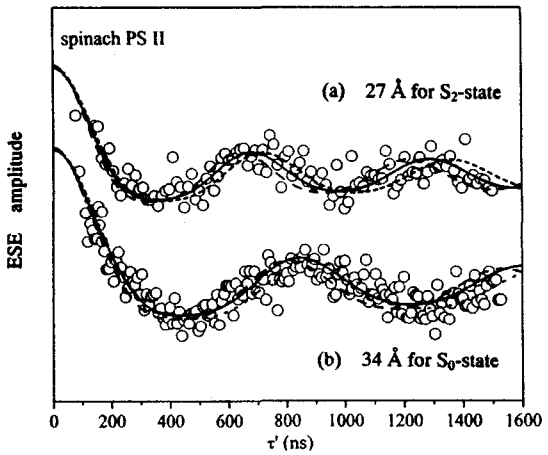

Fig. 1. PELDOR signals for the Mn-cluster in the S_2-state (a) and S_0-state (b). The partner is Y_D radical. The full lines show the simulation for the distance 27 and 34 Å respectively. The broken lines show the different simulations with ± 0.5 Å.

RESULTS AND DISCUSSION

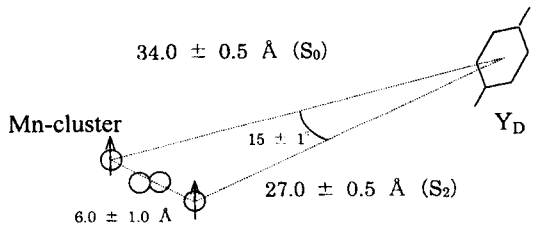

Fig. 2. The positions of spin centers of the S_2 and S_0-state Mn-cluster. The configuration of four manganese are shown based on the distance between the centers of the end of Mn-cluster 6 Å and the angles 15° derived from the X-ray data. The arrows show the spin center of S_0 and S_2 respectively.

Table 1. **The derived distances and angles of electron transfer cofactors in PS II studied by EPR**

Paramagnetic Pairs	Distances(Å)	Angles (°) from n axis	Methods
P680$-Q_A$	27.4 ± 0.3[2]	21 ± 5[3]	ESEEM
Y_D-Q_A	38.5 ± 0.8[4]	28 ± 5[5*]	'2+1' pulse
Y_Z-Q_A	34 ± 1[6]		PELDOR, ESEEM
Y_D-Y_Z	29.5 ± 0.5[7]	80 ± 2[8]	'2+1' pulse
Y_D-Chl_Z	29.4 ± 0.5[4]	50 ± 5[9]	'2+1' pulse
$Y_D-Mn_4(S_2)$	27.1 ± 0.2[10]	70 ± 2[8]	PELDOR
Q_A-Cyt b_{559}	40 ± 3[11]	78 ± 5[11]	PELDOR
Chl_Z- Cyt b_{559}	34 ±3***	58 ±5***	PELDOR
Y_D-non-heme Fe	42 ± 2[12]		Selective hole burn
P680$-Y_Z$	16 ± 2	74 ± 5	Graphic derived**
P680$-Y_D$	17 ± 2	58 ± 5	Graphic derived**
P680$-Mn_4(S_2)$	15 ± 5[12]		Time resolved

* The angle value in [4] was not correct and the corrected value is shown after re-calculation.
* * Drawing was carried out using the data 2, 3, 4, 5, 6, 7 and 8.
*** This work

Figure 1 shows PELDOR time profiles observed in the S_0 and S_2-states Mn-cluster with the partner $Y_D^{\bullet}$ radical, which shows that the spin center moved by oxidation as shown in Fig. 2.

Table 1 shows distances and orientations of the distance vectors relative to the membrane normal (crystal *c*-axis) so far obtained by EPR. Accurate values for distances were obtained by ESEEM and PELDOR. Other EPR methods give only approximate values comparable to the resolution of X-ray analysis.

The distances from P680 to Q_A and Mn cluster seem to be consistent with EPR results. The distance from Cyt b_{559} to Q_A obtained by EPR is much shorter than that by X-ray data. The values of distances for Y_D and Y_Z to P680 and to Mn-cluster by X-ray seem to be inaccurate, probably because of low resolution of 3.8 Å.

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