ELSEVIER

Biochimica et Biophysica Acta 1187 (1994) 89–93

BBA Biochimica et Biophysica Acta

Rapid Report

Distance between tyrosines Z^+ and D^+ in plant Photosystem II as determined by pulsed EPR

Andrei V. Astashkin [1], Yoshio Kodera, Asako Kawamori *

Faculty of Science, Kwansei Gakuin University, Uegahara 1-1-155, Nishinomiya 662, Japan

Received 11 April 1994

Abstract

A '2 + 1' electron spin echo method was applied to estimate the dipole interaction between tyrosines D^+ and Z^+ in Mn-depleted preparations of plant Photosystem II. The value of dipole interaction obtained corresponds to the distance of approx. 30 Å between the two types of tyrosine radical.

Key words: Photosystem II; Structural organization; Tyrosine D; Tyrosine Z; EPR, pulsed

The information about the structural organization of plant Photosystem II is essential for better understanding of the mechanism of the light-induced chemical reactions leading to water oxidation and release of free oxygen molecules. With the lack of X-ray data for PS II, the structural information can be obtained by alternative methods, including IR, EXAFS and EPR spectroscopies. In recent years EPR has been extensively applied to clarify the nature and function of various components of the charge transfer chain in PS II (for review see Ref. [1]) and to estimate the distances between them [2–8].

Two types of tyrosine radical in PS II, Y_D^+ and Y_Z^+, are known to give almost identical EPR spectra with a *g*-factor close to 2.0046, width of about 2 mT and partially resolved hyperfine structure of four lines [9–11]. These tyrosines belong to D_1 (Y_Z) and D_2 (Y_D) proteins and are thought to be located symmetrically with respect to the primary electron donor P680 [12–15]. Even with this symmetric arrangement, Y_D and Y_Z have different functions in PS II. Y_Z acts as an intermediate redox-active species in the electron transfer from the manganese cluster in OEC to the photooxidized P680. The other tyrosine, Y_D, does not directly participate in the reactions related to the oxygen evolution and its role in PS II is rather unclear. At room temperature, Y_D is oxidized with a characteristic time of one second and rereduced within tens of minutes [16,17], giving rise to easily observable EPR signal (Signal II_{slow}). The corresponding values for Y_Z in intact PS II are nanoseconds and hundreds of microseconds [10,18,19], and its EPR signal (Signal $II_{very\ fast}$) can be observed only with difficulty.

The difference in the properties of Y_D and Y_Z could probably be understood if more information about the structure of their local surroundings and about their spatial positions with respect to other electron carriers in PS II were available. The studies of Y_D^+ and Y_Z^+ by EPR, ENDOR and IR spectroscopies [11,20,21] have shown some differences in their local structures. However, these data are too scarce to explain the functional difference between the tyrosines. The measurement of relative positions of the tyrosines with respect to other electron carriers is a part of the problem of the global mapping of PS II. Some steps in this direction have already been taken and the distances between Y_D^+ and

* Corresponding author. Fax: + 81 798 510914.

[1] On leave from Institute of Chemical Kinetics and Combustion, Russian Academy of Sciences, 630090 Novosibirsk, Russia.

Abbreviations: PS II, Photosystem II; Chl, chlorophyll; P680, primary electron donor in Photosystem II; Y_Z, tyrosine Z electron donor in Photosystem II; Y_D, tyrosine D electron donor in Photosystem II; OEC, oxygen-evolving complex; Mops, morpholinopropanesulfonic acid; m.w., microwave; hfi, hyperfine interaction; EPR, electron paramagnetic resonance; ESE, electron spin echo; ENDOR, electron-nuclear double resonance; EXAFS, extended X-ray absorption fine structure; IR, infrared.

SSDI 0005-2728(94)00112-I

the Mn cluster in OEC (about 28 Å, [2,6]), between Y_Z^+ and $P680^+$ (15 Å, [3]) and between Y_D^+ and $Fe^{2+}Q_A^-$ acceptor complex (28 Å [6], less than 52 Å [7], more than 38 Å [8]) have been evaluated using continuous wave and pulsed EPR spectroscopies. This set of distances does not determine any unique structure of the charge transfer apparatus of PS II and additional structural information is necessary.

In Mn-depleted PS II Y_Z^+ can be trapped with a concentration comparable to that of Y_D^+ and studied by EPR [22]. In this work the '2 + 1' ESE method [23–25] is applied to estimate the distance between Y_Z^+ and Y_D^+ in Mn-depleted PS II.

The Mn-depleted PS II membranes were prepared from the oxygen-evolving PS II membranes (300–500 mmol O_2/mg Chl per h) isolated from spinach by the method of Kuwabara and Murata [26] and suspended in a buffer medium containing 0.2 M sucrose, 20 mM NaCl and 20 mM Mops-NaOH (pH 6.8). To remove manganese with a loss of oxygen evolution activity, the PS II membranes at the concentration of 0.2–0.3 mg Chl/ml were suspended in 0.8 M Tris buffer (pH 8.5), stirred for 30 min at 277 K under room light and centrifuged at $35\,000 \times g$ for 20 min. The final pellet was suspended in the same buffer as the oxygen-evolving PS II. Then the Mn-depleted PS II membranes with a concentration of 10 mg Chl/ml were transferred into Suprasil quartz tubes and equilibrated on ice in the dark for 2 h.

Before ESE experiments the Mn-depleted sample was illuminated for 15 s at 253 K by a 500 W tungsten-halogen lamp through a 10 cm – thick water filter. Immediately after illumination, the samples were put in liquid nitrogen in order to trap Y_Z^+ radicals.

After ESE experiments with the illuminated samples, the samples were dark-adapted for 30 min at 273 K and the measurements were repeated. To ensure the accurate measurement of the relative ESE signal amplitudes in the illuminated and dark-adapted samples, the dark-adapted samples were put into the cavity in the same position as the illuminated samples. In a separate experiment it was confirmed that in this case the changes of the cavity coupling, resonance frequency and quality factor were negligible and the error of the ESE signal amplitude measurements did not exceed 5%.

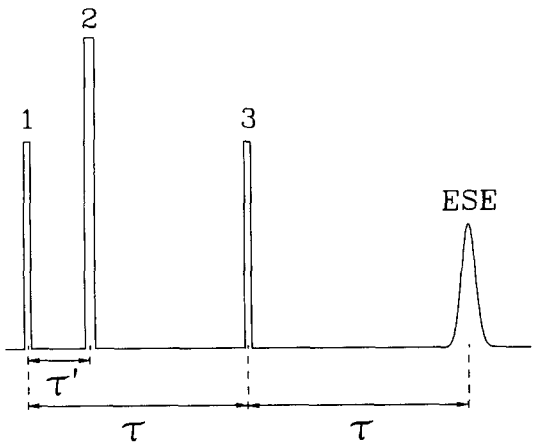

Fig. 1. The pulse sequence of '2+1' ESE method. The primary ESE signal is formed by the first and third m.w. pulses separated by the time interval τ. The amplitude of this signal is measured as a function of the second pulse position, τ'.

ESE measurements have been performed on a pulsed EPR spectrometer ESP-380 (Bruker) using a '2 + 1' ESE sequence (see Fig. 1). The spectrometer was equipped with a cylindrical dielectric cavity (ER4117DHQ-H, Bruker) and a nitrogen gas flow system (CF935, Oxford Instruments). The measurement temperature was about 80 K and microwave pulses of 16 ns, 24 ns and 16 ns duration were used. The m.w. magnetic field amplitude, H_1, in the three pulses was set to provide the spin rotation angles of 30°, 60° and 30°, respectively.

In the '2 + 1' ESE method the spin system is excited by three m.w. pulses (Fig. 1). The first and the third pulses, separated by the time interval τ, form the primary ESE signal. The second pulse, separated from the first one by the time interval τ' ($\tau' \leq \tau$), changes spin projections from $|\alpha\rangle$ to $|\beta\rangle$ and vice versa. If the magnetic dipole interaction between the pairwise-distributed radicals is noticeable, flip of one of the spins changes the local magnetic field for its partner in the pair. As a result, the magnetization after the third pulse cannot be completely refocused and the amplitude of the primary ESE signal exhibits the dependence on the second pulse position (i.e., on τ'). The following expression [24] describes this dependence in the case of pairwise-distributed radicals with similar g-values and EPR spectrum shapes:

$$V(\tau,\tau') \propto 1 - 2\langle S_3\rangle \sin^2(A\tau/2) - 2\langle S_2\rangle \sin^2(A\tau'/2) + \langle S_2S_3\rangle\left[\sin^2(A\tau'/2) - \sin^2(A(\tau\text{-}\tau')/2) + \sin^2(A\tau/2)\right] \quad (1)$$

In this expression $A = 2\pi D(1\text{–}3\cos^2\theta)$ is the secular component of the dipole interaction between the radical spins, S_2 and S_3 characterize the excitation of spins by the second and third m.w. pulses, respectively, and $\langle \ldots \rangle$ denotes averaging over the EPR spectrum shape. The values $S_{2,3}$ are calculated as:

$$S_{2,3} = \left[\omega_1^2/\omega_n^2\right] \sin^2\left(\omega_n^2 t_p/2\right) \quad (2)$$

where t_p is the duration of the corresponding m.w. pulse, $\omega_1 = 2\pi g\beta H_1$ is its amplitude, $\omega_n = (\omega_1^2 + \Delta\omega^2)^{1/2}$ is the nutation frequency of the electron spin during the pulse and $\Delta\omega = 2\pi g\beta\Delta H_o$ is a difference between the m.w. frequency and the resonance frequency of the spin.

As seen from Eq. (1), with increasing the time interval τ', the ESE amplitude oscillates with a frequency proportional to the value of magnetic dipole interaction at given orientation. The amplitude of these oscillations is proportional to the values $\langle S_2 \rangle$, $\langle S_3 \rangle$ and $\langle S_2 S_3 \rangle$ that depend on the EPR spectrum width. In a non-oriented system, Eq. (1) is to be averaged over the angle θ between the radius-vector **r** connecting radicals in the pair, and the direction of the external magnetic field H_0:

$$\langle V(\tau, \tau') \rangle \alpha \int_0^{\pi} V(\tau, \tau') \sin\theta \, d\theta \qquad (3)$$

Now let us consider the results of ESE experiments. The field-sweep primary ESE spectra measured at $\tau = 280$ ns for Mn-depleted PS II are shown in Fig. 2. Spectrum 1 corresponds to the illuminated sample (containing Y_D^+ and Y_Z^+ radicals) and spectrum 2 corresponds to the dark-adapted sample (Y_D^+ only). Spectrum 3 represents the difference between the first and second spectra and refers to Y_Z^+ radicals. A stick diagram obtained by numerical simulations and qualitatively explaining the structure of the Y_Z^+ spectrum is given at the bottom of Fig. 2. The lower part of this diagram shows splittings due to the hyperfine interactions of two equivalent protons at 3 and 5 ring positions. The hfi constant of 0.62 ± 0.02 mT obtained by the simulation agrees well with the data presented in [27]. The hyperfine splitting due to the β-methylene proton (upper part of the stick diagram) is found to be

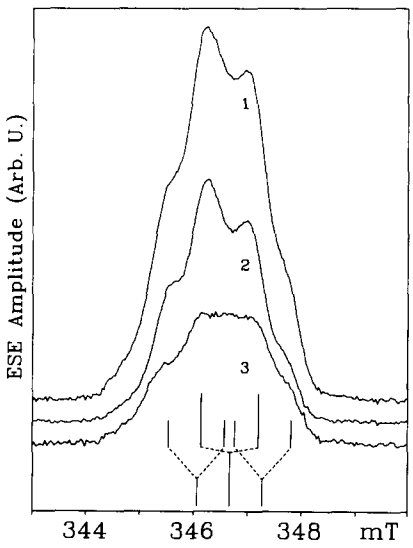

Fig. 2. The field-sweep primary ESE spectra of oxidized tyrosine radicals in Mn-depleted PS II. 1 – illuminated sample (Y_D^+ and Y_Z^+), 2 – dark-adapted sample (Y_D^+ only), 3 – difference (Y_Z^+ only). In these experiments the m.w. pulses of 16 ns (90°) and 24 ns (180°) length were used and the ESE signal intensity was monitored by the boxcar averager with the integration gate of 180 ns. A stick spectrum at the bottom shows the isotropic splittings due to 3.5 ring protons and one β-methylene proton.

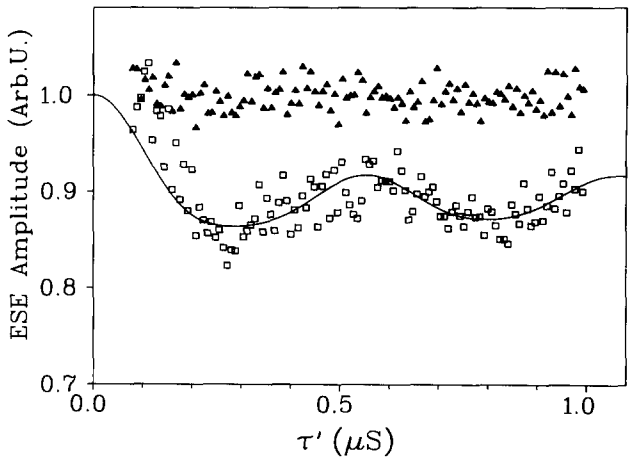

Fig. 3. The dependences of the primary ESE signal of tyrosine radicals on τ'. Open squares: obtained in the illuminated sample of Mn-depleted PS II. Filled triangles: obtained in the same sample after dark-adaptation. Solid line: calculated using Eqs. (1–3) for the dipole interaction constant $D = 2$ MHz. The trace for the illuminated sample was corrected by subtracting a constant value as described in the text. All traces are normalized to unity at $\tau' = 0$.

1.08 ± 0.02 mT. This value is close to that determined in [22]. The shape of the Y_Z^+ field-sweep ESE spectrum is thus fully explained by the diagram presented and we may suggest that the contribution of other (contaminating) paramagnetic species is negligible.

The '2 + 1' ESE experiment was performed at a fixed $\tau = 1080$ ns, with τ' varying from 80 ns to 992 ns. The dependence of the primary ESE amplitude on τ', recorded for the Mn-depleted PS II sample with trapped Y_Z^+ radicals, is shown in Fig. 3 by open squares. This dependence reveals about two periods of low-frequency oscillations and differs remarkably from that obtained in the same sample after dark adaptation (filled triangles). In the dark-adapted sample, only Y_D^+ radicals survive. As their concentration is very low (one radical per one reaction center), there is no detectable dipole interaction between them, and the experimental '2 + 1' trace shows no oscillatory behavior. The difference between these two traces allows one to ascribe the oscillations, observed for the sample with trapped Y_Z^+ radicals, to the dipole interaction between Y_Z^+ and Y_D^+.

The primary ESE amplitude in the dark-adapted Mn-depleted sample was about 60% of that in the illuminated sample. This decrease of the ESE amplitude allows one to estimate the amount of trapped Y_Z^+ radicals as approx. 70% of the amount of the Y_D^+ radicals. Accordingly, about 82% of the total ESE signal intensity in the illuminated sample is determined by pairwise-distributed radicals responsible for the low-frequency oscillations in the dependence of the ESE signal on τ'. The other 18% of the ESE signal is determined by 'unpaired' Y_D^+ radicals that do not give the oscillations. Thus, to obtain a pure contribution of

the pairwise-distributed radicals, the '2 + 1' ESE trace for the illuminated sample (open squares in Fig. 3) was corrected by subtraction of a constant background with an amplitude 30% of that observed in the dark-adapted sample.

As stated in the beginning and as seen from Fig. 2, the EPR spectra of Y_D^+ and Y_Z^+ radicals are similar (at least from the point of view of the m.w. excitation conditions). Hence, one can approximately consider them to be a single pairwise-distributed species and use Eq. (1) to describe the results of '2 + 1' ESE experiment obtained for them.

To determine the value of the dipole interaction between these two radical species, D, calculations were done using Eqs. (1–3). To estimate the quantities $\langle S_2 \rangle$, $\langle S_3 \rangle$ and $\langle S_2 S_3 \rangle$, the EPR spectrum shape of Y_D^+ was used. These calculations allowed one to determine the value of dipole interaction $D = 2 \pm 0.1$ MHz. The dependence of the primary ESE amplitude on τ', calculated for $D = 2$ MHz, is represented in Fig. 3 by a solid line. It shows a reasonable agreement with the experiment.

In a point dipole approximation:

$$D = (g\beta)^2/hr^3 \quad (4)$$

the distance between Y_Z^+ and Y_D^+, estimated from the obtained value of D, is $r = 29.6 \pm 0.5$ Å. The error of 0.5 Å has no physical meaning, since it is much smaller than the size of the tyrosine molecule. Hence, the round value $r \sim 30$ Å can be considered as a good approximation for the distance between Y_Z^+ and Y_D^+ radicals in plant Photosystem II.

The distances of 14 Å from Y_D and Y_Z to the closest Mg atoms of P680 were predicted in Ref. [14] with the aid of computer modelling of the structure of the donor side of PS II. With a symmetric arrangement of Y_D and Y_Z with respect to P680, the distance between the tyrosines must be close to 30 Å in agreement with the result of the present study.

Finally, it has to be mentioned that oscillations in the '2 + 1' ESE experiment can be caused also by electron-nuclear magnetic interactions [28]. Let us consider this possibility for the system studied. The 2-pulse ESEEM patterns we recorded for Y_D^+ and Y_Z^+ radicals (not shown) are similar to those described in Refs. [22,29]. The most intense line in the Fourier transform spectrum of the Y_D^+ ESEEM is caused by hfi with one of the β-methylene protons and has a frequency of 1.52 MHz. The corresponding line in the spectrum of the Y_Z^+ ESEEM has a frequency of 1.95 MHz and about 2.5-times smaller amplitude. This frequency is very close to that observed in the '2 + 1' experiment (Fig. 3, open squares). However, with the hfi constants of the order of 30 MHz (10 mT) typical for β-methylene protons of the studied tyrosine radicals, the situation of practically exact cancellation of nuclear Zeeman and hyperfine interactions arises for the $S = +1/2$ spin projection of the unpaired electron ($\nu_I - a/2 \approx 0$ where $\nu_I = 14.75$ MHz is a proton Zeeman frequency in the applied static magnetic field of 346.5 mT and a is a hfi constant). For an inhomogeneously broadened EPR spectrum, this situation corresponds to the condition of the complete excitation of the 2-pulse ESEEM by m.w. pulses. A direct calculation using a density matrix formalism shows that no nuclear ESEEM occurs in the '2 + 1' ESE method in this case for the m.w. pulses with $g\beta H_1 \approx 7$ MHz used in our experiments. Indeed, the experimental '2 + 1' trace of Y_D^+ (filled triangles in Fig. 3) shows no low-frequency modulation. We therefore conclude that the oscillation observed in the '2 + 1' trace for the illuminated PS II sample is not caused by hfi of the β-methylene proton of the Y_Z^+ radical.

The authors thank Dr. V.V. Kurshev (University of Houston, USA) for useful advice. A.V.A. and Y.K. are grateful to the Japan Society for the Promotion of Science (JSPS) for awarding them postdoctoral fellowships to conduct research in Kwansei Gakuin University. A.V.A. is also greatly indebted to Prof. Yu.D. Tsvetkov (Novosibirsk, Russia) for giving him the chance to conduct research in Japan.

References

[1] Miller, A.-F. and Brudvig, G.W. (1990) Biochim. Biophys. Acta 1056, 1–18.
[2] Evelo, R.G., Styring, S., Rutherford, A.W. and Hoff, A.J. (1989) Biochim. Biophys. Acta 973, 428–442.
[3] Hoganson, C.W. and Babcock, G.T. (1989) Biochemistry 28, 1448–1454.
[4] Innes, J.B. and Brudwig, G.W. (1989) Biochemistry 28, 1116–1125.
[5] Isogai, Y., Itoh, S. and Nishimura, M. (1990) Biochim. Biophys. Acta 1017, 204–208.
[6] Kodera, Y., Takura, K. and Kawamori, A. (1992) Biochim. Biophys. Acta 1101, 23–32.
[7] Kawamori, A., Kodera, Y., Astashkin, A.V., Dzuba, S. and Tsvetkov, Yu.D. (1993) Kwansei Gakuin University Annual Studies XLII, 105–130.
[8] Hirsh, D.J., Beck, W.F., Innes, J.B. and Brudwig, G.W. (1992) Biochemistry 31, 532–541.
[9] Commoner, B., Heise, J.J. and Townsend, J. (1956) Proc. Natl. Acad. Sci. USA 42, 710–718.
[10] Hoganson, C.W. and Babcock, G.T. (1988) Biochemistry 27, 5848–5855.
[11] Boerner, R.G. and Barry, B.A. (1993) J. Biol. Chem. 268, 17151–17154.
[12] Debus, R.J., Barry, B.A., Babcock, G.T. and McIntosh, L. (1988) Proc. Natl. Acad. Sci. USA 85, 427–430.
[13] Vermaas, W.F.J., Rutherford, A.W. and Hansson, O. (1988) Proc. Natl. Acad. Sci. USA 85, 8477–8481.
[14] Svensson, B., Vass, I., Cedergren, E. and Styring, S. (1990) EMBO J. 7, 2051–2059.
[15] Ruffle, S.V., Donnelly, D., Blundell, T.L. and Nungent, J.H.A. (1992) Photosyn. Res. 34, 287–300.
[16] Babcock, G.T. and Sauer, K. (1973) Biochim. Biophys. Acta 325, 483–503.

[17] Styring, S. and Rutherford, A.W. (1987) Biochemistry 26, 2401–2405.
[18] Babcock, G.T. (1987) in New Comprehensive Biochemistry: Photosynthesis (Amesz, J., ed.), pp. 125–158, Elsevier, Amsterdam.
[19] Blankenship, R.E., Babcock, G.T., Warden, J.T. and Sauer, K. (1975) FEBS Lett. 51, 287–293.
[20] MacDonald, G.M., Bixby, K.A. and Barry, B.A. (1993) Proc. Natl. Acad. Sci. USA 90, 11024–11028.
[21] Mino, H., Kodera, Y. and Kawamori, A. (1992) in Research in Photosynthesis (Murata, N., ed.), Vol. II, pp. 61–64, Kluwer, Dordrecht.
[22] Kodera, Y., Takura, K., Mino, H. and Kawamori, A. (1992) in Research in Photosynthesis (Murata, N., ed.), Vol. II, pp. 57–60, Kluwer, Dordrecht.
[23] Kurshev, V.V., Raitsimring, A.M. and Tsvetkov, Yu.D. (1989) J. Magn. Reson. 81, 441–454.
[24] Kurshev, V.V., Raitsimring, A.M. and Ichikawa, T. (1991) J. Phys. Chem. 95, 3564–3568.
[25] Raitsimring, A., Peisach, J., Lee, C.H. and Chen X. (1992) J. Phys. Chem. 96, 3526–3531.
[26] Kuwabara, T. and Murata, N. (1982) Plant Cell Physiol. 23, 533–539.
[27] Barry, B.A., El-Deeb, M.K., Sadusky, P.O. and Babcock, G.T. (1990) J. Biol. Chem. 265, 20139–20143.
[28] Kurshev, V.V., Astashkin, A.V. and Raitsimring, A.M. (1988) J. Struct. Chem. 29, 62–68.
[29] Evelo, R.G., Dikanov, S.A. and Hoff, A.J. (1989) Chem. Phys. Lett. 157, 25–30.