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ENDOR study on the position of hydrogens close to the manganese cluster in S_2 state of photosystem II

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Proton matrix ENDOR of the manganese multiline in the S_2 state of photosystem II membranes from spinach has been investigated. The spectral structure over a range of frequencies of ± 2 MHz centered on the position for a free proton was analyzed through employing a simulation method based on the dipolar interaction between electron and proton magnetic moments. The 6 pairs of lines resolved were attributed to the surrounding proton populations at distances varying within the range 2.7–6.0 Å from the putative manganese center. Two of the 6 pairs, namely those corresponding to protons at distances of 2.7 and 3.2 Å from the manganese center were eliminated on washing of the membranes with deuterated buffer. This suggests that these protons belong to the water molecules coordinating to the manganese cluster. The presence of ethylene glycol instead of glycerol and sucrose in the buffer broadened both EPR and ENDOR spectra. This suggests that ethylene glycol molecules are also accessible to the two-manganese cluster. A possible model of water association to the manganese ions in the photosynthetic water-oxidation system is proposed.

ENDOR; Manganese; ESR signal, multiline; Water coordination; Deuterium substitution; State S_2; Photosystem II; (Spinach)

1. INTRODUCTION

Since the initial discovery of a multiline EPR signal from manganese in the S_2 state of PS II by Dismukes and Siderer [1], various physicochemical and theoretical methods have been applied in order to obtain information about the structure of the manganese cluster functioning in water cleavage [2–5]. Based on analysis of the temperature dependence of the multiline signal intensity, De Paula et al. [3] have proposed that various EPR species can be explained theoretically by assuming that varying degrees of exchange coupling take place within a manganese tetramer. EXAFS [4] and optical absorption [5] studies also provide data on the valence state of the manganese ions in each S-state. A recent ESEEM (electron spin echo envelope modulation) study of NH_4C-treated PS II membrane fragments has shown that nitrogen atoms are coordinated to the manganese cluster [6]. Hansson et al. [7] observed line broadening of the multiline signal on washing PS II membranes with buffer enriched in $H_2{}^{17}O$ and proposed that a water molecule is located near the manganese cluster. Nugent [8] examined the line shape of the multiline signal obtained with improved resolution by employing PS II membranes incubated in deuterated buffer. However, no information has been yielded by the results as concerns the position of the water molecule coordinated to the manganese cluster. We report here information on the position of hydrogens surrounding the manganese cluster in the S_2 state as deduced by means of proton matrix ENDOR including deuterium substitution. Matrix ENDOR followed by analysis of the pure dipolar interaction between

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Abbreviations: PS, photosystem; ENDOR, electron nuclear double resonance; Mes, 4-morpholineethanesulfonic acid; Chl, chlorophyll; Mops, 4-morpholinepropanesulfonic acid, rf, radiofrequency

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an unpaired electron and the surrounding nuclei in the matrix yields information regarding the positions of atomic nuclei, while local ENDOR together with subsequent analysis of hyperfine interactions provides further data concerning the characteristics of the electronic state and chemical bonding within an atom or a molecule [9]. With the aid of a simulation method based on dipolar interactions between electron and proton magnetic moments we could determine tentatively the distance of protons from the putative center of the maganese cluster. It was observed that some proton signals, probably those arising from the hydrogen atoms of water molecules coordinated directly to manganese ions, were eliminated on replacement by deuterium.

2. MATERIALS AND METHODS

PS II membranes were prepared from market spinach by the method of Kuwabara and Murata [10]. The PS II membranes were incubated with a deuterated buffer (10 mM NaCl/5 mM $CaCl_2$/30 mM Mes-NaOD (pH 6.2), and then centrifuged at 30000 × g for 20 min at 8°C. This procedure was repeated twice. Finally, 50 vol.% deuterated ethylene glycol was added to give a concentration of 10–15 mg Chl/ml. The sample thus prepared will henceforth be referred to as EG-D. Control PS II membranes (EG-H) were prepared via the same method in undeuterated buffer solution. PS II membranes suspended in 30 vol.% glycerol buffer solution [0.2 M sucrose/20 mM NaCl/20 mM Mops (pH 6.8)], denoted GS-H, were also used for comparison. All procedures were performed under a dim green safe light. PS II membranes were transferred to Suprasil quartz tubes of 3 mm inner diameter. After dark adaptation for 2 h at 0°C, samples were illuminated for 6 min at 195 K in a methanol/solid CO_2 bath, quickly cooled in liquid N_2 in darkness and then stored at 77 K until use in ENDOR measurement.

Multiline EPR signals were recorded at 4.2–5.5 K using a Varian E109 spectrometer equipped with an Oxford flow cryostat. The microwave power for half-saturation at 4.2 K was 0.2 mW for samples EG-H and EG-D, whereas it amounted to 2 mW for GS-H. The multiline ENDOR signals were measured with 10 kHz modulation of the NMR frequency. The rf power of 250 W from an ENI 3200L power amplifier was supplied to 8 lines of ENDOR coils parallel to the cylindrical axis of a TE_{011} cavity, and terminated with a 50 Ω dummy load. The rf voltage across the dummy load was monitored on an oscilloscope as an indicator of rf magnetic field produced by current through the coils.

Optimum resolution of the ENDOR spectrum was obtained at 5.5 K for samples EG-H and EG-D, and at 4.5 K for GS-H. ENDOR-induced EPR [11] was measured by sweeping the magnetic field at various fixed NMR frequencies between 10.5 and 17.5 MHz to discriminate the ENDOR of the maganese multiline signal from overlapping ENDOR spectra due to other radical species such as Signal II_s, Q_A^- and Cyt. b-559.

3. RESULTS AND DISCUSSION

Fig. 1a–c shows ENDOR-induced EPR spectra of sample GS-H recorded at three different frequencies of 12.8, 13.9 and 15.6 MHz, respectively. Each

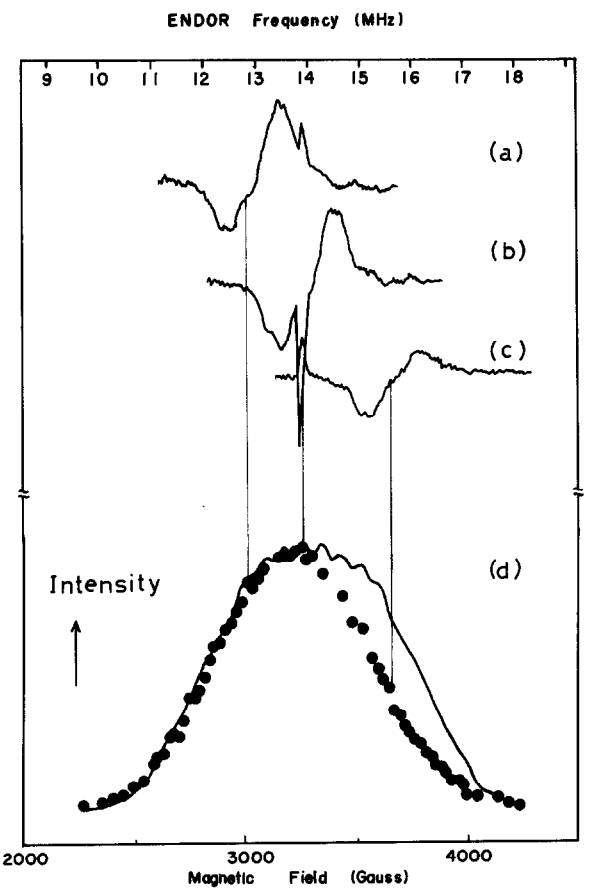

Fig. 1. ENDOR-induced multiline EPR signals for the glycerol/sucrose-containing sample (GS-H) observed at fixed frequencies: (a) 12.8, (b) 13.9 and (c) 15.6 MHz. The sharp signal of 20 G half-width at a magnetic field strength of 3240 G is assigned to Signal II_s and the broader signals peak-to-peak width approx. 200 G, to a component of the multiline. Instrumental conditions; microwave frequency, 9.12 GHz; microwave power, 1.8 mW; modulation frequency 10 kHz; FM depth, 120 kHz; rf power, 250 W (about 4 G) temperature, 4.5 K. (d) Plot of ENDOR-induced EPR intensities measured by peak-to-peak height at various constant frequencies from 9.8 to 17.5 MHz. Magnetic field strength was calculated from the resonance condition for free protons. The line shape shown by a solid curve was calculated using the spin Hamiltonian given in [1] for the antiferromagnetically coupled manganese(III–IV) dimer where parameters g = 1.96, A_1 = 172 G and A_2 = 87 G, and Lorentzian broadening of each line width of about 160 G were used.

of the spectra obtained was composed of a broad line of about 200 G in peak-to-peak width accompanied by a sharp line of about 20 G half-width. Since the sharp signal remained at a constant field of 3240 G, which is identical with the field position for Sig. II_s, it was assigned to ENDOR of Sig. II_s. On the other hand, the broad signal shifted its resonance position over 1500 G. This magnetic field range coincides with that for the multiline signal. When the peak height thus obtained was plotted as a function of rf frequency or resonance field for free protons, the ENDOR-induced EPR pattern depicted in fig.1d was obtained. Along with the plots, the calculated intensities based on the spin Hamiltonian given by Dismukes and Siderer [1] are shown by a solid curve, which coincides with our measured data except for those above 14 MHz. The discrepancy in intensity at high magnetic fields can be explained by our instrumental features, i.e. a decrease in rf field above 14 MHz as monitored on the oscilloscope. The derivative-like line shape in fig.1a–c could not be explained exactly, due to various plausible reasons, such as inhomogeneous broadening and anisotropic interactions between electron and nuclear moments [12]. We observed the same ENDOR line shape throughout this field range, showing that the same nuclei contributed to the ENDOR spectrum of each of the multiline spectra.

In order to minimize disturbance by the overlapping lines due to Signal II_s, we measured the ENDOR signal at 3440 G which is 200 G distant from the center of Signal II_s. The spectra in fig.2a,b show the light-minus-dark ENDOR signals of samples EG-H and EG-D, respectively, measured at 5.5 K. We also show the spectrum in fig.2c, as a reference, which represents the ENDOR of sample GS-H. As shown by trace a or more clearly by trace c, the ENDOR signal is composed of several pairs of lines (a–a′ to f–f′) symmetrically displaced from the center of the derivative curve. Among the six pairs of lines, two pairs, e–e′ and f–f′, disappeared on deuteration, the others changing only slightly in intensity.

The presence of glycerol and sucrose instead of ethylene glycol in suspension induces marked changes in line shape, however, it was notable that these changes did not involve the loss of the two line pairs, e-e′ and f-f′, although the component lines were appreciably narrowed. The change in

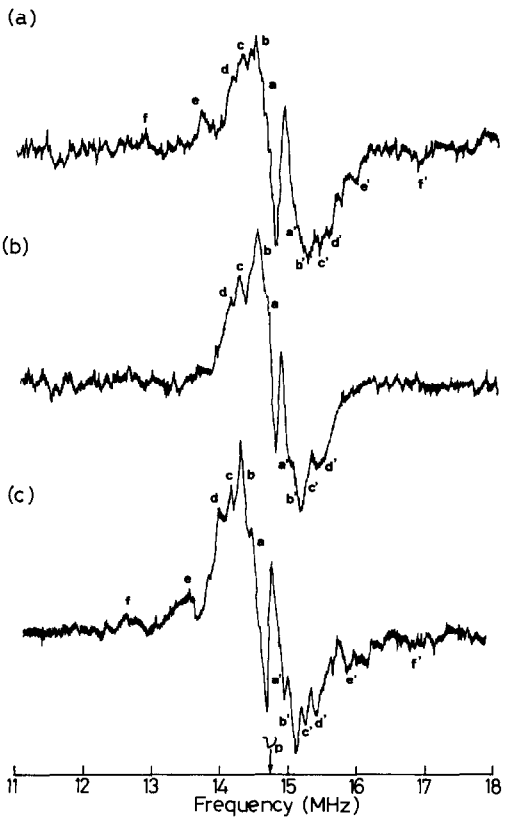

Fig. 2. Light-minus-dark ENDOR signals of the multiline in control(EG-H) (a), deuterated(EG-D) (b) and GS-H (c) samples. Positions of each pair of lines corresponding to two nuclear spin orientations are indicated a-a′ to f-f′. Approximate distance, r, can be estimated from eqn 1: $\nu_{sep} = 79.0/r(A)^3$ MHz. Instrumental conditions: Fm depth, 60 kHz; temperature, 5.5 K for samples EG-H and EG-D; magnetic field, 3440 G; all other details as given in the legend to fig.1. The trace represents the result of γ-fold data accumulation.

line shape may suggest that some of the hydrogen atoms, probably those of proteins, underwent configurational changes due to substitution of buffer containing ethylene glycol by containing one glycerol/sucrose. Also, one may consider the alternative interpretation that the number of protons close to the manganese cluster decreased due to a size effect.

Assuming that the manganese spins are concentrated at the center of gravity, we can calculate the dipolar interaction of the manganese magnetic moment $\gamma_e\hbar$ with one of the surrounding protons, $\gamma_p\hbar$.

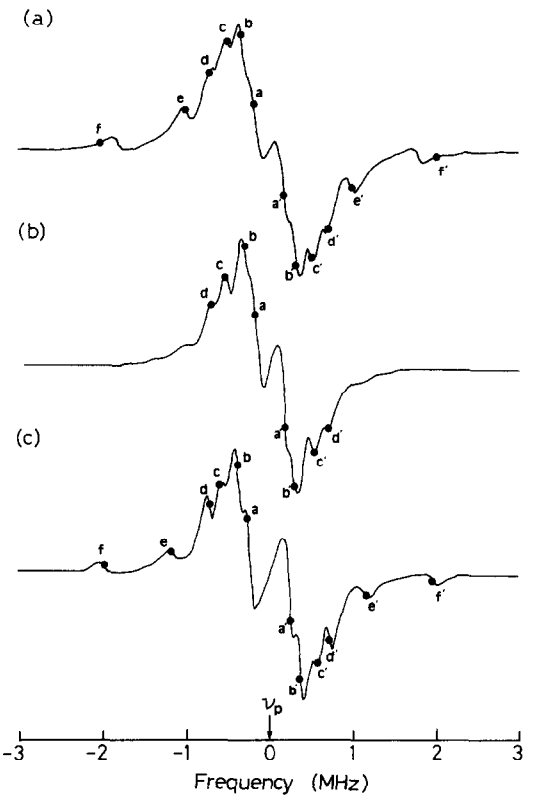

Fig. 3. Simulated line shape of ENDOR spectra of the multiline in samples, EG-H (a), EG-D (b) and GS-H (c), using the best-fit values for the distances and intensities listed in table 1 and the linewidth given in the text. Predicted ENDOR separations $79.0/r(A)^3$ MHz using the distances in table 1 are denoted a-a′ to f-f′.

The separation in frequency between a pair of ENDOR lines is expressed by:

$$2\pi\nu_{sep} = \gamma_e\gamma_p\hbar(1/r^3)[3\cos^2\theta-1] \quad (1)$$

where r denotes the distance between the manganese center and the position of the proton, and θ, the angle between the position vector and the direction of the magnetic field. The observed separation is approx. $79.0/r(A)^3$ MHz, since protons with $\theta = 90°$ (usually denoted A_1) mostly contribute to ENDOR separation.

For exact spectral simulation, contributions by various numbers of protons at different distances and angles should be summed up. By assuming Gaussian broadening of width 117 kHz for each line in samples EG-D and GS-H, and a width of 140 kHz for sample EG-H, we took as variables the distance and intensity to be the fitting parameters, averaging thoughout the angular distribution as given in [13], to determine the best fit with experimental spectra. The simulated line shapes thus obtained are shown in fig. 3 for samples EG-H(a), EG-D(b) and GS-H(c). Contributions from protons with $\theta = 0°$ could not be resolved owing to the considerable line broadening of about 120 kHz for each line. The values employed for the distances and intensities in best-fit simulations are also listed in table 1 together with the predicted values for ENDOR separations, ν_{sep}.

Although the relative intensities, a priori, do not reflect the number of protons, the loss or marked

Table 1

ENDOR frequency separations (MHz) and the parameters, distance (Å) and intensity, used for line shape simulation in the PS II samples

Samples		Peak position					
		a	b	c	d	e	f
Control	separation	0.370	0.693	1.067	1.407	2.011	4.016
(EG-H)	distance	5.98	4.85	4.20	3.83	3.40	2.70
	intensity	4.0	6.5	7.5	2.5	2.0	1.0
Deuterated	separation	0.366	0.672	1.067	1.407		
(EG-D)	distance	6.00	4.90	4.20	3.83		
	intensity	4.0	6.3	4.8	1.6		
Glycerol/sucrose-	separation	0.531	0.761	1.190	1.441	2.412	4.016
-containing	distance	5.30	4.70	4.05	3.80	3.20	2.70
(GS-H)	intensity	4.0	2.4	5.0	1.0	0.6	1.0

Intensity relations between samples adjusted at positions a and f

change in signal intensity at the corresponding frequency indicates that some protons at the relevant distances are replaced by deuterons. The tentatively assigned values for the distances in table 1 are not necessarily correct in every case due to ambiguity in the structure of the manganese cluster, but may at least be relevant to the assumption that the order of 2 or 3 Å in distance originates from protons of the water molecule directly coordinated to the manganese center, since no proton can be assumed to be accessible within a distance of 2 Å from a manganese cation. Recently, McDermott et al. [4] established a value for the distance of 2.75 Å for Mn–Mn and 1.75 Å for Mn–0 in their two-manganese-cluster model. Our present results are roughly consistent with theirs. Similarly, the presence of three protons near the two manganese ions has been proposed in one of the models based on XANES analysis [4]. If we assume that one proton is at a closest distance of 2.4 Å from each of the two manganese ions with a 50% spin density, the calculated value of the ENDOR separation accounts for the distance of 2.7 Å in table 1. The next near-neighbor protons, 3.2 Å distant, may be those attached to the two oxygen atoms coordinated to the two manganese ions in their model.

Judging from the ENDOR data in table 1, the first and second nearest-neighbor protons of the manganese center appear to be completely deuterated. As shown in fig. 2, the ENDOR spectra of samples GS-H and EG-D are of greater resolution vs sample EG-H. This suggests that fewer protons contribute to the line broadening in the former samples than in the latter. As depicted by fig. 4, our ENDOR study measured the contributions by all protons of water and proteins within about 6 Å from the putative center of the manganese cluster. The lower degree of broadening of ENDOR lines in glycerol- or glycerol/sucrose-containing medium vs that in ethylene glycol-containing medium may be ascribed to size discrimination at the manganese site: the diameter of the entrance to the water-cleaving hole would be of a size intermediate between those of glycerol and ethylene glycol molecules (~ 5 Å) larger molecules such as glycerol and sucrose being inaccessible to this hole. From the marked difference in signal intensity between samples EG-H and EG-D as shown in table 1, we may also consider the ENDOR signals remaining in sample EG-D to be mostly the result of the contributions from a larger number of protons in the surrounding proteins compared with the smaller numbers that can be substituted by deuterons. Only a small number of hydrogens belonging to some proteins might have been substituted by deuterium, which one may scarcely consider to lie close to the putative manganese center within a distance of 3 Å.

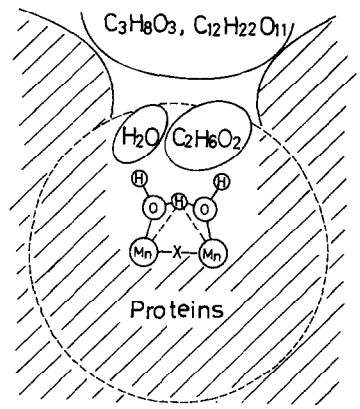

Fig. 4. Proposed model for water-cleaving hole including two manganese cluster. One of the protons may be located equidistantly at 2.4 Å from both managanese ions. Distances for other protons were estimated from the assumed center shown by a cross. The dashed circle represents the radius of 6 Å within which proton matrix ENDOR was observed. The entrance to the water-cleaving region has a diameter of about 5 Å, thus excluding large molecules such as glycerol and sucrose hatched area: approximately indicates the region where no buffer solution is included.

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