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The nature of the Fe(III) EPR signal from the acceptor-side iron in photosystem II

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The EPR spectrum at both X- and S-band (3.94 GHz) of the oxidized acceptor-side iron in photosystem II from spinach shows two absorption-type peaks at $g=8.0$ and 5.6. The intensities of these peaks have been measured at X-band in the temperature range 2–10 K. All results can be fully described assuming that the EPR spectrum arises from high-spin Fe(III) with $D=1.0\pm0.3$ cm^{-1} and $E/D=0.10\pm0.01$. Quantifications show that the spectrum in our case represents 0.4–0.5 Fe(III) per reaction center. The EPR parameters are consistent with the iron having bicarbonate and/or tyrosine as ligands in addition to four imidazoles.

Photosystem II; Acceptor-side iron; EPR; (Spinach)

1. INTRODUCTION

The reducing side of PS II is visualized as a complex in which a ferrous ion is situated near the two plastoquinones, Q_A and Q_B. It is likely that it is built in analogy with the acceptor side of the reaction center in photosynthetic purple bacteria [1,2]. This hypothesis is based on the spectroscopic similarities between the two systems [3] and the observation of sequence homologies between proteins D1 and D2 in PS II and the L and M subunits in the bacterial reaction center [4,5].

The function of the two quinones is quite similar in the two systems [6], but several properties of the ferrous ion differ which probably reflects slightly different iron environments [3]. In bacterial reaction centers oxidation of the iron has not been observed even under strongly oxidizing conditions [3], whereas in PS II the iron can be oxidized in the dark with ferricyanide [7] or in the light in the presence of some exogenous quinone-type acceptors [8,9].

The ferric form of the iron can be observed by EPR spectroscopy and shows a series of resonances around $g = 6$ [7]. Normally, two signals at $g = 8$ and 5.6 dominate the spectrum in PS II preparations from spinach. These resonances are sensitive to the occupancy of the Q_B site and to chemical species bound to this site [9,10].

In the present work the EPR spectrum of the oxidized iron in PS II has been studied at two EPR frequencies and at sub-helium temperatures. From these measurements a model is derived which is in accordance with that presented by Petrouleas and Diner [11]. An integration of the EPR spectrum shows that it represents oxidized iron in a large fraction of the PS II reaction centers.

2. MATERIALS AND METHODS

2.1. *Preparation of photosynthetic material*

PS II-enriched thylakoid membranes from spinach were prepared as described in [12] and suspended at 10–15 mg Chl/ml in 20 mM Mes-NaOH, pH 6.3, containing 15 mM NaCl, 5 mM $MgCl_2$ and 400 mM sucrose.

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Abbreviations: Chl, chlorophyll; PS, photosystem; PPBQ, phenyl-*p*-benzoquinone

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The acceptor-side iron was oxidized using the illumination-thawing procedure described in [9], with PPBQ (2 mM, added dissolved in ethanol) as an electron acceptor or using 5 mM $K_3Fe(CN)_6$ as an oxidant [7]. Photoreduction of the oxidized iron was accomplished by illumination of the X-band EPR samples at 200 K for 8 min.

2.2. *EPR spectroscopy*

EPR spectra at X-band (9.4 GHz) were recorded with a Bruker ER 200 D-SRC spectrometer and either an ESR-9 or an ESR-10 helium cryostat from Oxford Instruments. For spectra at S-band (3.9 GHz) a Bruker ER 061 SR microwave bridge and ER 6102 SR reentrant cavity were used. The ESR-9 cryostat was used also at S-band but with a home-made quartz insert allowing sample tubes with 12 mm outer diameter. A typical sample volume was 1.5 ml.

The sample temperature at X-band was determined utilizing the high-spin Fe^{3+} EPR signal from metmyoglobin essentially as described earlier [13]. However, the metmyoglobin could not be dissolved directly in the sample tube due to interference of its $g = 6$ signal with the sample signals.

3. RESULTS AND DISCUSSION

Fig.1 shows X-band EPR spectra of the oxidized acceptor-side iron in spinach produced by two different methods as described in section 2. Both spectra show two absorption peaks with g values of 8.0 and 5.6. Furthermore, repeated experiments with different preparations always gave the same area ratio between the two peaks at the same temperature.

Fig.2 shows the S-band EPR spectrum of the ferricyanide-oxidized iron. Within experimental errors, the g values are the same as in X-band, which is a strong indication that both peaks arise from Kramers' doublets separated by more than the X-band Zeeman energy. Otherwise one does not expect a linear field dependence of the energy levels.

Fig.3 shows the spectra obtained at two temperatures and fig.4 illustrates an extended temperature variation of the two peaks. The inset shows the g values calculated with $D \gg g\mu_B B$, i.e. essentially at zero magnetic field. At X-band this condition is not completely fulfilled at high magnetic fields which means that the smaller g values, if observed, are expected to differ somewhat from the values given in fig.4. However, this does not influence our analysis. It is clear from figs 3 and 4 that the experimental g values of these peaks and their temperature dependence can be explained assuming that they arise from high-spin Fe^{3+} with the spin-Hamiltonian zero-field parameters $D = 1.0 \pm 0.3$ cm^{-1} and $E/D = 0.10 \pm 0.01$. The two peaks at $g = 8.0$ and 5.6 are also ex-

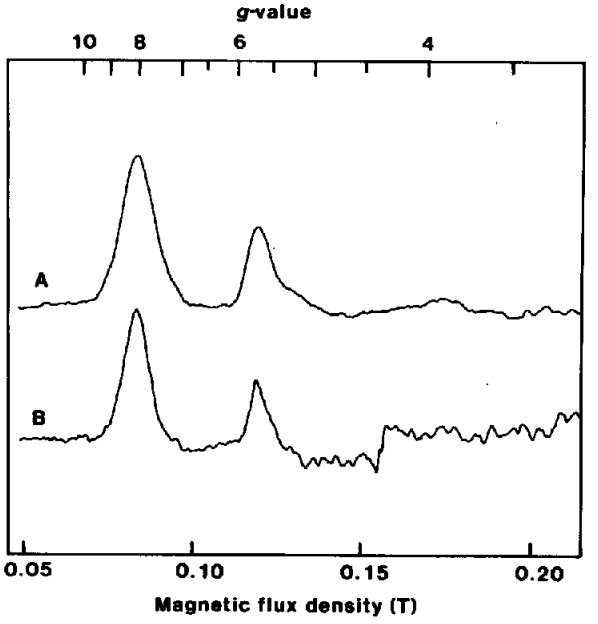

Fig.1. X-band EPR difference spectra (oxidized minus reduced) of the PS II acceptor-side iron. The oxidation was accomplished either by illumination in the presence of PPBQ (A) or by ferricyanide (B) as described in section 2.1. Conditions for EPR: microwave frequency, 9.46 GHz; power, 32 mW; modulation amplitude, 2.5 mT; temperature, 4.6 K.

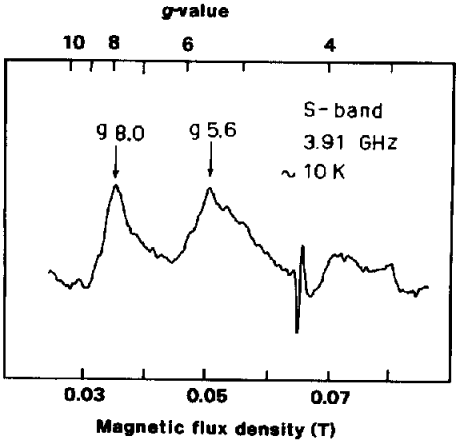

Fig.2. S-band EPR difference spectrum (ferricyanide-oxidized minus sample without ferricyanide) of the acceptor-side iron (10 mg Chl/ml). Conditions for EPR: microwave frequency, 3.91 GHz; power, 10 mW; modulation amplitude, 0.4 mT; temperature, about 10 K.

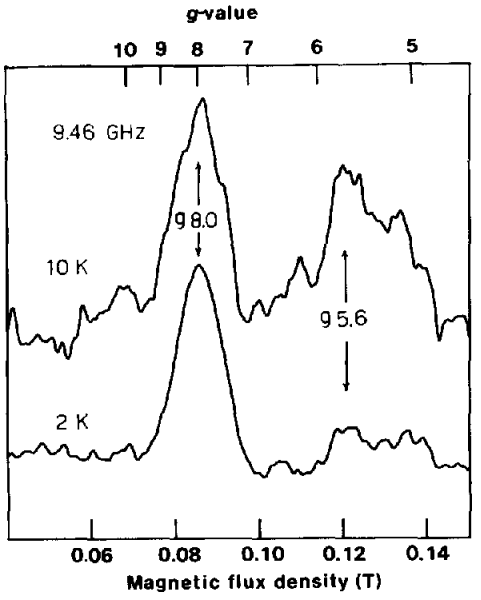

Fig.3. X-band EPR difference spectra of the acceptor-side iron (15 mg Chl/ml) recorded at two temperatures. The sample was treated as in fig.1A. EPR conditions as in fig.1.

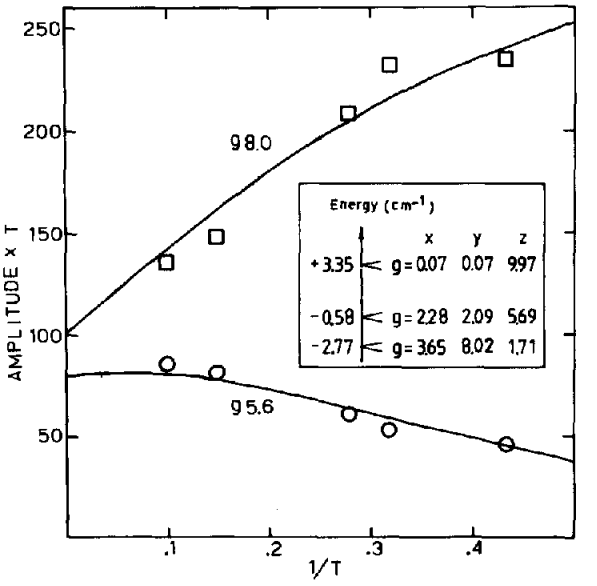

Fig.4. Temperature variation of the peaks at $g = 8.0$ (□) and $g = 5.6$ (○). The amplitude (arbitrary units) at non-saturating power was used as a measure of intensity. The sample was the same as in fig.3. The lines are theoretical curves assuming that the two peaks arise from the ground and middle doublets, respectively. The insert shows the energy diagram and zero-field g values for high-spin Fe^{3+} ($S = 5/2$) calculated with $D = 1.0\ cm^{-1}$ and $E/D = 0.10$.

pected to have absorption-type shapes in agreement with the experiments. The peaks connected to the other g values of the two doublets and to the upper-most doublet are either too weak or too broad to be detected, possibly with the exception of the $g_x = 3.6$ signal from the ground doublet (X-band at about 0.18 T, see fig.1).

An important question is if the EPR signals at $g = 8.0$ and 5.6 correspond to substantial amounts of Fe^{3+} or not. As the most important donors and acceptors can be quantified from their EPR spectra, one approach is to compare the number of electrons that have left the donor side and appeared at the acceptor side of PS II after photoreduction of the oxidized iron. In one experiment (cf. fig.1A) the iron was oxidized using a 200 K illumination (in order to transfer one electron to the acceptor side) and a subsequent thawing cycle in the presence of PPBQ. Then, a second illumination at 77 K was applied which results in photoreduction of both Fe^{3+} and Q_A [8,9]. The electrons are taken from cytochrome *b*-559 and/or a chlorophyll molecule in PS II [14,15], while the oxygen-evolving system is inhibited [16]. Normalizing to the reaction center concentration (obtained from EPR signal II_{slow}, run directly after the first thawing at non-saturating conditions), we found that 0.65 cytochrome *b*-559 and 0.58 chlorophyll had been oxidized whereas only 0.73 Q_A had been reduced. Thus, the remaining $0.65 + 0.58 - 0.73 = 0.50$ electrons should have rereduced the iron, which means that the recorded EPR spectrum corresponded to 0.50 Fe^{3+} per reaction center.

With the spin-Hamiltonian parameters known we can also evaluate the concentration of Fe^{3+} by direct integration of the EPR signals simply by measuring the areas under the isolated $g = 8.0$ and 5.6 peaks ([17], eqns 13 and 14). The peaks can be used independently and give slightly different values probably due to uncertainties in the base lines. Note, the ratio of the areas under the $g = 8.0$ and 5.6 peaks is predicted [17] to be 1.3 at high temperatures (>10 K). After corrections for the population distribution among the three doublets using the measured zero-field splittings the final value was 0.37 Fe^{3+} per reaction center in fair agreement with the electron counting. In any case,

a substantial fraction of the iron can be detected as Fe^{3+}.

The coordination geometry of the acceptor-side Fe in PS II probably resembles that in bacteria as the spectroscopic properties are very similar [3]. *Rps. viridis* shows a distorted octahedral coordination with four nitrogen atoms from histidines and two oxygen atoms from glutamic acid as ligands [18]. The iron coordination cannot, however, be identical because of the apparently much higher reduction potential for the bacterial center and the absence [19] in PS II of the corresponding glutamic acid. On the other hand, the four histidines are conserved [18] also in PS II.

Some non-heme dioxygenases produce EPR spectra quite similar to those of the oxidized PS II iron. The yellow oxidized form of lipoxygenase from soybean has a similar zero-field splitting but the symmetry is somewhat more axial [20]. A recent EXAFS study [21] points to 4 ± 1 histidines and 2 ± 1 carboxylate oxygen as iron ligands in this enzyme.

Complexes with protocatechuate 3,4-dioxygenase have been formed with E/D spanning the whole theoretical range 0–1/3 [22,23]. In its native form it has a rhombic spectrum but in some complexes with inhibitors [23] the EPR spectrum can be very similar to the PS II iron spectrum. The zero-field splittings are also very much the same but D is negative which produces an inverted diagram compared to fig.4 with the $g \approx 8$ peak arising from the uppermost doublet. Mössbauer [22], resonance Raman and EXAFS [24] studies strongly suggest two histidines and two tyrosines as ligands. The involvement of tyrosines is interesting because model studies have shown that coordinating phenolates lower the reduction potential [25].

Bicarbonate strongly influences both the $Fe^{2+}Q_A^-$ EPR signal [26] and the pH obtained after successive light flashes [27] and has been suggested as an iron ligand [28]. An interesting comparison can be made with the transferrins in which bicarbonate (or carbonate) is very likely directly coordinated [29] to the strongly bound ferric iron. Only one histidine but two tyrosines have been established as ligands [29] which fits well with the highly stabilized ferric valence state [30] in these proteins.

Obviously, more work is required before one understands in which way the iron coordination in PS II differs from the bacterial one. However, our EPR data are consistent with those where the glutamic acid in bacteria has been replaced by tyrosine or bicarbonate (or both).

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REFERENCES

[1] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1984) J. Mol. Biol. 180, 385–398.
[2] Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1987) Proc. Natl. Acad. Sci. USA 84, 5730–5734.
[3] Rutherford, A.W. (1987) in: Progress in Photosynthesis Research (Biggins, J. ed.) vol.1, pp.277–283, Martinus Nijhoff, Dordrecht.
[4] Youvan, D.C., Bylina, E.J., Alberti, M., Begush, H. and Hearst, J.E. (1984) Cell 37, 949–957.
[5] Rochaix, J.D., Dron, M., Rahire, M. and Malnoe, P. (1984) Plant Mol. Biol. 3, 363–370.
[6] Crofts, A.R. and Wraight, C. (1983) Biochim. Biophys. Acta 726, 149–185.
[7] Petrouleas, V. and Diner, B.A. (1986) Biochim. Biophys. Acta 849, 264–275.
[8] Zimmermann, J.-L. and Rutherford, A.W. (1986) Biochim. Biophys. Acta 851, 416–423.
[9] Petrouleas, V. and Diner, B.A. (1987) Biochim. Biophys. Acta 893, 126–137.
[10] Diner, B.A. and Petrouleas, V. (1987) Biochim. Biophys. Acta 893, 138–148.
[11] Petrouleas, V. and Diner, B.A. (1988) Biochim. Biophys. Acta, in press.
[12] Franzén, L.-G., Hansson, Ö. and Andréasson, L.-E. (1985) Biochim. Biophys. Acta 808, 171–179.
[13] Aasa, R., Andréasson, L.-E., Lagenfelt, G. and Vänngård, T. (1987) FEBS Lett. 221, 245–248.
[14] De Paula, J.C., Innes, J.B. and Brudvig, G.W. (1985) Biochemistry 24, 8114–8120.
[15] Visser, J.W.M. (1975) Thesis, University of Leiden, The Netherlands.
[16] Styring, S. and Rutherford, A.W. (1988) Biochim. Biophys. Acta 993, 378–387.
[17] Aasa, R. and Vänngård, T. (1975) J. Magn. Res. 19, 308–315.
[18] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Nature 318, 618–624.
[19] Trebst, A. and Draber, W. (1986) Photosynth. Res. 10, 381–392.
[20] Slappendel, S., Veldink, G.A., Vliegenthart, J.F.G., Aasa, R. and Malmström, B.G. (1981) Biochim. Biophys. Acta 667, 77–86.

[21] Navaratnam, S., Feiters, M.C., Al-Hakim, M., Allen, J.C., Veldink, G.A. and Vliegenthart, J.F.G. (1988) Biochim. Biophys. Acta 956, 70–76.
[22] Que, L., jr, Lipscomb, J.D., Zimmermann, R., Münck, E., Orme-Johnson, N.R. and Orme-Johnson, W.H. (1976) Biochim. Biophys. Acta 452, 320–334.
[23] Que, L., jr, Lipscomb, J.D., Münck, E. and Wood, J.M. (1977) Biochim. Biophys. Acta 485, 60–74.
[24] Felton, R.H., Barrow, W.L., May, S.W., Sowell, A.L. and Goel, S. (1982) J. Am. Chem. Soc. 104, 6132–6134.
[25] Pyrz, J.W., Roe, A.L., Stern, L.J. and Que, L., jr (1985) J. Am. Chem. Soc. 107, 614–620.
[26] Vermaas, W.F.J. and Rutherford, A.W. (1984) FEBS Lett. 175, 243–248.
[27] Van Rensen, J.J.S., Tonk, W.J.M. and De Bruijn, S.M. (1988) FEBS Lett. 226, 347–351.
[28] Michel, H. and Deisenhofer, J. (1988) Biochemistry 27, 1–7.
[29] Anderson, B.F., Heather, M.B., Dodson, E.J., Norris, G.E., Rumball, S.V., Waters, J.M. and Baker, E.N. (1987) Proc. Natl. Acad. Sci. USA 84, 1769–1773.
[30] Kretchmar, S.A., Reyes, Z.E. and Raymond, K.N. (1988) Biochim. Biophys. Acta 956, 85–94.