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Complete EPR Spectrum of the S_3-State of the Oxygen-Evolving Photosystem II

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The light-driven water oxidation that is catalyzed by Photosystem II (PSII) is not only responsible for the presence of O_2 on Earth but also a key reaction in photosynthesis, the process that has produced both the biomass and the fossil fuels. Despite refined three-dimensional X-ray structures at 3.5−3.0 Å resolution,[1,2] the mechanism by which water is oxidized and O_2 is produced remains unclear.[3−5] In the enzyme cycle a $\mathrm{Mn_4(oxo)}_n\mathrm{Ca}$-cluster goes through five sequential redox states denoted S_n, where n varies from 0 to 4, upon the absorption of four successive photons.[6] In the S_4 state two molecules of water are oxidized, the S_0-state is regenerated, and O_2 is released. The oxidation states of the Mn-cluster are not certain, but the majority view at this time favors $\mathrm{Mn^{III}}_3\mathrm{Mn^{IV}}$ for S_0, $\mathrm{Mn^{III}}_2\mathrm{Mn^{IV}}_2$ for S_1, and $\mathrm{Mn^{III}Mn^{IV}}_3$ for S_2. EPR has contributed to understanding the structure, valence, and function of the Mn complex.[7] This report describes for the first time the complete S_3 EPR signal. Simulations strongly suggest that it arises from a spin $S = 3$ state.

One of the first indications that the S-state cycle involved the oxidation of Mn was the observation of a so-called multiline EPR signal upon formation of the S_2-state.[8] This signal is centered at $g = 2$ arising from an $S = {}^1/_2$ ground state. Most recent simulations of this signal based on either cw X-band EPR or pulse Q-band ENDOR spectroscopy had the following features in common: (a) a 3 + 1 magnetic structure (which proved to be consistent with the 3D structure), (b) 3 $\mathrm{Mn^{IV}}$ and 1 $\mathrm{Mn^{III}}$, and (c) at least one di-μ-oxo $\mathrm{Mn^{III}Mn^{IV}}$ motif.[9−11] The magnetic couplings between each of the Mn ions and the location of the $\mathrm{Mn^{III}}$ differed in the models proposed.[9−11] Under certain conditions, the structural conformation of the Mn-cluster is modified so that the S_2-state exhibits EPR signals with g values from 4.1 to 10 corresponding to spin values $\geq {}^5/_2$.[7,12]

The S_0-state also exhibits an $S = {}^1/_2$ EPR multiline signal centered at $g = 2$.[13,14] From the Mn hyperfine values obtained by using pulse Q-band ENDOR spectroscopy[11] it has been proposed that the S_0 multiline signal arises from a cluster containing 3 $\mathrm{Mn^{III}}$ and 1 $\mathrm{Mn^{IV}}$.

Since both S_0 and S_2 have noninteger spin values and since the Mn-cluster is oxidized in the S_1 to S_2 transition, it is expected that the S_1-state exhibits an integer spin value. Indeed, by using parallel polarization EPR spectroscopy signals were detected in the S_1-state.[15−17] One of these consists of a multiline signal centered at $g = 12$. This signal has been proposed to be compatible with a $\mathrm{Mn^{III}}_2\mathrm{Mn^{IV}}_2$ motif.[18]

Little is known for the S_3-state and the S_4-state. The S_4-state has escaped detection for the following reasons (however, see refs 19 and 20): (i) the reduction of $\mathrm{Tyr_Z}^{\bullet}$ is the limiting step for water oxidation in the native enzyme and thus the S_4-state is kinetically indistinguishable from $S_3\mathrm{Tyr_Z}^{\bullet}$;[19] (ii) the rate constant of the $S_3\mathrm{Tyr_Z}^{\bullet}$ to S_0 transition is rapid ($t_{1/2} \approx 1$ ms);[3−5] and (iii) experimental methods for trapping potential intermediate states are lacking. The chemical nature of the S_3-state itself is under debate. The lack of a strong shift in the X-ray absorption edge in the S_2 to S_3 transition has led to a model being proposed in which a ligand centered oxidation (possibly a bridging O) occurs instead of a Mn oxidation[3] (see however ref 21). EPR signals in the S_3-state have been detected in plant PSII only at g values ≥ 4 by using both perpendicular (at $g \geq 6.7$) and parallel (at $g = 8-12$) modes.[22,23] These signals were proposed to originate from an $S = 1$ spin state,[22] but a preliminary cw Q-band EPR study suggested that a spin $S = 1$ could not explain the signals observed at 34 GHz.[24] Conditions are described here in which a large number of EPR features are detected at 9 GHz in the S_3-state.

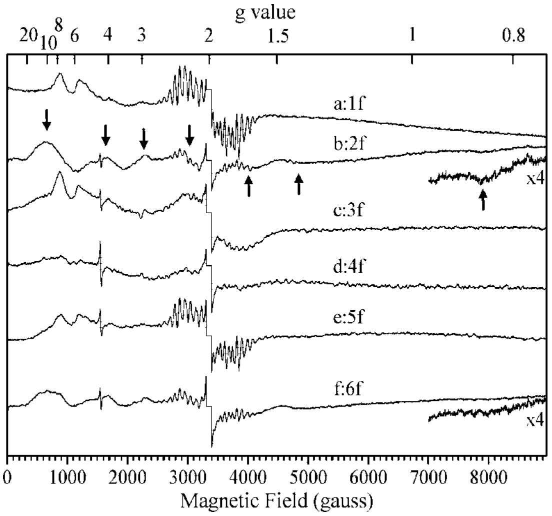

Figure 1. EPR spectra recorded after a series of laser flashes (1 Hz) in the presence of PPBQ dissolved in dimethylsulfoxide. Instrument settings: modulation amplitude, 25 G; microwave power, 20 mW; microwave frequency, 9.4 GHz; modulation frequency, 100 kHz, temperature, 8.5 K. The $\mathrm{Tyr_D}^{\bullet}$ spectral region at $g \approx 2$ was deleted. The arrows indicate the main features associated with the S_3-state (see Figure 2).

PSII core complexes from a thermophilic cyanobacterium *Thermosynechococcus elongatus* strain lacking the *psbA1* and *psbA2* genes[25] were purified in the absence of glycerol as described earlier.[26] EPR spectroscopy and sample preparation were done exactly as described earlier.[26]

Figure 1 shows the difference spectra “after-*minus*-before” flash illumination. After one flash and five flashes, the characteristic S_2 multiline signal is detected (see Supporting Information). From the flash dependence of the amplitude of the S_2 multiline signal the miss parameter was estimated to be ~6%. In addition to the S_2 multiline signal, signals at $g = 7.6$ and $g = 5.5$, which originate from the oxidized nonheme iron, are also detected.[27] A narrow signal at $g = 4.3$ from a contaminant $\mathrm{Fe^{III}}$ is also present in the

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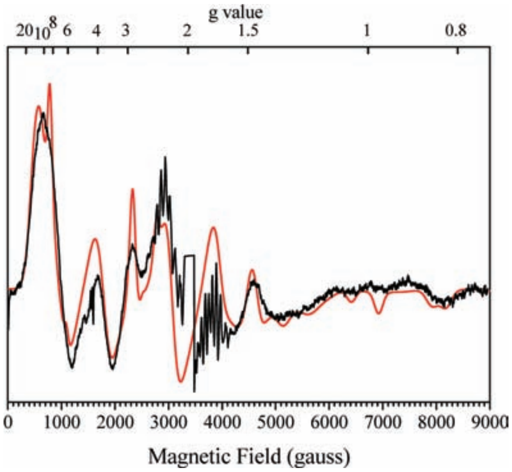

Figure 2. S_3-experimental spectrum averaged from four experiments after subtraction of a cubic baseline (black trace) and simulated spectrum (red trace) for $S = 3$ with $g_{\text{iso}} = 2$, $|D| = 0.175\ \text{cm}^{-1}$, and $E/D = 0.275$.

difference spectra.[29] After three flashes the characteristic S_0 multiline signal[13,14] is detected.

After two flashes, i.e., in the S_3-state, in addition to the already known signals at $g \approx 8$ and $g \approx 4$,[22,23] several other previously unreported features oscillate with a period of 4, i.e., with a maximum after the sixth flash. Although the signal at $g \approx 4$ is complex with two parts which behave differently (see Supporting Information) all the other features behave similarly as a function of the temperature. This indicates that all the signals detected belong to the same spin state.

The signal at $g \approx 8$ was previously fitted assuming a spin value of $S = 1$ with the ZFS parameters $D = \pm\ 0.435\ \text{cm}^{-1}$ and $E/D = -0.317$.[22] These parameters predict a signal at $g \approx 0.85$ but not the other signals detected between 2000 and 5000 gauss.[30] Therefore, higher spin values were tested to account for these additional spectral features. Simulations were performed by using XSophe[33] and EasySpin.[34] The Hamiltonian components retained for the simulations were the electronic Zeeman interaction and the zero-field splitting. The routine "esfit" from EasySpin was used to find the zero-field splitting parameters which best fitted the experimental spectrum for spin states $S = 1$, 2, or 3.

For $S = 1$, no set of ZFS parameters can account for all the observed features here. Moreover, preliminary Q-band EPR data obtained in plant PSII[24] seemed also to exclude this spin state value for S_3. An $S = 2$ spin state yields additional resonances in the field range studied here; however, the experimental S_3 spectrum is still not satisfactorily reproduced and some resonances are missing (see Supporting Information). For $S = 3$, a satisfying fit reproducing all the features observed experimentally was obtained (Figure 2). This indicates that the S_3 experimental EPR spectrum is associated with a pure septuplet spin system and not with a triplet state as reported previously.[22]

In the S_2 to S_3 transition an oxidation occurring outside the first coordination sphere of the Mn ions would likely yield an $S = {}^1/_2$ radical weakly magnetically coupled to the $S = {}^1/_2$ ground state of the $Mn_4(oxo)_nCa$ cluster in the S_2-state, resulting in a split signal which is not observed here. The fact that an $S = 3$ spin state is observed indicates that all the oxidized species are strongly magnetically coupled. A direct consequence is that the oxidation likely occurs at the level of the cluster, i.e., on either a Mn ion or a ligand in the first coordination sphere (a bridging O atom[3] or an amino acid ligand). The complete S_3 EPR spectrum shown here provides us with a new basis for future structural investigations on the S_3-state.

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Supporting Information Available: Sample preparation, spectral simulations, and temperature dependencies are detailed. This material is available free of charge via the Internet at http://pubs.acs.org.

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