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High-field (94-GHz) EPR spectroscopy on the S_2 multiline signal of photosystem II

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Abstract The multiline signal of the S_2 state in Photosystem II was measured both in frozen-solution and single-crystal preparations from the cyanobacterium *Thermosynechococcus elongatus*. The frozen-solution EPR spectrum shows a gaussian-like line shape without any resolution of Mn hyperfine couplings. This line shape can be understood on the basis of the single-crystal spectra, where a strong orientation dependence of partially resolved hyperfine structures appears. Simulation of the frozen-solution spectrum on the basis of Mn hyperfine couplings taken from published pulse-ENDOR data yields a fully rhombic *g*-matrix for the multiline signal with principal components 1.997, 1.970, and 1.965. The resulting isotropic *g*-value $g_{iso} = 1.977$ is surprisingly small compared to other manganese complexes containing manganese ions in the formal oxidation states three and four.

Keywords: Photosystem II; Oxygen evolving complex; Manganese cluster; EPR; Multiline signal

1. Introduction

Photosystem II (PSII) is the protein complex in green plants and cyanobacteria responsible for light-driven water oxidation and concomitant oxygen evolution (see e.g. [1]). X-ray crystallography [2,3] shows that the metal center, supposed to be the catalytic site where the water-splitting reaction takes place, is composed of four manganese ions and a calcium ion (Mn_4Ca-cluster). In close vicinity to the metal cluster a redox-active tyrosine is placed which couples the light-induced electron transfer chain of organic cofactors within PSII to the metal cluster.

The manganese–calcium cluster cycles during the enzymatic reaction through five states (S_0–S_4) [4] and accumulates the four redox equivalents necessary for water oxidation. The dark stable ground state is the S_1-state. The S_2 state can either be populated by a saturating laser flash at room temperature or trapped by continuous illumination at low temperatures and is paramagnetic with a half-integer spin state. Therefore, it is accessible to conventional EPR spectroscopy. Two EPR signals were detected from the S_2-state: the multiline signal (MLS) composed of 18–20 lines centered at $g \approx 2$ [5], and the $g \approx 4.1$ signal [6,7]. Here we focus on the MLS. This signal is the result of antiferromagnetic exchange coupling between the manganese ions which leads to a $S = 1/2$ ground state. In the past, a controversy regarding the number of manganese ions responsible for the S_2-MLS existed. While Åhrling and Pace [8] favor a model including only two Mn ions, more common are models invoking four Mn ions [9–13]. The model using only two Mn ions contradicts the published ^{55}Mn-ENDOR data [11,13], and based on the current X-ray crystallographic model of the Mn_4Ca cluster with all five ions within a very tight pocket [2,3], the model assuming four interacting Mn ions seems more realistic.

Since the discovery of the MLS [5] a number of different parameter sets for the hyperfine couplings (hfcs) of the manganese nuclei and the *g*-matrix of the $S = 1/2$ ground state have been derived from EPR spectroscopy at S-, X-, and Q-band frequencies [8–10,12]. Additional information on the manganese hfcs has been gained from X- and Q-band pulse ENDOR spectroscopy [11,13]. However, the complicated structure of the S_2-MLS arising from the exchange coupling between the manganese ions, the high nuclear spin ($I = 5/2$) of Mn with anisotropic hyperfine coupling, and *g*-anisotropy make a distinction of the quality of the different parameter sets extremely difficult. In the case of binuclear Mn model complexes a multifrequency approach including W-band (94-GHz) EPR has proven successful for resolving such ambiguities [14,15]. The first pioneering step towards high-field/high-frequency EPR of the Mn_4Ca cluster of PSII are experiments at W-band presented by Kawamori et al. [16] at the 2004 Photosynthesis Congress. There, using the cw EPR technique the S_2-MLS signal was observed in PSII single crystals but no MLS from frozen-solution samples has been reported so far.

Here we report pulse EPR measurements at 94 GHz on both frozen solution and single crystals of PSII from *Thermosynechococcus* (*T.*) *elongatus*. Based on the hyperfine data from Q-band ^{55}Mn-ENDOR [13] we deduce from the 94-GHz frozen-solution spectrum the principal components of the rhombic *g*-matrix for the MLS spectrum of the S_2-state of PSII.

2. Materials and methods

Preparation and crystallization of PSII core complexes (PSIIcc) from *T. elongatus* are described elsewhere [17]. PSIIcc in 100 mM PIPES buffer at pH 7.0, including 5 mM $CaCl_2$ and 0.03% β-DM, with a chlorophyll concentration of 13 mM were filled into quartz tubes (0.7 mm i.d., 0.87 mm o.d.) and frozen in liquid nitrogen. The frozen

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solution was then illuminated about 2 min at 200 K with a 180 W halogen lamp immediately before the EPR experiments. The sample tube was transferred after illumination into the cryostat pre-cooled to 80 K. Single crystals of PSIIcc soaked in 100 mM PIPES buffer at pH 7.0, including 5 mM $CaCl_2$, 10% w/w PEG 2000 and 25% w/w glycerol, were sucked with a drop of soaking buffer into quartz capillaries and rapidly frozen in liquid nitrogen in an arbitrary orientation. Pulse-EPR experiments were done at 5 K as the MLS could be detected only at temperatures below 10 K. Field-swept echo (FSE) spectra were acquired using a two pulse sequence $\pi/2$–τ–π–τ-echo with pulse lengths of 28 ns and 56 ns for the $\pi/2$- and π-pulses, respectively and $\tau = 300$ ns on an Elexsys E680 (Bruker, Rheinstetten, Germany) including an E680-PU power upgrade and a Bruker Teraflex EN600-1021H ENDOR resonator. The large spectral width of the signal exceeded the field range covered by the room temperature coils, therefore the measurements had to be performed by sweeping the main coils. For field calibration, the signal of the $Y_D^{\cdot}$ radical with well-known g-values was used as an internal standard. Derivative-type spectra were obtained from the FSE spectra by the pseudo-modulation function of the Bruker Xepr software.

To obtain the g-matrix principal components from the frozen-solution spectra, simulations were done using a self-written computer programme that calculates the resonance positions by second-order perturbation theory. This programme takes into account a spin Hamiltonian including the electron Zeeman and the hyperfine interaction terms. As shown by Haddy et al. [18], already at 9 GHz third-order corrections are smaller than 0.5% and thus the second-order treatment is well-sufficient for 94 GHz. In order to account for the large quadrupole couplings in the parameter set of Åhrling and Pace [8] the corresponding simulation was performed using EasySpin [19].

3. Results and discussion

FSE EPR spectra of frozen PSIIcc solutions are shown in Fig. 1. The spectrum after illumination (a), before illumination (b), and the light–dark difference spectrum (c) are presented. The main difference between spectra (a) and (b) and the only prominent signal in the difference spectrum (c) is a very broad line assigned to the light-induced MLS of the S_2-state. In the $g = 2.005$ region the strong contribution of the $Y_D^{\cdot}$ radical is visible in the spectrum after illumination and the dark spectrum. Due to the optimization of the acquisition parameters for the MLS and changes in the $Y_D^{\cdot}$ intensity upon sample illumination, this signal is also present in the difference spectrum. Furthermore six troughs (negative intensities) can be seen in the spectrum after illumination and the dark spectrum. These belong to Mn^{2+} traces within the sample. Such Mn^{2+} signals sharpen and show up with high intensity at high-field/high-frequency EPR (see e.g. [20]). The negative signal sign of the Mn^{2+} contribution relative to that of $Y_D^{\cdot}$ and the MLS is due to the different transition moment of the $S = 5/2$ state of Mn^{2+} compared to the two $S = 1/2$ states. The flip angle of the microwave pulses was optimized for the $S = 1/2$ systems and, consequently, not for Mn^{2+}. This effect can be used to separate the echo signals arising from different spin states [21] by appropriate choice of the integration window. However, since the Mn^{2+} signal contribution is almost absent in the difference spectrum, no such separation was performed here. Additionally, a much broader signal contribution is detected centered at about 3265 mT in the spectrum after illumination and the dark spectrum. Like the Mn^{2+} signal, this intensity vanishes in the light–dark spectrum and remains unassigned here. The main light-induced signal is centered at $g_{center} = 1.976$ with an approximate width (full-width at half-maximum) of 85 mT and a full-width of about 202 mT which is roughly 25 mT broader compared to X-band (data not shown). The signal shape can be well-described as gaussian and no resolution of ^{55}Mn hyperfine couplings is visible in contrast to spectra recorded at X-band frequencies where the S_2-MLS shows prominent hyperfine features (about 18 resolved lines in cw mode). Rather drastic changes in the appearance of EPR spectra at different microwave frequencies are common for manganese clusters [14,15] and the S_2-MLS shows already at Q-band frequencies [13] reduced hyperfine resolution compared to X-band spectra. Strongly orientation-dependent hyperfine structure of the S_2-MLS has been reported by Kawamori et al. [16] for cw EPR experiments at 94 GHz on PSII single crystals. At all orientations of the PSII single crystal with respect to the magnetic field [16] the intensity of the hyperfine lines seems smaller compared to the broad underlying signal than in experiments on frozen solution at X-band frequencies.

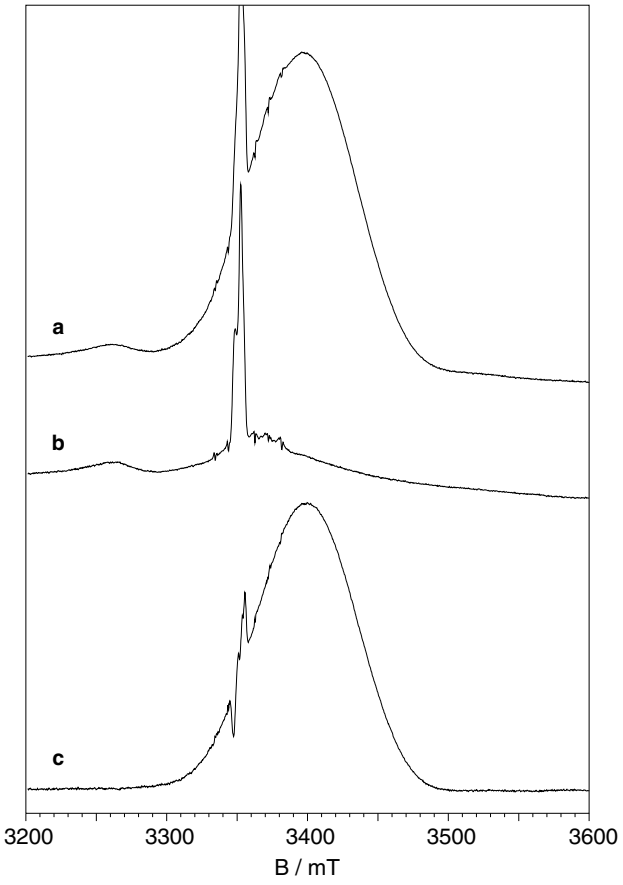

Fig. 1. 94-GHz FSE spectra of frozen solution PSIIcc samples from *T. elongatus*. (a) Spectrum after sample illumination, (b) prior to illumination, and (c) light–dark difference spectrum. $T = 5$ K; 4096 accumulations per field point; 204 μs shot repetition time.

To correlate the broad featureless light-induced signal in our 94-GHz pulse EPR experiments on frozen solution to the earlier observation of the S_2-MLS at 94 GHz by cw EPR on PSII single crystals [16] we have also recorded corresponding spectra for PSIIcc single crystals. Two single-crystal FSE spectra are shown in Fig. 2. These are spectra recorded after illumination and no light–dark correction has been performed. Both spectra have a slightly smaller width than the frozen-solution spectrum and are centered at different effective g-values as expected for an anisotropic g-matrix but correspond in general to the broad signal observed for the frozen-solution sample. The