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Intermediates of the S_3 to S_2 transition in the Oxygen Evolving Complex of Photosystem II

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Introduction

The oxygen evolving complex (OEC) of Photosystem II (PSII) catalyses the light-driven oxidation of water to dioxygen. The OEC comprises a tetranuclear Mn cluster, its cofactors Ca^{2+} and Cl^-, and a redox active tyrosine, Y_z (BBA vol. 1503). During catalysis it cycles through 5 redox states denoted S_i (i=0-4). At the unstable S_4 state the Mn cluster has accummulated 4 oxidising equivalents, and thus 2 water molecules are oxidised, dioxygen is released and the cycle starts again from the S_0 state.

EPR spectroscopy has been crucial in studying the oxygen evolving complex (Britt, 1996). Recently, signals were detected from the S_3 state using perpendicular and parallel mode EPR (Matsukawa et al., 1999; Ioannidis and Petrouleas, 2000). Interestingly, these signals were found to be sensitive to NIR illumination (Ioannidis and Petrouleas, 2000) reminding the similar sensitivity of the S_2 state (Boussac et al., 1998). NIR light excitation of the S_3 state produces, in addition to other signals that appear to be associated with excited state configurations of S_3, a derivative-shaped EPR signal at g ~ 5 (Ioannidis and Petrouleas, 2000). This signal bears unexpected similarities to a signal observed earlier by Nugent et al. (1997), in samples that had undergone multiple turnovers above S_1 and subsequently stored at 77 K for a week or longer. In the present study we compare the NIR effect on the S_3 with the decay of the same state at 77 K.

Materials and methods

PS II-enriched thylakoid membranes were isolated from market spinach by standard methods. Samples for EPR measurements were suspended in 0.4 M sucrose, 15 mM NaCl and 40 mM MES, at pH 6.5 and at a concentration of 6-8 mg Chl/ml. The S_3 state was formed as previously described (Ioannidis and Petrouleas, 2000). For illumination purposes the light from a 340 W projector lamp was used. Excitation of the S_3 state by near infrared light (NIR) was done at 4.2 K, using the same source but the light was filtered through a RG715 SCHOTT filter. White light illumination of S_3 samples which were incubated at 77 K for long time periods was done in the same manner but using a BG 39 SCHOTT filter. EPR measurements were done with a Bruker ER-200D-SRC spectrometer interfaced to a personal computer and equipped with an Oxford ESR 900 cryostat, an Anritsu MF76A frequency counter and a Bruker 035M NMR Gaussmeter.

Results and Discussion

In our previous study of the S_3 state we discovered that NIR illumination at 50 K induced significant changes (Ioannidis and Petrouleas, 2000). In the course of the present studies we found that NIR illumination at 4.2 K is also efficient in producing the same changes and in addition it allows for a better resolution. This effect is examined in fig. 1 using both perpendicular- and parallel-mode EPR . The S_3 state is characterized by a perpendicular-mode broad signal at g=10 and a parallel-mode signal at g=17 (spectra a). Upon NIR illumination at 4.2 K these signals diminish (spectra b) and new signals at g=5, 2.9 appear, accompanied by

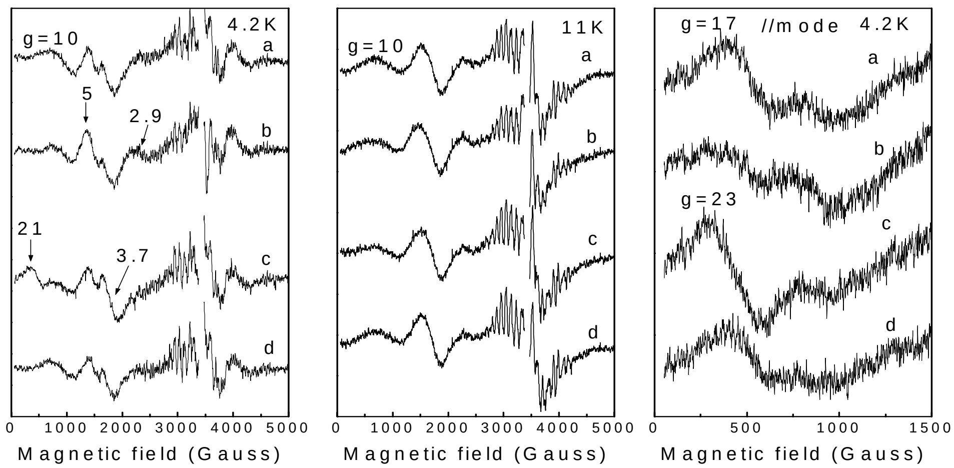

Fig. 1. NIR excitation of the S_3 state. From all spectra the dark S_1 spectrum was subtracted. See text for details. EPR conditons: microwave freq. 9.59 GHz (⊥ mode), 9.3 GHz (// mode), microwave power 35 mW (⊥ mode), 8.3 mW (// mode), mod. amplitude 10 Gpp.

a broad radical at g=2 (perpendicular-mode). Incubation at –80C for 2 min causes the diminution of the g=5, 2.9 species, the disappearance of the g=2 radical and the growth of a new species, characterized by signals at g=21, 3.7 (perpendicular-mode, spectra c) and g=23 (parallel-mode, spectrum c). This species is attributed to an excited S_3* state, which is induced by NIR illumination of the S_3 state at liq. He temperatures and can be trapped at –80C. Further incubation at –50C for 2 min results in the relaxation to the initial S_3 state, but with diminished intensity (spectra d), probably due to charge recombination with Q_A^-. This occurred during the step (b) to (c) judging from the observed decrease of the Q_A^- signal in the spectra at 4.2 K. The details of these experiments are examined in a forthcoming study. It is argued there that the g = 21/3.7/23 and g = 5/2.9 represent different relaxation branches of the NIR-excited S_3 state. In the following we will concentrate on the g 5/2.9 signals.

The g=5 signal reported above shows unexpected similarities to a signal that was induced earlier in samples that had undergone multiple turnovers above S_1 and subsequently stored at 77 K for a week or longer (Nugent et al., 1997). The signal was assigned to a new form of S_2 trapped during the decay of S_3 at cryogenic temperatures. We have confirmed the observations of Nugent et al. (1997) and have found a correlation between the decay of S_3 and the evolution of the g=5 signal at 77 K, with the aid of the recently detected EPR signature of S_3 (Matsukawa et al., 1999). The details of the experiments will be published elsewhere, but a summary of the results is shown in fig. 2. Spectrum (a) represents the S_3 state. After several weeks of incubation at 77 K, the S_3 (g=10) and S_2 (from centers which had not advanced to the S_3 state) signals have diminished due to recombination with Q_A^-, and a large g=5 signal has grown (b). Visible light illumination at 4.2 K induces a broad radical g=2 signal, very similar to the one shown above, diminution of the g=5 signal accompanied by the formation of a weak g = 2.9 signal (discernible only in difference spectra) and an increase in the Q_A^- signal (c) (Nugent et al., 1997). Incubation of this state at 4.2 K for 1 hr results in the decay

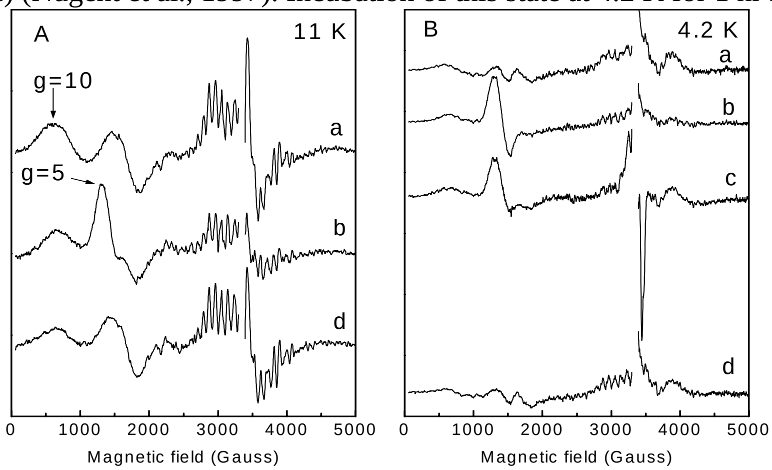

Fig. 2. Prolonged incubation of the S_3 state at 77 K. Spectra minus the S_1 dark. See text for details. EPR conditions: microwave frequency 9.4 GHz, microwave power 35 mW, mod. amplitude 25 G.

of the radical and partial restoration of the g=5 signal, which becomes maximal again at 77 K (not shown). Incubation at –50C for 2 minutes causes the loss of the g=5 signal and an increase in the S_2 signals, i.e. multiline and g=4.1 derivative (d).

Fig. 3 (A – C) focuses on the g = 5, 2.9 region of spectra similar to the ones shown above. Spectrum A was obtained following NIR excitation and storage at 4.2 K until the g~2 radical decayed, and B after subsequent storage for 5 min at 77 K. The S_3 spectrum prior to NIR excitation has been subtracted from the spectra. Since the S_3 signal at g=10 is sensitive to NIR excitation the difference creates an artifact in the baseline at low fields. Spectrum C is from

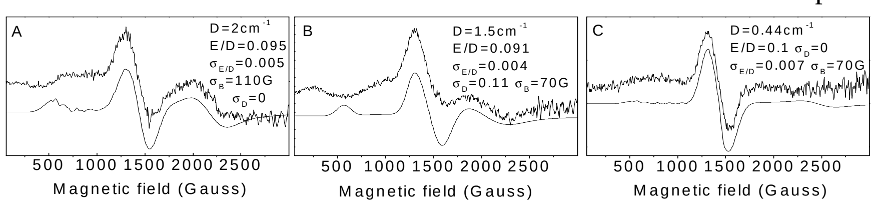

Fig. 3. Theoretical simulations of the g=5 signal induced by NIR excitation (A), 5 min incubation of the NIR excited state at 77 K (B), and after several weeks storage of the untreated S_3 state at 77 K (C). The negative intensity at about 500 G in spectra A and B is an artifact due to subtraction.

an experiment similar to Fig. 2b after subtraction of the spectrum corresponding to Fig. 2d. Inspection of the spectra shows that the g=5 feature has a derivative shape with an inflection point which varies between 4.65 and 4.75 among the spectra and appears to be nearly isotropic. Derivative features with these g values can only result from a S = 7/2 manifold (see also Sanakis et al., 2001). The g=5 resonance results from the "±3/2" doublet while a weaker derivative feature at variable position (around g=3) and intensity is predicted from the "±1/2" doublet. Continuous lines in Fig. 3 represent theoretical simulations with the included values of parameters. It is interesting to note the progressive change in the parameters. Presumably the S = 7/2 state forms concomitantly to the NIR-induced formation of the radical, which is assumed to be centered on Y_Z. The tyrosyl radical decays at liq. He temperatures presumably by recombination with Q_A^- and leaves the cluster in a frozen S = 7/2 configuration which at 77 K relaxes to a more isotropic configuration, approximately the same one that is obtained by the prolonged storage of the non-excited S_3 state at 77 K. The S = 7/2 configuration is very stable (several months storage at 77 K) but it decays quickly to the S_2 (multiline and g = 4.1) configuration at –50°C. The S = 7/2 will be accordingly called S_2' and it may represent a deprotonated S_2 state, as originally suggested by Nugent et al. (1997). It is interesting that white light excitation of S_2' produces again the Y_Z radical, a slight distortion of the symmetry and appearance of the g=2.9 contribution.

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