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The *g* = 2 doublet signal does not correlate with the multiline signal in Ca^{2+}-depleted PSII

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Introduction

Calcium is an indispensable metal cofactor for the normal function of OEC. Oxygen evolution is inhibited by selective depletion of Ca^{2+} and is restored by reconstitution of Ca^{2+}. The formation of the S_2 state in Ca^{2+}-depleted PS II has been depicted by the generation of a multiline EPR signal, the spectral features of which depend on the procedures conducted for Ca^{2+}-depletion. Upon illuminating the Ca^{2+}-depleted PS II in the S_2 state, another EPR signal with a splitting linewidth of 160 Gauss is generated around g = 2 by CW EPR. The split signal arises from an alternate redox reaction in OEC due to an interruption in the normal oxidation process beyond the S_2 state in the absence of Ca^{2+}. The signal has been thought to arise from organic radical with S = 1/2 interacting with the S_2-state Mn-cluster, where an oxidized histidine or Y_Z tyrosine is attributed to the putative radical. Support for the involvement of the Y_Z radical in the split signal has been given by pulsed ENDOR and ESEEM studies, in which an interaction between the Y_Z radical and the S_2-state Mn-cluster with S = 1/2 accounts for the ESE field-swept spectrum in acetate-treated PS II. The involvement of the Mn-cluster in the split signal has been furthermore supported by the results of high frequency pulsed ENDOR study that shows the presence of manganese ion signal in the split signal in acetate-treated PS II (Peloquin, J. M. et al. 1998).

In these studies, the split signal has been analyzed by assuming that the signal arises from a single magnetic species. However, it has been revealed that the split signal consists of two different signals that overlap at the g =2 region: a symmetric doublet signal and a singlet-like signal (Astashkin, A.V. et al. 1997). The former signal has a splitting of approximately 150 G at g =2, and is induced by illuminating the Ca^{2+}-deplted PS II in the S_2 state for a short period at 273 K, while the latter signal is induced by a longer illumination period. Distinctly, the presence of DCMU did not inhibit the formation of the doublet signal but inhibited that of the singlet-like signal, indicating that the latter signal requires more than two turnovers of PS II beyond the S_2

state. Pulsed ENDOR-induced EPR study indicates that the Y_Z radical is associated with the doublet signal, but not with the singlet-like signal.

In this paper, we report the decay and recovery kinetics of the doublet signal in association with those of the multiline signal. The obtained results support that the doublet signal does not correlate with the multiline.

Materials and methods

For Ca^{2+} depletion, the PS II membranes were suspended in a medium containing 400 mM sucrose, 20 mM NaCl, 10 mM citric acid/NaOH (pH 3.0) at 273 K for 5 min, and then, 10% vol. of a solution containing 400 mM sucrose, 20 mM NaCl and 500 mM MOPS /NaOH (pH 7.5) was added to adjust the final pH at about 6.5. The treated membranes were washed and resuspended in a final buffer solution containing 400 mM sucrose, 20 mM NaCl and 20 mM Mes/NaOH (pH 5.5-6.5). For the formation of the dark-stable S_2-state, the Ca^{2+}-depleted membranes (0.5 mg Chl/ml) were illuminated for 1 min followed by dark-adaptation for 30 min, and then DCMU (0.05 mM) was added to the suspension to ensure a single turnover event beyond the S_2 state when indicated.

CW EPR measurements were performed using a Bruker ESP-580E X-band spectrometer, equipped with a ER4116 DM X-band (TE_{102}) dual mode resonator. For ESE measurements, a cylindrical dielectric cavity (ER4117DHQ-H, Bruker) was used.

Results and Discussion

Figure 1 shows the CW (a, b) and field-swept ESE spectra (c, d) of the Ca^{2+}-depleted PS II membranes. Illuminating the EPR samples in the S_2-state for 1 min at 273 K induced a pronounced doublet signal at $g\sim2$ with a concomitant decrease of the multiline signal (a, c). The doublet signal decayed completely during dark incubation for 70 min at 273 K, concurrent with a recovery of the multiline (b, d).

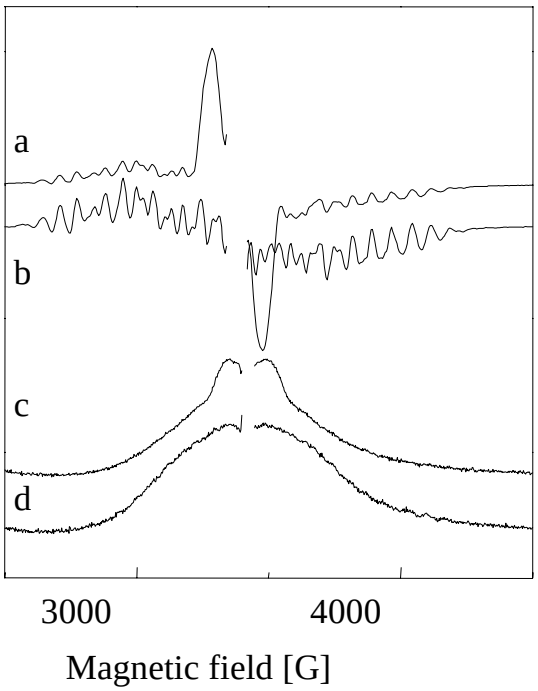

Fig. 1: CW and primary ESE spectrum of Ca^{2+}-depleted PS II. S_2-state samples were illuminated at 273 K for 60 s in the presence of DCMU (a, c), and then dark adapted for 70 min at 273 K (b, d).

Figure 2 shows the decay course of the doublet signal and the recovery course of the multiline in the present of DCMU (panel A) and in the absent of DCMU (panel B). As clearly shown in panel A, there is no correlation between the recovery of the multiline signal and the decay of the doublet signal; the doublet signal decays rapidly ($t_{1/2}$~ 2 min)

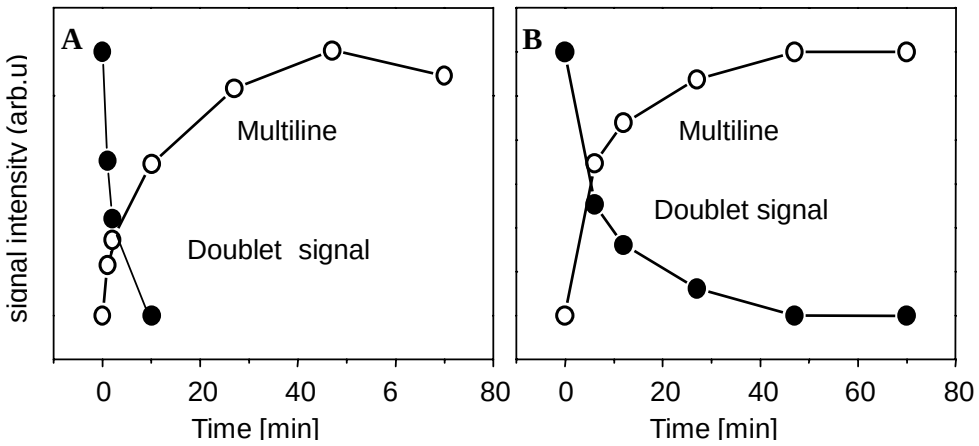

Fig. 2: Effects of dark incubation on the doublet signal (●) and multiline signal (○). S_2-state Ca^{2+}-depleted PS II was illuminated in the presence (panel A) and absence of DCMU (panel B), then dark-incubated at 273 K.

by recombination with Q_A^-, while the recovery of the multiline is the much slower process. In the absence of DCMU (panel B), the decay of the doublet signal and the recovery of multiline signal apparently show similar course of $t_{1/2}$~ 10 min, which

explain the early report by Sivaraja, M. et al. (1989).

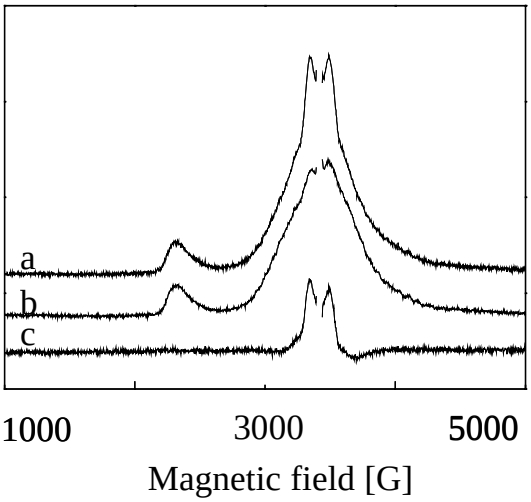

Fig. 3: Primary ESE field sweeps of Ca^{2+}-depleted PS II. S_2-state samples were illuminated at 273 K for 60 s in the presence of DCMU (a); and dark-stored at 77 K for 6 weeks (b). Spectrum c was obtained by subtracting spectrum b from spectrum a.

Figure 3 shows the effect of a long storage at 77 K on the field-swept ESE spectra of the doublet signal. The doublet signal, induced by illuminating the S_2-state Ca^{2+}-delpeted PS II at 273 K in the presence of DCMU (spectrum a), decayed significantly after dark storage at 77 K for 6 weeks (spectrum b). As clearly shown in the difference spectra c (before minus after 77 K storage), the decay of the doublet signal is not accompanied with the recovery of the multiline signal. The apparent non-correlated behaviors found between the two signals suggest that these two signals arise from the different origin, and it is compatible with the view that the doublet signal arises from the interaction between Y_Z radical and unknown amino acid (Astashkin et al. 1997). In fact, the difference spectrum c shows that the decayed signal is typically Pake's doublet of $S = 1/2$ typical of the magnetic dipole interaction of the two radicals.

Recombination between Q_A^- and doublet species leads to a thermoluminescence band at 7° at pH 5.5 and at 17° at pH 7.0. This may be the reflection of the pH dependent properties change of Y_Z radical (Mino et al. 2000), suggesting that Y_Z radical is a counterpart for recombination with Q_A^-.

Reference

Astashkin, A.V., Mino, H., Kawamori, A. and Ono, T. (1997) *Chem. Phys. Lett.*, **272**, 506-516.

Mino, H., Kawamori, A.and Ono, T. *Biochimica et Biophysica Acta* 1457 (2000) 157-165

Sivaraja, M., Tso, J. and Dismules, G. C. (1989) *Biochemistry*, **28**, 9459-9464.

Peloquin, J. M., Campbell, K. A. and Britt, R. D. (1998) *J. Am. Chem. Soc.*, **120**, 6840-6841.