

EPR properties of a $g=2$ broad signal trapped in an S_1 state in Ca^{2+} -depleted Photosystem II

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Abstract

We investigated a new EPR signal that gives a broad line shape around $g=2$ in Ca^{2+} -depleted Photosystem (PS) II. The signal was trapped by illumination at 243 K in parallel with the formation of Y_Z^{\cdot} . The ratio of the intensities between the $g=2$ broad signal and the Y_Z^{\cdot} signal was 1:3, assuming a Gaussian line shape for the former. The $g=2$ broad signal and the Y_Z^{\cdot} signal decayed together in parallel with the appearance of the S_2 state multiline at 243 K. The $g=2$ broad signal was assigned to be an intermediate S_1X^{\cdot} state in the transition from the S_1 to the S_2 state, where X^{\cdot} represents an amino acid radical nearby manganese cluster, such as D1-His337. The signal is in thermal equilibrium with Y_Z^{\cdot} . Possible reactions in the S state transitions in Ca^{2+} -depleted PS II were discussed.

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1. Introduction

Photosynthetic oxygen evolution is carried out by a machinery termed oxygen-evolving complex (OEC). OEC is composed of four manganese ions, one calcium ion, chloride, and Y_Z tyrosine and is located at the luminal surface of the Photosystem (PS) II D1 reaction center protein. Recently, PS II structures with 3.5–3.7 Å resolutions have been reported [1–3]. The four Mn ions form a tetranuclear cluster that accumulates oxidized equivalents generated in the PS II reaction center by the successive absorption of four photons to catalyze water oxidation. Kinetic analyses have revealed that a molecular oxygen is produced by a series of reactions with five distinct intermediate states labeled S_i ($i=0-4$), in which S_1 is thermally stable in the dark. As

photons are absorbed by the reaction center, the S state advances stepwise to reach the S_4 state, which is the highest oxidation state. S_4 decays spontaneously to S_0 by releasing molecular oxygen [as reviewed in Refs. [4–7]].

EPR is a powerful tool that provides structural and functional information about the OEC. After the first discovery of the S_2 state multiline signal [8], many EPR signals from the different states of the OEC have been reported. The EPR signal of the S_0 state, which shows a hyperfine structure that is similar to the multiline signal in the S_2 state but with a broader overall width, was assigned to the antiferro-magnetically coupled $S=1/2$ system in the ground state [9,10]. S_1 and S_3 state EPR signals with broad linewidths were also identified in spinach PS II and assigned to a first excited state $S=1$ of an integer spin system [11–13]. On the other hand, in PS II preparations of *Synechocystis* sp. and spinach, which are depleted of 23 and 17 kDa extrinsic polypeptides, a different S_1 state signal with a hyperfine structure was observed [14,15]. Nugent et al. have reported an EPR signal at $g=2$ that is produced by illumination below 20 K of the S_1 state in untreated PS II and ascribed it to S_1X^{\cdot} [16]. The S_1X^{\cdot} signal decays after dark annealing at 77 K with the appearance of a multiline signal. Illumination at 5

Abbreviations: CW, continuous-wave; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; EDTA, ethylene diamine tetraacetic acid; EPR, electron paramagnetic resonance; ENDOR, electron nuclear double resonance; MES, 2-morpholinoethanesulfonic acid; MOPS, 2-(*N*-morpholino)ethanesulfonic acid; OEC, oxygen-evolving complex; PS, photosystem; Y_Z , tyrosine-Z, the D1-Tyr161; Y_D , tyrosine-D, the D2-Tyr161

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K, on the other hand, induced two different types of $g=2$ EPR signals, depending on the S state [17]. A model for the interaction of the S_1 state and the $Y_2\dot{Z}$ radical has been proposed on the basis of the analogy with a model, in which the “split signal” arises from the interaction between the S_2 state and the $Y_2\dot{Z}$ radical [18].

Calcium is indispensable for the normal function of OEC. The oxygen evolution is inhibited by the selective depletion of Ca^{2+} and is restored by the reconstitution of Ca^{2+} [19]. Ca^{2+} has been assumed to play a structural role, such as the stabilization and optimization of the Mn-cluster, and/or to participate directly in the water-oxidation reaction. The depletion of Ca^{2+} or its replacement by other metal cations modifies the properties of the Mn-cluster in the S_2 state, depending on the metal ions in the process of Ca^{2+} depletion [20–23]. The illumination of the Ca^{2+} -depleted PS II in the S_2 state generates another EPR signal with a splitting linewidth of 160 gauss around $g=2$ with complete or partial disappearance of the S_2 state multiline signal [24]. This split signal has been denoted as the “ S_3 -state signal.” The S_3 -state signal was ascribed to be formed by an auxiliary reaction in PS II, in which the $Y_2\dot{Z}$ radical was stabilized by the interruption of the normal oxidation process to the S_3 state in the absence of Ca^{2+} . There have been two alternative interpretations for the origin of the split signal. The first is that the $g=2.0$ split signal arises from an organic radical with $S=1/2$ that interacts with an oxidized Mn-cluster with $S=1/2$ (S_2 state). In this case, an oxidized histidine [25,26] or $Y_2\dot{Z}$ [27] has been attributed to the putative radical. The signal has been interpreted to arise from the interaction between the $Y_2\dot{Z}$ radical and the oxidized Mn-cluster ($S=1$), based on a simulation of the ESE-field swept spectrum in Ca^{2+} -depleted PS II by assuming a 4.5 Å inter-distance between these species [28]. It is alternatively assumed that the signal arises from the interaction between the $Y_2\dot{Z}$ radical and the S_2 -state Mn-cluster ($S=1/2$). This is based on the simulation of the ESE field-swept spectrum measured in acetate-treated PS II by assuming a distance of 3.5 Å between $Y_2\dot{Z}$ and $(Mn)_4$ [29]. The involvement of the $Y_2\dot{Z}$ radical in the split signal has also been suggested by the pulsed ENDOR and ESEEM results obtained in acetate-treated PS II [28,30]. It is notable that the split signal has been assumed to arise from a single magnetic species in these studies.

The other interpretation of the split signal has been based on the finding that the split signal consists of two overlapping different signals: a symmetric doublet signal and an asymmetric singlet-like signal [31]. The doublet signal has a splitting of approximately 150 G at $g=2$ and is induced by the illumination of the Ca^{2+} -depleted PS II in the S_2 state in the presence of DCMU or by a short period of illumination at 273 K in the absence of DCMU. The singlet-like signal is induced by illumination for a longer period and suppressed in the presence of DCMU. The formation of this signal, therefore, requires more than two turnovers of the PS II light reaction, which advances OEC beyond the S_2 state.

The doublet signal has been simulated by assuming $D_0=130$ G and $J=(-) 40$ G for a typical interaction, called Pake’s doublet, between paired organic radicals [31]. The anisotropy of the doublet signal that is expected for the paired organic radicals was also confirmed by ESE measurements in oriented PS II membranes [32]. The results of a pulsed ENDOR-induced EPR study indicate that the $Y_2\dot{Z}$ radical is associated with the doublet signal but not with the singlet-like signal [31]. These results suggest that the doublet signal arises from a dipole interaction between the $Y_2\dot{Z}$ radical and another organic radical [31], while the origin of the singlet-like signal is not clear yet. The $g=11$ – 15 signal with an $S=2$ spin state was also observed in parallel with a singlet-like signal [33]. The intensity of the doublet signal is independent from that of the multiline signal; thus, the doublet signal cannot be directly related to the manganese cluster [34]. Based on these observations, the relative locations of the two radicals responsible for the doublet signal were estimated [32].

In the present study, we report a new EPR signal at a $g=2$ region detected in the illuminated S_1 state in Ca^{2+} -depleted PS II. Based on the structure of the PS II reaction center [3], we discuss the origin of the so-called “split signal.”

2. Materials and methods

Oxygen-evolving PS II membranes were prepared from spinach as described previously [35] with some modifications [36]. After being thawed, the membranes were preilluminated and relaxed by incubation for 12 h in the dark. The membranes were washed twice in a medium containing 400 mM sucrose, 20 mM NaCl, and 0.1 mM MES/NaOH (pH 6.5) and were resuspended in the same medium. For Ca^{2+} depletion, the membranes were suspended in a medium containing 400 mM sucrose, 20 mM NaCl, and 10 mM citric acid/NaOH (pH 3.0) for 5 min at 273 K; then, 10 vol.% of a medium containing 400 mM sucrose, 20 mM NaCl, and 500 mM MOPS/NaOH (pH 7.5) was added to adjust the final pH to approximately 6.5, as described previously [37]. The membranes were washed twice in a medium containing 400 mM sucrose, 20 mM NaCl, 20 mM Mes/NaOH (pH 6.5), and 0.5 mM EDTA. Unless otherwise stated, all procedures were carried out in the dark or under a dim green light to maintain the OEC in the S_1 state. DCMU (dissolved at 10 mM in dimethyl sulfoxide) was added to give a final concentration of 0.05 mM in the dark to the S_1 state PS II.

For the EPR measurement, the membranes were precipitated by centrifugation at $35,000\times g$ for 20 min and transferred to suprasil quartz EPR tubes with a 4-mm diameter. The tubes were purged with Ar gas, sealed, and then stored in liquid N_2 until use. The PS II sample in an ESR tube was illuminated for 10 s with a 500 W halogen-tungsten lamp in an ethanol bath at 243 K and then rapidly frozen in liquid N_2 just after the illumination.

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