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Can we trap the $S_3Y_Z^{\cdot}$ metalloradical intermediate during the S-state transitions of Photosystem II? An EPR investigation

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ABSTRACT

We report the trapping of two metalloradical intermediates corresponding to the transitions S_2 to S_3 and S_3 to S_0 of the oxygen evolving complex (OEC) of Photosystem II (PSII), in preparations containing methanol, at temperatures near that of half inhibition of the respective S-state transitions. The first intermediate, with an EPR width of 160 G, is assigned to $S_2Y_Z^{\cdot}$, based on its similarity to the one previously characterized after trapping at 10 K. The second with a splitting of ~80 G is tentatively assigned to $S_3Y_Z^{\cdot}$. The $S_3Y_Z^{\cdot}$ EPR signal is weaker than the $S_2Y_Z^{\cdot}$ one, and both are stable at cryogenic temperatures.

1. Introduction

Photosystem II (PSII) couples the light-induced charge separation to the splitting of water. The catalytic site of water oxidation, called Oxygen Evolving Complex (OEC) undergoes periodically four one-electron oxidation steps, S_0–S_1,..., S_3–$(S_4)S_0$. The oxidizing equivalents are stored at/or in the immediate vicinity of a Mn_4Ca cluster, the site of water oxidation. Oxygen evolves during the S_3 to $(S_4)S_0$ transition, the S_4 being a transient state [1,2] and references therein. The transitions S_0–S_1, S_2–S_3 and S_3–S_0 have half-inhibition temperatures of ~230 K [3,4], while the S_1 to S_2 transition proceeds at temperatures as low as 135–140 K [3,4] or even lower [5 and our unpublished observations]. Tyrosine Z (TyrZ) of Photosystem II mediates electron transfer between the primary electron donor in PSII (P_{680}), a specialized chlorophyll (chl) moiety, and the Mn_4CaO_5 cluster during the S-state transitions of the OEC in a proton-coupled reaction.

Trapping of the Mn-TyrZ· metalloradical intermediates has been at the focus of considerable interest in recent years. Various intermediates have been identified and characterized by EPR spectroscopy, including the $S_0Y_Z^{\cdot}$, $S_1Y_Z^{\cdot}$, $S_2Y_Z^{\cdot}$ and variants of them obtained after treatments that alter the electronic configuration of the OEC but preserve the oxygen evolution (addition of a few percent (v/v) methanol, replacement of Ca with Sr) [5–11]. These signals are produced by direct visible-light illumination at liquid helium temperatures [5–11] with the exception of the $S_2Y_Z^{\cdot}$ in the untreated sample, which is trapped by visible-light illumination at 77–190 K [9]. The notable missing metalloradical intermediate is that of the S_3 to S_0 transition. The notion of removal of protons and electrons from the Mn cluster, in an alternate fashion at each S-state transition, formed the basis of an extended S-state cycle proposed in [12]. Based on experimental evidence obtained at ambient temperatures, and with respect to the S_2 to S_3 and S_3 to S_0 state transitions, formation of $Y_Z^{\cdot}$ results in proton expulsion from the cluster (or close to it), with a τ of about 30 μs in the former transition [13] and about 200 μs in the latter transition [14]. This is followed by the electron transfer step from the cluster to the $Y_Z^{\cdot}$ radical, which concludes both transitions. The reaction rates, activation energies and kinetic isotope effects (KIEs) have been obtained for the proton and electron transfer steps of both transitions ([13], see [15] for the activation energy of S_3 to S_0 state transition).

The time-resolved room temperature EPR spectrum of $S_3Tyr_Z^{\cdot}$ was recently reported in PSII preparations from *Thermosynechococcus elongatus* in which the D1 subunit was coded for by genes *psbA₂* and *psbA₃* (under normal laboratory conditions the *psbA₁* gene is expressed). It was shown that the $Tyr_Z^{\cdot}$ spectrum from PsbA3-PSII was very similar to that of $Tyr_D^{\cdot}$, while that from PsbA2-PSII was somewhat different. [16].

Abbreviations: PSII, Photosystem II; OEC, oxygen evolving complex; S-states, S_0,..., S_4 oxidation states of the OEC; Tyr Z or Y_Z, tyrosine 161 of the D2 polypeptide chain; tyrD or Y_D, tyrosine 160 of the D1 polypeptide chain; P_{680}, the primary electron donor in PSII; Q_A, Q_B, the primary secondary plastoquinone electron acceptors of PSII; MES, 2-[*N*-morpholineethanesulfonic acid]; Chl, chlorophyll; DCBQ, di-chloro-*p*-benzoquinone; PpBQ, phenyl-*p*-benzoquinone

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There are different constraints for the trapping of the various intermediates. These have been discussed in [17] and refined in subsequent studies [18]. While all intermediates can be trapped at temperatures well below the half-inhibition temperature for the respective transitions, induction of the $S_3Y_Z^{\cdot}$ intermediate requires presumably temperatures above the half inhibition temperature for the S_3 to S_0 transition.

In this paper we investigated the possibility of trapping the $S_2Y_Z^{\cdot}$ and $S_3Y_Z^{\cdot}$ intermediates at temperatures near and above the half inhibition temperature for the S_2 to S_3 and S_3 to S_0 transitions which is ~230 K [3,4]. Preliminary evidence for the trapping of the former intermediate in untreated samples at these elevated temperatures has already been published [19]. We restrict the present investigation to methanol containing samples, as the presence of 3–5% methanol appears to enhance the efficiency of trapping the respective intermediates.

It is found that visible-light illumination of the $S_2\ldots Q_A$ state at 233 K results in a 160 G wide signal, which we assign to the $S_2Y_Z^{\cdot}$ metalloradical intermediate, based on its similarity to the one produced by direct illumination at liquid helium temperatures [9]. Furthermore, visible-light illumination of the $S_3\ldots Q_A$ state at 250 K results in the formation of a new EPR signal with a splitting of 80 G, which we attribute to the $S_3Y_Z^{\cdot}$ metalloradical intermediate. The latter signal is weaker than the former and both are notably stable at cryogenic temperatures.

2. Materials and methods

2.1. PSII sample isolation

PSII-enriched thylakoid membranes were isolated from spinach [20,21]. Samples for EPR measurements were suspended in 0.4 M sucrose, 15 mM NaCl, 40 mM 2-[*N*-morpholineethanesulfonic acid] (MES), pH 6.5, at about 6–8 mg chl/mL and stored in liquid nitrogen. Methanol 5% (v/v) was added to all samples. All samples prior to the EPR experiments were given a pre-flash at −10 °C and were subsequently dark adapted for 40 min at 0–4 °C. This poised all centers to the S_1 state. The samples were subsequently supplemented with 1 mM of an exogenous electron acceptor, either di-chloro-*p*-benzoquinone (DCBQ), or Phenyl-*p*-benzoquinone (PpBQ), [22,23].

2.2. Illumination conditions

A studio photographic flash unit, Elinchrom Style RX 1200, with variable flash power up to 1200 Ws, and pulse duration (half width at half height) of 1–2 ms was used for flash excitation of the samples. At temperatures below 273 K double hits occur with low frequency, but misses are increased and thus flashes with long duration are needed. In this manner, the S-state cycle can proceed efficiently and good oscillation patterns are obtained [19]. The unit was equipped with a flexible fiber light extension for illumination inside the cavity. The S_2 state was produced typically by single flash illumination of the S_1 state at temperatures in the range 230–250 K. The S_3 state was formed by flash illumination of the S_2 state at 253 K. For the trapping of the metalloradical intermediates, samples in the S_2 or S_3 states were flash-excited at the appropriate temperatures, and within 0.5–1 s, transferred to liquid nitrogen, and from there into the EPR cryostat at 10 K. Formation of the intermediates at 10 K was done by excitation with 3 flashes spaced by 2 s.

2.3. EPR measurements

EPR measurements were obtained with an extensively upgraded former Bruker ER-200D spectrometer interfaced to a personal computer and equipped with an Oxford ESR 900 cryostat, an Anritsu MF76A frequency counter, a Bruker 035 M NMR gaussmeter and a SR830 digital lock-in amplifier by Stanford Research. The perpendicular 4102ST cavity was used and the microwave frequency was 9.41 GHz. For the approximate spin quantification, the doubly-integrated intensity of the present signals was compared to the doubly-integrated intensity of the multiline signal of the S_2 state. Comparison was also made with the maximum size of the $Y_D^{\cdot}$ signal.

3. Results and discussion

3.1. The S_2 to S_3 state metalloradical intermediate

In a recent investigation of transient changes occurring in the S_2 state EPR signals, during advancement to S_3 state, it was found that the $S_2Y_Z^{\cdot}$ intermediate could be trapped at 223 K [19], albeit at a smaller intensity compared to the same intermediate trapped in the range 77–190 K. We examined the possibility of trapping this intermediate in methanol containing samples.

The presence of few percent methanol, which does not inhibit O_2 evolution [24], induces a homogeneous conformation of the S_2 state [25], and probably slows down the EPR transitions.

Panel (A) of Fig. 1 shows the changes in the $S_2\ldots Q_A$ state signal (trace a) after flash illumination at 233 K followed by rapid cooling to 10 K (trace b). The temperature of 233 K is at the lower end of the threshold for the S_2 to S_3 state transition in 5% MeOH containing samples. Therefore the efficiency of S_3 state formation is very low. From the difference spectrum (b)–(a) of panel (A) we observe that a prominent split signal is trapped and it is accompanied by a small decrease in the intensity of the multiline signal. The changes are reversed by 3 min incubation at 233 K in the dark (trace c). The small reversible decrease of the multiline signal in spectrum (b) is attributed to the magnetic interaction of TyrZ$^{\cdot}$ with Mn, which presumably broadens the Mn spectrum in the fraction of centers, where TyrZ$^{\cdot}$ is formed [9]. A higher resolution recording of the split signal is shown in panel (B) (continuous trace) overlaid on the $S_2Y_Z^{\cdot}$ signal (dashed trace) formed by flash illumination at 10 K [9]. The two signals are practically identical apart from small differences in the $g \sim 2$ region, which cannot be evaluated at present. Both signals show very little saturation at the highest microwave power used, 200 mW, a fact that is compatible with their metalloradical character. Double integration of the $S_2Y_Z^{\cdot}$ signal trapped at elevated temperatures indicates that 18–25% of centers can be trapped in this metalloradical state. This is 4–5 times larger than the size of the signal trapped at cryogenic temperatures.

We reported that the $S_2Y_Z^{\cdot}$ in control samples could only be produced by illumination at temperatures higher than 77 K [9]. This was attributed to an energy barrier likely concerning proton translocation from TyrZ to His190 (and from there also to Asn298) [26]. The presence of methanol probably affects this barrier, but formation of $S_2Y_Z^{\cdot}$ in this case shows a complicated temperature profile (which will be examined in a separate study). A high activation energy of 0.46 eV (with KIE of 5.7) was reported for proton expulsion from the cluster to the lumen, caused by the formation of $S_2Y_Z^{\cdot}$ at room temperature [13]; these authors argued that this proton removal step likely occurs via a path originating from TyrZ-His190.

3.2. The S_3 to S_0 state metalloradical intermediate

We have argued earlier that production of the $S_3Y_Z^{\cdot}$ intermediate is inhibited below the onset of the S_3 to S_0 transition [17]. The only option is the trapping of the intermediate at or above the onset of this transition. There are however difficulties in detecting this intermediate by EPR spectroscopy. The S_3 state has