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The pH dependence of the donor side reactions in Ca^{2+}-depleted Photosystem II: A role for Ca in the proton expulsion pathway?

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Introduction

During oxidation of water the photosystem II complex cycles through 5 intermediate S-states (S_0-S_4). The catalytic water oxidation is performed with the involvement of four Mn-ions, a redox active tyrosine, Y_Z, situated 7-8 Å away, and one essential Ca^{2+} ion (Diner & Babcock, 1996; Nugent, 2001). Depletion of Ca^{2+} inhibits oxygen evolution, alters the proton release in the S-cycle and perturbs the S_2 state leading to the generation of a stable modified S_2 multiline EPR signal from Mn. In addition, Ca^{2+} depletion stalls the S-state cycle in the $S_2Y_Z^{\bullet}$ state during illumination and generates a characteristic "split" EPR signal (Ono & Inoue 1988; Boussac *et. al.*, 1989, 1990; Sivaraja *et al.* 1989; MacLachlan & Nugent, 1993; Gilchrist *et al.*, 1995; Lakshmi *et. al.*, 1999). The $Y_Z^{\bullet}$ in this state is very long-lived (Andréasson *et. al.*, 1995). The question then is what the function of this Ca ion is in photosynthetic water oxidation.

We recently reported that the S_3 state when exposed to high pH, gives rise to the "split" EPR signal from S_2 $Y_Z^{\bullet}$ (Geijer *et. al.*, 2001). Thus it was proposed that the redox potential of $Y_Z^{\bullet}/Y_Z$ decreased at high pH thereby making Y_Z directly available for *oxidation* by the Mn-cluster. This finding clearly shows that Y_Z is in contact with the bulk medium through a H-bonding network and is further experimental support for the H-atom transfer mechanism in photosynthetic water oxidation (Tommos & Babcock, 1998). In this current work we have sought to address the role of Ca^{2+} in the context of the H-atom transfer mechanism. The specific question to address is whether a proton deficiency in the Mn/substrate water ensemble could prevent reduction of $Y_Z^{\bullet}$ under conditions of Ca^{2+} depletion. This could then suggest a role for the Ca^{2+} cofactor in the proton currents of PSII.

Materials and Methods

Ca^{2+}-depleted PSII membranes from spinach were prepared by citrate treatment at pH 3.0 (Ono *et. al.*, 1988). The oxygen evolution was 0-10% in the absence and 75-80% of the control in the presence of 20 mM $CaCl_2$. The membranes were finally resuspended in a low buffer medium (0.25 mM MES pH 6.5, 400 mM sucrose, 15 mM NaCl) to allow the pH jump. The Ca^{2+} depleted membranes were illuminated for 1 min with 0.1 mM EDTA to induce the stable S_2 state and then dark-adapted for 5 min (Boussac *et. al.*, 1989). Then the pH was changed by the addition of stronger buffers (final conc. 14 mM): DL-glutamic acid (4.0 – 5.0), MES (5.0-6.5) and HEPES (6.5-7.5). The $S_2Y_Z^{\bullet}$ state was induced quantitatively by 30s illumination at 0°C (Boussac *et. al.*, 1989). Low temperature EPR measurements were performed as in (Geijer *et. al.*, 2001) and kinetic EPR measurements were performed at the low field maximum of $Y_Z^{\bullet}$ with laser flash induction (Andréasson *et. al.*, 1995). To allow decay of $Y_Z^{\bullet}$ between the flashes we used a 1 min dark interval and only 20 flashes were given to each individual sample.

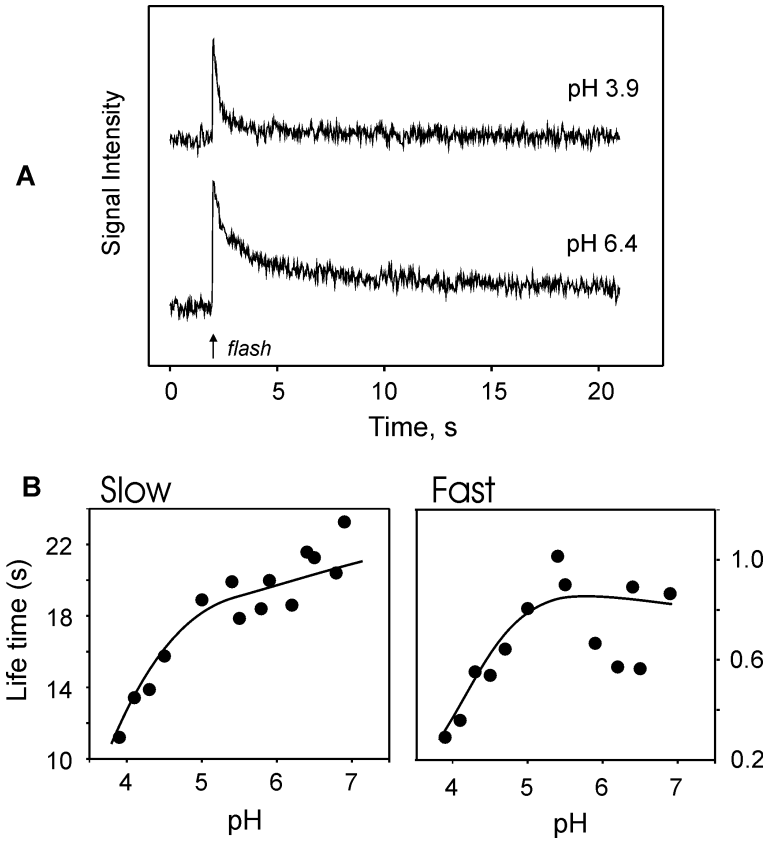

Figure 1: Decay kinetics of the flash-induced EPR signal from $Y_Z^\bullet$ in Ca^{2+}-depleted PSII membranes at different pH. A) Decay kinetics at pH 3.9 (upper trace) and pH 6.4 (lower trace). B) pH dependence of the slow and fast decay kinetics of $Y_Z^\bullet$. PSII conc. 2.2 mg Chl/ml, T 293 K, frequency 9.78 GHz, power 20 mW, mod. amp. 5 G, conversion time 20ms.

Results

The decay kinetics of $Y_Z^\bullet$ at pH 6.4 and 3.9 are shown in Fig. 1A. The decay follows two life-times. At pH 6.4, the slow phase encompasses a large fraction of $Y_Z^\bullet$; whereas, at pH 3.9 the $Y_Z^\bullet$ decay is almost entirely contained within a faster reaction. The biphasic decay behavior and the amount of flash-induced $Y_Z^\bullet$ at pH 6.4 are similar to that reported earlier (Andréasson *et. al.*, 1995; Lydakis-Simantiris *et. al.*, 1998). We also determined that the same amount of $Y_Z^\bullet$ was oxidized by the flash (20±3%) over the entire pH interval through comparison with the $Y_D^\bullet$ signal. The strong pH dependence of the $Y_Z^\bullet$ decay was apparent with both kinetic phases as shown in Fig 1B. At pH 6.4 the fast phase decays with 0.8s and the slow phase with 22s time constants. At pH 3.9 the time constants for the decay of $Y_Z^\bullet$ are 0.3 s and 11s. Between pH 6.4-7.0, about 65% of $Y_Z^\bullet$ decays with slow kinetics (life times 20s) while the fast phase dominates completely at pH 3.9. Both phases are insensitive to the pH above pH 6 but become faster below pH ~5.5. We have not measured below pH 3.9 but at this pH the slow phase is almost absent. Thus, it seems that the p*K* for the changes in the decay time constants (at least of the slow phase) is around pH 4.5. In presence of 20 mM $CaCl_2$, 30-35% of the flash-induced signal of $Y_Z^\bullet$ remained as observed earlier (Andréasson *et. al.*, 1995). This corresponds to 7-10% of all centers.

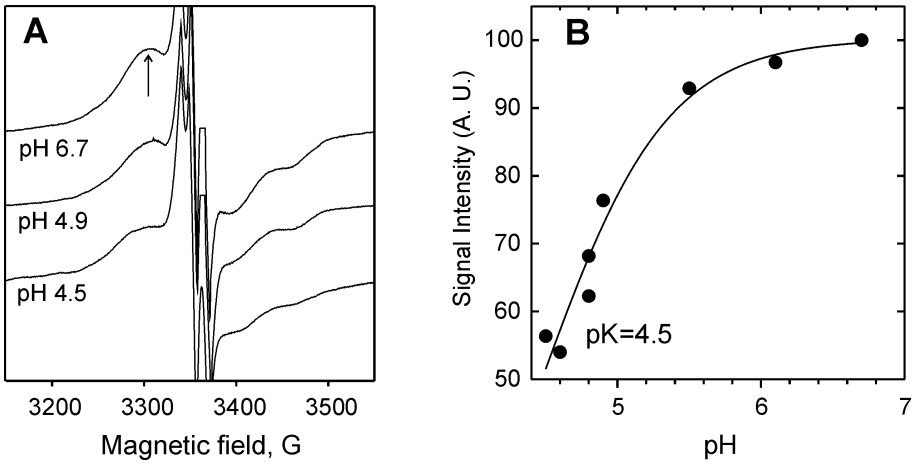

Figure 2: A. EPR spectra of the "split" EPR signal from the $S_2 Y_Z^\bullet$ state induced at different pH. B. pH dependence of the amplitude of the $S_2 Y_Z^\bullet$ signal and fitting of the amplitude to a pH dependence with a single pK of 4.5. EPR conditions: T 10 K, frequency 9.41 GHz, power 20 mW and mod. ampl. 10 G.

We also measured the induction of the $S_2Y_Z^\bullet$ split EPR signal at differing pH values. The spectra for differing pH values is shown in Fig. 2A. The maximum signal is formed at pH 6.5-7.0 and the signal is smaller at low pH. The pH dependence of the $S_2Y_Z^\bullet$ signal is shown in Fig. 2B and corresponds to an apparent p*K* of ~4.5. In Ca^{2+}-depleted PSII, S_2 gives rise to a stable, modified multiline EPR signal. At pH < 4.5 the entire stable multiline signal was lost during the illumination at 0ºC (not shown) indicating complete turnover of the S_2 state. However, only 54% of the maximal signal amplitude of the $S_2Y_Z^\bullet$ signal was induced at this pH (Fig 2B). Thus, it seems the S_2 state turned over quantitatively during the illumination although we observed the $S_2Y_Z^\bullet$ signal in only about 50% of the centers. It should also be mentioned that there was no increased induction of the radical EPR signals from chlorophyll or carotenoid molecules and no signs of enhanced oxidation of cyt b_{559} at the lower pH's where we have the diminished induction of the $S_2Y_Z^\bullet$ signal.

Discussion

Our measurements provide insight into the mechanism behind the inhibitory effects of Ca^{2+}-depletion in PSII. We find that the pH of the bulk medium has large effects with the Ca^{2+}-depleted PSII. Specifically, the $Y_Z^\bullet$ radical decays much faster at low pH than at high pH and the amplitude of the inducible $S_2Y_Z^\bullet$ signal is decreased by low pH in the medium.

We see two mechanisms that can give rise to these pH dependent effects. The fast decay of $Y_Z^\bullet$ at low pH could reflect faster recombination with acceptor quinones. Mechanistically this is unlikely to involve pH changes to the Q_A acceptor as it is not involved in protonation/deprotonation reactions. However, $Y_Z^\bullet$ is de-protonated upon oxidation. Thus, an increased recombination rate could manifest from an increase in the midpoint potential of the $Y_Z^\bullet/Y_Z$ couple. In the intact PSII system the $Y_Z^\bullet/Y_Z$ redox couple can be increased by pH in the S_3 state (Geijer *et al.*, 2001). At pH 4-4.5 the midpoint potential can reach almost 1000 mV, which in the Ca^{2+}-depleted system would increase the driving force in the recombination.

However, we see also another explanation to our data. These are built on our observation that the stable S_2 multiline signal is abolished to a larger extent than the S_2 $Y_Z^\bullet$ signal is formed by illumination at low pH. Thus, the entire S_2 population disappears when the Ca^{2+}-depleted PSII

centers are illuminated. In contrast, only 50-60% of the centers are found in the S_2 $Y_Z^\bullet$ state at pH 4.5. Which is the state for the remaining 40-50% of the centers? It is unlikely that they did not turn over at all. In this case a large fraction of the stable S_2 multiline signal should have remained. Probably we should also have observed oxidation of one or other of the alternative electron donors in PSII in the 40-50% of the centers that did not form the S_2 $Y_Z^\bullet$ signal. This was not observed. Instead we propose that the WOC actually is able to convert the S_2 state to the S_3 state in the Ca^{2+} depleted PSII membranes at low pH. Probably the formed S_3 does not turn over further since this transition is blocked at low pH (Bernat *et al*., these proceedings).

Why should the inhibition induced by Ca^{2+}-depletion be abolished by low pH? We suggest that this is a consequence of the mechanism for the reduction of $Y_Z^\bullet$. The coordination of Ca^{2+} by PSII is likely to involve carboxylic residue(s). When the pH is lowered to pH 3.0 during Ca^{2+}-depletion these carboxylic groups become protonated leading to release of Ca^{2+}. We then postulate that the loss of the Ca^{2+} ion results in the disruption of a H^+ pathway. Specifically the Ca^{2+} ion may be involved in the formation of a neutral proton pathway from the Mn cluster. However, following pH 3 treatment and loss of Ca^{2+}, the carboxyl groups are deprotonated when the pH is moved back to pH 6-7. This disrupts H^+ movement leading to inhibition. Consequently the requirement of coupled transfer of both an electron and a proton from the Mn-cluster to reduce $Y_Z^\bullet$ is likely to be much slowed down (or even completely inhibited) as proton transfer is inhibited. We suggest then, as the bulk pH is decreased the charged carboxylic groups become neutralized upon protonation, and H^+ transfer is restored facilitating $Y_Z^\bullet$ reduction and S-state advance. The pH-induced changes reported here occur with a pK ~ 4.5 which is normal for titration of carboxylic side-chains. To conclude, the Ca^{2+} ion in PSII may function in a H^+ relay mechanism and the loss of this cofactor may prevent the reduction of $Y_Z^\bullet$ by the Mn in water oxidation.

Acknowledgements

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