

# The split $S_3$ EPR signal from the water oxidizing complex in Photosystem II – pH dependence and possible mechanistic implications

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The electrons extracted from the  $\text{CaMn}_4$  cluster during water oxidation in photosystem II (PSII) are transferred to  $\text{P}_{680}^+$  via the redox active tyrosine D1-Tyr161 ( $\text{Y}_Z$ ). Upon  $\text{Y}_Z$  oxidation a proton moves in a hydrogen bond towards D1-His190 ( $\text{His}_Z$ ). The deprotonation and re-protonation mechanism of  $\text{Y}_Z\text{-O}/\text{Y}_Z\text{-OH}$  is of key importance for the catalytic turnover of photosystem II. By illumination at liquid helium temperatures ( $\sim 5$  K)  $\text{Y}_Z$  can be oxidized forming the neutral radical,  $\text{Y}_Z^\bullet$ . This can be followed by the induction of a split EPR signal from  $\text{Y}_Z^\bullet$  in a magnetic interaction with the  $\text{CaMn}_4$  cluster, offering a way to probe  $\text{Y}_Z$  oxidation in active PSII. In the  $S_3$  state, light in the near-infrared region induces the split  $S_3$  EPR signal,  $S_2'\text{Y}_Z^\bullet$ . The  $S_2'\text{Y}_Z^\bullet$  state is suggested to be formed by a “backward” electron transfer from  $\text{Y}_Z$  induced by the excitation of one of the Mn-ions in the  $\text{CaMn}_4$  cluster (1).

The pH dependence of the split EPR signal formation at 5 K has previously been investigated in the  $S_1$  state (2) and the  $S_0$  state (3). Here we present the pH dependence of the split EPR signal induced in the  $S_3$  state by NIR illumination. At acidic pH the split  $S_3$  EPR signal decrease with the apparent  $\text{p}K_a \sim 4.1$ . In conclusion, the pH dependence of the split EPR signals investigated so far,  $S_1\text{Y}_Z^\bullet$ ,  $S_2'\text{Y}_Z^\bullet$ , and  $S_0\text{Y}_Z^\bullet$  have similar pH dependencies in the low pH region ( $\text{p}K_a \sim 4.1\text{-}4.9$ , (2-3)). In all cases the decrease is assigned to the disruption of the essential H-bond in the  $\text{Y}_Z\text{-His}_Z$  motif, possibly by a direct protonation of the  $\text{N}_\epsilon$  in the imidazole ring of  $\text{His}_Z$  that acts as a hydrogen bond acceptor, and it can be concluded that this is independent whether the split EPR signal formation occurs via “forward” or “backward” electron transfer.

At alkaline pH, the split  $S_3$  EPR signal decrease with the apparent  $\text{p}K_a \sim 7.5$ . At pH above  $\sim 7.5$  the formation of the split  $S_3$  EPR signal is hindered by the induction of another split EPR signal,  $S_2''\text{Y}_Z^\bullet$ . This state is induced by a change in the redox potential of  $\text{Y}_Z$  (4) and also implies that there is no major inhibition of  $\text{Y}_Z$  oxidation at high pH values. Interestingly, such change in redox potential of  $\text{Y}_Z$  is not affecting the formation of the split EPR signal induced from the  $S_1$  state at high pH (2). This might reflect the charge compensating proton release taking place in the  $S_2 \rightarrow S_3$  transition.

Our results provide further evidence that the radical involved in the split EPR signals is indeed  $\text{Y}_Z^\bullet$  and allows discussion about the hydrogen bond patterns around  $\text{Y}_Z$  in the  $S_3$  state.

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- (3) Sjöholm, J., Havelius, K.G.V., Mamedov, F., and Styring, S. (2009) The  $S_0$  state of the water oxidizing complex in photosystem II: pH dependence of the EPR split signal induction and mechanistic implications, *Biochemistry* 48, 9393–9404.
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