

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 CaMn_4 cluster during water oxidation in photosystem II (PSII) are transferred to P_{680}^+ via the redox active tyrosine D1-Tyr161 (Y_Z). Upon Y_Z oxidation a proton moves in a hydrogen bond towards D1-His190 (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 (~ 5 K) Y_Z can be oxidized forming the neutral radical, Y_Z^\bullet . This can be followed by the induction of a split EPR signal from Y_Z^\bullet in a magnetic interaction with the CaMn_4 cluster, offering a way to probe 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 Y_Z induced by the excitation of one of the Mn-ions in the 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 N_ϵ in the imidazole ring of 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 ~ 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 Y_Z (4) and also implies that there is no major inhibition of Y_Z oxidation at high pH values. Interestingly, such change in redox potential of 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 Y_Z^\bullet and allows discussion about the hydrogen bond patterns around 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.
- (4) Geijer, P., Morvaridi, F., and Styring, S. (2001) The S_3 state of the oxygen-evolving complex in photosystem II is converted to the $S_2\text{Y}_Z$ state at alkaline pH, *Biochemistry* 40, 10881-10891.