

# New insight into the electronic structure of the $\text{Mn}_4\text{O}_x\text{Ca}$ cluster in photosystem II based on $^{55}\text{Mn}$ ENDOR spectroscopy and Ca/Sr exchange

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The electronic structures of the native  $\text{Mn}_4\text{O}_x\text{Ca}$  cluster and the biosynthetically substituted  $\text{Mn}_4\text{O}_x\text{Sr}$  cluster of the oxygen evolving complex (OEC) of photosystem II (PSII) of PSII core complexes isolated from *Thermosynechococcus elongatus*, poised in the  $\text{S}_2$  state, were studied by X- and Q-band CW-EPR and by pulsed Q-band  $^{55}\text{Mn}$ -ENDOR spectroscopy. Both wild type and tyrosine D less mutants grown autotrophically in either  $\text{CaCl}_2$  or  $\text{SrCl}_2$  containing media were measured. The obtained CW-EPR spectra of the  $\text{S}_2$  state displayed the characteristic pronounced differences in the hyperfine pattern of the multiline EPR signal. In sharp contrast, the manganese ( $^{55}\text{Mn}$ ) ENDOR spectra of the Ca and Sr forms of the OEC were remarkably similar. Global simulations of the X- and Q-band cw EPR and  $^{55}\text{Mn}$  pulsed ENDOR spectra using the Spin Hamiltonian formalism were performed to investigate this surprising result. It is shown that:

- (i) all four manganese ions contribute to the  $^{55}\text{Mn}$ -ENDOR spectra;
- (ii) only small changes are seen in the fitted isotropic hyperfine values for the Ca and Sr containing OEC, suggesting that there is no change in the overall spin distribution (electronic coupling scheme) upon Ca/Sr substitution;
- (iii) the changes in the CW-EPR hyperfine pattern can be explained by a more than 2-fold increase in the anisotropy of the onsite hyperfine tensor of one Mn ion;
- (iv) this Mn ion is assigned to the Mn center of highest spin multiplicity and is therefore readily identified with the only remaining  $\text{Mn}^{\text{III}}$  in the  $\text{S}_2$  state.

DFT calculations confirm the above conclusions. It is shown that for several recently proposed structural models of the  $\text{Mn}_4\text{O}_x\text{Ca}$  cluster the exchange of Ca with Sr has little or no influence on the electronic couplings between the Mn ions. The strong similarities between the results presented here for the  $\text{Mn}_4\text{O}_x\text{Sr}$  cluster and literature data on the ammonia modified multiline signal, suggests that both Ca/Sr exchange and ammonia binding affect the  $\text{Mn}^{\text{III}}$  ion in the  $\text{S}_2$  state in a similar manner. Collectively these results give strong support to previous suggestions that Ca has predominantly a functional as opposed to a structural role in water splitting catalysis.