EPR Evidence for ^{13}C-Carbonate and Calcium Binding to Mn during Photo-assembly of the Photosynthetic Water Oxidizing Complex

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Biogenesis (or repair) of the water-oxidizing complex of photosynthetic organisms (WOC-PSII) requires (re)assembly of the active-site inorganic cluster ($Mn_4O_xCa_1Cl_y$) from free inorganic cofactors and cofactor depleted apoWOC-PSII, by a light-dependent process called photoactivation. Using perpendicular- and parallel-mode EPR spectroscopy, we characterize the high-affinity Mn site in spinach PSII both in the Mn^{2+} and the photo-oxidized Mn^{3+} redox states. In the absence of bicarbonate and Ca^{2+}, the EPR-active form $[Mn^{3+}(OH^-)]$ exhibits pronounced pH-induced variation in the ligand field symmetry (derived from g_{eff}) and the ^{55}Mn hyperfine interaction A_Z, reflecting coordination changes at the Mn^{3+} site. Upon addition of bicarbonate in absence of Ca^{2+}, the pH dependence of g_{eff} disappears yielding a single value, consistent with carbonate coordination at the Mn^{3+} site (see Figure). Unequivocal evidence for direct coordination of ^{13}C-carbonate to high affinity Mn^{2+} is provided by ESEEM spectroscopy. Binding of Ca^{2+} to its effector site in absence of added bicarbonate, also eliminates the pH dependence of g_{eff} and A_Z yielding a single coordination environment indicative of an alkaline form. These data are consistent with a model, shown in the Figure, in which Ca^{2+} binds to the high affinity Mn^{3+} via an ionizable ligand (XH) which could be either $H_2O/OH^-/(H)CO_3^-$. This work is supported by NIH and HFSP.