13C ENDOR Reveals That the D1 Polypeptide C-Terminus Is Directly Bound to Mn in the Photosystem II Oxygen Evolving Complex


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Extended research efforts have focused on characterizing the structure and ligand environment of the [Mn3Ca] cluster that makes up the catalytic core of the oxygen-evolving complex (OEC) in photosystem II (PSII). The OEC catalyzes the light-induced four-electron oxidation of two molecules of H2O to O2 and 4 H+ via a five-step reaction pathway referred to as the “S state” or “Kok” cycle. When the OEC is poised in the S2 state, EPR reveals a g = 2 “multiline” signal with ≈19 55Mn hyperfine lines resolved in the X-band CW spectrum. This signal is attributed to an S = 5/2 ground state arising from antibiskeynetic coupling of the four manganese ions and is reminiscent of EPR spectra of oxidized bridged MnIIIMnIV dimers. One such spectroscopic model of the OEC was the subject of a pulse EPR study in which electron nuclear double resonance (ENDOR) and electron spin−echo envelope modulation (ESEEM) spectroscopies probed water and methanol ligation to the Mn dimer core. Those results provided a basis for the exploration of water and methanol binding to biological Mn clusters.

In the present study, ENDOR spectroscopy is used to characterize the interaction of carboxylate ligands with the Mn ions of exchange-coupled clusters. Figure 1A presents the Q-band (34 GHz) Mims ENDOR spectrum of 13C(l = 1/2) in the carboxylate position of an acetate bridge between a MnIII and MnIV in [MnIII(MnIV(µ-O)2µ-OAc(TACN)](BPh4)2 (TACN = 1,4,7-triazacyclononane). This acetate moiety is invoked as a model for protein-derived carboxylates thought to coordinate the OEC in PSII.3 We compare these 13C−I results to ENDOR spectra of PSII from Synechocystis sp. PCC 6803 for which (i) the C-terminal α-COO− group (Ala344) of D1 and all alanine-derived C=O groups are labeled with 13C (α-13C-Ala PSII; Figure 1B) and (ii) all C atoms are uniformly labeled with 13C (Figure 1C).

The CW EPR spectra of the 13C and 12C acetate isotopologues of I are essentially identical (Figure S1). While 12C-I gives no ENDOR intensity around the 13C Larmor frequency (νL(13C) = 13.0 MHz at B0 = 1218.0 mT), the 13C−I spectrum possesses a pair of structured peaks centered at νL(13C) and split by 1.9 MHz (Figure 1A). The dipolar (adip) and isotropic (aso) hyperfine interactions govern the magnitude of the splitting between the ENDOR peaks. adip is defined as the site-specific through-space interaction of the 13C nuclear spin with the electron spin on each Mn ion. adip originates from the unpaired electron spin population in the valence s-orbital of the 13C atom. For multimetal clusters such as I, exchange interactions between the metal ions lead to an effective spin (S = 1/2) that is different from the spins of the isolated ions (Siso = 2 and Siso = 1/2). Thus the observed hyperfine (asoI + adip) must be scaled by the appropriate projection factors to give adip before deriving both the covalent character of the bond and the distance between the unpaired spin for each site and the magnetic ligand nucleus. The projection factors for the MnIII and MnIV ions in I are +2 and −1, respectively. The site adip representing each Mn ··· 13C interaction was computed using the coordinates from the high-resolution X-ray crystallographic structure.6 These two hyperfine contributions were then scaled by the projection factors and transformed into a common frame to give adip. With the effective adip contribution fixed, the value for aso was varied from +4 to −4 MHz until a satisfactory simulation of the experimental data was achieved. To resolve the structure evident in the ENDOR features of all spectra in Figure 1, the simulations required an isotropic line width parameter no larger than 0.2 MHz. We also accounted for τ-dependent blind spots inherent to Mims ENDOR spectra.8 Using this procedure, the simulation parameters that gave the best fit to the experimental data of 13C−I were adip = [−2.4, −0.8, 3.2] MHz and aso = −1.0 MHz (Figure 1A). This value for aso is small compared to that found for a carbon bond directly to a paramagnetic center,9 as the α-C of the acetate is two bonds away from each Mn ion.

Biochemical and spectroscopic studies5 and X-ray crystallography10–12 identify a number of residues as possible ligands to the Mn ions in the OEC. However, these studies give somewhat conflicting results. For example, D1-Ala344 is positioned differently in each X-ray structure. The Ferreira structure shows the COO− group of the D1 polypeptide bound to the Ca ion, while structures

Figure 1. Q-band Mims ENDOR spectra (black) of (A) 13C−I; (B) α-13C-Ala PSII; (C) 13C−PSII. Experimental parameters: νMW = 33.9 GHz; B0 = 1218−1220 mT; selected τ values from 180 to 300 ns were used (see Supporting Information); t(π/2) = 20 ns; t(π) = 64 µs; T = 12 K (13C−I) and 5 K (PSII). Simulation (blue) parameters for 13C−I spectrum: adip = [−2.5, −0.8, 3.2]; aso = −1.0 MHz; line width 0.2 MHz.

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The ENDOR spectrum of uniformly $^{13}$C-labeled PSII (Figure 1C) also shows prominent peaks split by $\approx 1.7$ MHz, a slightly smaller splitting than observed for the $^{13}$C-Ala PSII sample (Figure 1B). We note that if the intensity of the uniformly labeled sample is appreciably more intense than that for the $^{13}$C-Ala sample. This implies that there are multiple $^{13}$C-containing moieties with couplings in the $1.7-1.9$ MHz range. The slightly larger splitting in the $^{13}$C-Ala PSII spectrum indicates the OEC Mn interaction with the D1-Ala344 $^{13}$C is one of the strongest (see red line Figure 1). An additional set of peaks with a resolved splitting of $0.9$ MHz arises from another group of $^{13}$C nuclei with appreciably weaker couplings. A number of mutagenesis, FTIR difference and X-ray crystallographic studies suggest additional amino acid derived carboxylate ligands to the OEC (see Figure S3). $^{10,11}$ Further $^{13}$C ENDOR studies are currently underway to identify which of these amino acids are responsible for these more weakly coupled features.

In conclusion, $^{13}$C ENDOR data indicate that multiple classes of $^{13}$C-containing moieties are coupled to the [MnCa] cluster. In particular, these data conclusively show that the C-terminal carboxylate of D1-Ala344 is bound to at least one Mn ion.

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Supporting Information Available: Supporting spectra, simulations, and experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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