



Advanced EPR Investigations of Spin-Coupled Mn/Fe Intermediates Formed During Oxygen Activation in a Heterobimetallic Oxidase

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Introduction

Heterobimetallic Mn/Fe proteins make up a new class of metalloenzymes that are overrepresented in bacterial pathogens and archaeal extremophiles, indicative of conferred resistance to life under hostile conditions.¹ These proteins perform hydrolytic, radical, and two-electron chemistry, though the molecular basis underlying this diverse reactivity remains unknown. In this project, we are investigating R2lox to understand the mechanism of O₂ activation and C-H bond oxidation.² High-field pulsed EPR offers a valuable method to probe the electronic structure of distinct intermediates, providing structural information on the active site and ligands bound to the metal centers in great detail.^{3,4}

Experimental

R2lox samples were prepared as described previously.² 94 GHz EPR experiments were performed on the HiPER instrument within the EMR facility of the NHMFL. Field-swept EPR spectra were obtained using a Hahn stimulated echo sequence of $\pi/2 - \tau - \pi - \tau - \text{echo}$ with $\pi = 200$ ns and $\tau = 600$ ns. ELDOR-detected NMR (EDNMR) spectra were measured with the following pulse sequence: $\pi_{\text{HTA}} - T - \pi/2 - \tau - \pi - \tau - \text{echo}$. The high turning angle pulse (π_{HTA}) of 7500 ns was applied at 94 GHz with an estimated amplitude of 1×10^7 rad/s. τ was set to 1500 ns in the EDNMR experiments.

Results and Discussion

The resting state of R2lox is a Mn^{III}(μ -OH)Fe^{III} species with unique magnetic properties, including weak antiferromagnetic exchange between the two metals that results in a ground spin state of $S_T = 1/2$. The O₂ activation pathway proceeds via a series of two-electron intermediates that are also spin-coupled. We have been able to resolve the high-field EPR spectra of one of these intermediate species and compare it to the assembled product (Fig.1). The spectral breadth is significantly greater for the freeze-trapped intermediate, with the increased *g*-tensor anisotropy suggesting that the intermediate species has weaker exchange coupling between the two metals. This measurement confirms that the species derives from a Mn^{III}/Fe^{III} state.

To identify the nature of the ligands bound to the metal centers, ELDOR-detected NMR (EDNMR) spectroscopy was used. The transient intermediate exhibits slightly anisotropic coupling to two exchangeable protons (Fig.1, inset). The coupling to one of the protons is greater at high fields, while the other remains relatively constant across the spectral envelope. The magnitude of the coupling as well as the modest field dependence is inconsistent with one of those protons residing in the bridging position between the two metal centers;³ thus, we can now assign the protonation state of the Mn-bound terminal ligand to a fully protonated water species.

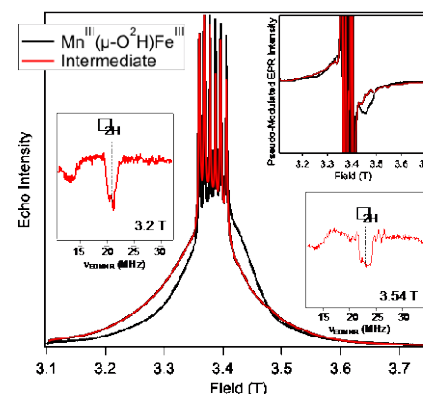


Fig.1 Field-swept echo-detected and pseudo-modulated (*inset, top*) EPR spectra of the R2lox product (black) and the freeze-trapped assembly intermediate (red). ED-NMR spectra of the intermediate at the indicated field positions also shown.

Conclusions

The pulsed EPR experiments performed thus far have enabled us to propose that the Mn/Fe spin-coupled species observed during assembly that exhibits large *g*-tensor anisotropy is a Mn^{III}/Fe^{III} state with a water ligand bound to the Mn^{III} center. That the water ligand remains protonated during assembly highlights the reduced Lewis acidity of a Mn^{III} center relative to a Fe^{III} center, guiding our understanding of potential *in vivo* roles for the metal center.

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