SPring. 8

Nuclear resonance vibrational spectroscopy definition of intermediate Q in methane monooxygenase

Non-heme iron enzymes play a key role in the activation of dioxygen in Nature [1]. Among the strategies for the four-electron activation of O2, some mononuclear enzymes (e.g., syringomycin halogenase SyrB2) use two electrons from the Fe^{II} center and two electrons from the cofactor α -ketoglutarate, producing a 5-coordinate Fe^{IV}=O intermediate that cleaves the C-H bond of activated substrates (Fig. 1(a)). Alternatively, the two Fe^{II} centers of the binuclear enzyme soluble methane monooxygenase (sMMO) provide all four electrons, producing an Fe^{IV}₂ site able to cleave the strong C-H bond of methane to produce methanol. This high valent intermediate Q has been long studied as its structure informs the design of catalysts for methane oxygenation. Early results assigned Q as a closed core $Fe^{IV}_{2}(\mu - O)_{2}$, later supported by resonance Raman (rR) spectroscopy [2]. In 2010, an open core complex O=Fe^{IV}-O-Fe^{IV}-OH was found to oxidize dihydroanthracene at a rate 100× higher than its closed core $Fe^{IV_2}(\mu-O)_2$ complex [3] and subsequent high energy resolution fluorescence detected X-ray absorption spectroscopy (HERFD-XAS) studies favored an open core structure [4]. Our study [5] utilized ⁵⁷Fe Nuclear Resonance Vibrational Spectroscopy (NRVS) to characterize the geometric and electronic structure of intermediate Q, providing insight into the closed vs. open core debate (Fig. 1(b)).

⁵⁷Fe NRVS is a synchrotron-based technique that detects vibrational modes containing iron motion. The NRVS data are interpreted using Density Functional Theory (DFT) to interrogate the structure of Q. ⁵⁷Fe NRVS was performed at SPring-8 **BL09XU**, Nuclear Resonant Scattering beamline, using a nuclear resonance energy of 14.4 keV, monochromated to 1.0 meV using both a high-heat-load and highresolution monochromator. Vibrational sidebands were collected 100 meV above the elastic peak.

The NRVS spectrum of Q exhibited time-dependent changes in the beam. Using Mössbauer spectroscopy, we assigned this decay to cryoreduction of Q. Selective binning of scans from the first 10 hours and after 30 hours of measurement gave the vibrational spectra of Q (early) and cryoreduced Q (late). Figure 2 presents the NRVS spectra of early and late ${}^{16}O_2$ Q and late ${}^{18}O_2$ Q. The early Q spectrum shows features at 605, 420, 375, 330, 300 and 275 cm⁻¹, where late Q shows loss of the 605, 375 and 275 cm⁻¹ features and growth of new features at 580, 365, 240 and 220 cm⁻¹. Further, two decay features 580 and 420 cm⁻¹ show isotope sensitivity (Fig. 2(b) inset).





90 closed and open core DFT optimized structures of Q were generated and used to simulate the NRVS and prior spectroscopic data. The closed core models contained two bridging $O(H)_x$ and one terminal ligand, while the open core models all contained one oxo bridge, with differing levels of protonation of carboxylate and oxygenic ligands. Comparison of the simulated vibrational spectra of each model to past rR data [2] showed that both open and closed core models could reproduce the experimental data, but eliminated some models. Further, time-dependent DFT analysis investigated which models would show rR enhancement of a 690 cm⁻¹ feature by excitation at 351 nm. This analysis excluded all open core models except for those with two terminal hydroxides (OH-Fe^{IV}–O–Fe^{IV}–OH) or both a terminal hydroxide and an oxo ($O=Fe^{IV}-O-Fe^{IV}-OH$). For the remaining structural models, the full set of NRVS data (early and late ¹⁶O₂, late ¹⁸O₂) were simulated and compared to experimental NRVS data.

Figure 3 shows the NRVS simulations of open (a) and closed (b) core models that were the best simulations of the experimental data. The top panels display the experimental data, the middle panels show simulations of the early and late ¹⁶O₂ data, and the bottom panel compares simulations of the ¹⁶O₂ and ¹⁸O₂ late data. Figure 3(a) shows simulations for the closed core model containing a bis-µ-oxo core and a terminal hydroxide (bis(µO)–OH-12). Simulations to the low energy region (<450 cm⁻¹) are in good agreement with the experimental data. Further, the small ^{16/18}O₂ isotope sensitivity of the experimental decay data is well reproduced due to the longer Fe-OH bond which dictates the magnitude of this isotope shift. Figure 3(b) displays simulations for the best open core model, O=Fe^{IV}-O-Fe^{IV}-OH model (OC16). Comparison of the low energy region again shows good agreement to experiment, however, the simulations fail to reproduce



Fig. 2. NRVS spectra of Q: (a top) ${}^{16}O_2$ early (red), late (black) and Fe^{II}Fe^{II} reactant species (yellow), (a bottom) spectra corrected for residual Fe^{II}Fe^{II}. (b) ${}^{16}O_2$ (black) and ${}^{18}O_2$ (blue) late Q with isotope sensitive features (inset).

the small magnitude of the isotope shift in the late data and this and other open core structural models were eliminated. Thus, the NRVS data on Q from SPring-8 show that the closed but not the open core structure is supported by the experiment.

Experimentally, intermediate Q in the binuclear sMMO enzyme performs hydrogen atom abstraction (HAA) from methane with a ΔG^{\dagger} of 10.3 kcal/mol, while the Fe^{IV}O intermediate of the mononuclear enzyme SyrB2 has a ΔG^{\dagger} of 17.7 kcal/mol for its substrate threonine. Correcting for the difference in bond

strength of the substrates, SyrB2's ΔG^{\pm} for methane would be 24 kcal/mol. DFT calculations on the Q models give a barrier for a closed core of 12.4 kcal/mol (close to experiment), while the open core $O=Fe^{IV}-O-Fe^{IV}-OH$ and mononuclear $Fe^{IV}O$ sites are calculated to be 25.0 and 22.1 kcal/mol respectively. The low barrier for the closed core is due to the greater thermodynamic driving force associated with the reorganization of the resulting $Fe^{III}Fe^{IV}(\mu-O)(\mu-OH)$ closed core, enabling efficient reactivity of sMMO in the conversion of methane to methanol.



Fig. 3. Model simulations: top panel: experimental data, middle panels: simulations of the early (red) and late (black) ${}^{16}O_2$ data, bottom panel: simulations of the ${}^{16}O_2$ and ${}^{18}O_2$ late (blue) data (**a**) representative closed core model (bis(μ O)–OH-12) (**b**) Best open core model (OC16).

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