NRVS definition of the non-heme $Fe^{IV}=O$ intermediate in a halogenase and its control of reactivity

Mononuclear non-heme iron (NHFe) enzymes catalyze a wide array of biologically-relevant reactions, including H-atom abstraction, hydroxylation, halogenation, desaturation, and aromatic ring cleavage. They are important in neurotransmitter, antibiotic, and natural product biosynthesis, bioremediation, hypoxic regulation, and DNA cleavage in anticancer activity [1]. An α-ketoglutarate (αKG) dependent NHFe enzyme, syringomycin halogenase (SyrB2), which has an active site with 2-histidine 1-halide (Br^- or Cl^-) facial triad ligation rather than the more common 2-histidine 1-carboxylate facial triad found in most of NHFe enzymes, is involved in the biosynthesis of the phytotoxin syringomycin E. SyrB2 catalyzes chlorination of L-threonine (L-Thr) through a key, highly reactive, chloroferryl (Cl-Fe^{IV}=O) intermediate that activates the unreactive aliphatic C-H bond. For the non-native substrate L-norvaline (L-Nva) this enzyme was found to hydroxylate rather than halogenate (Fig. 1) [2]. Therefore, understanding the nature of this Fe^{IV}=O intermediate and its reaction mechanism for halogenation *versus* hydroxylation is key in the development of efficient catalysts for a range of important chemistries.

The first nuclear resonance vibrational spectroscopy (NRVS) study for an Fe^{IV}=O intermediate in a non-heme Fe enzyme is reported in Ref. [3]. The combination of NRVS coupled to density functional theory (DFT) calculations was applied in order to elucidate the geometric structure of the Fe^{IV}=O intermediate in SyrB2 and determine the factors that govern its reactivity and selectivity. NRVS is a technique that probes the vibrational sidebands of the 14.4 keV ^{57}Fe Mössbauer transition using third-generation synchrotron radiation. It is selective only for vibrations involving iron displacement, making it an ideal tool for studying the vibrational modes of a biological NHFe enzyme active site.

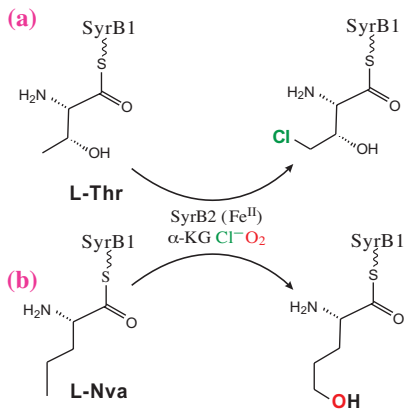

Fig. 1. SyrB2 catalyzes both chlorination of the native substrate L-Thr **(a)** and hydroxylation of a non-native substrate L-Nva **(b)**. [2]

The SyrB2 Fe^{IV}=O intermediate was generated and trapped with both Br^- and Cl^- ligated to the Fe (for a mass perturbation to aid in the assignment of the NRVS data). This intermediate could be trapped at the necessary purity and concentration by using a slow non-native substrate (L-cyclopropylglycine [L-Cpg]) attached to the non-native carrier protein CytC2. NRVS data for both samples were collected at beamline **BL09XU** at SPring-8 (as well as beamline 3-ID-D at APS). The partial vibrational density-of-states spectra for both halide bound forms of the intermediate are shown in Fig. 2(a) [3]. Both spectra show three peaks, with a shift in intensity from the higher energy modes to the lower energy modes when Cl^- is replaced by Br^-.

NRVS data on structurally-defined model complexes were used to calibrate DFT calculations to determine the computational method that best reproduces experiment. This experimentally-calibrated DFT method was then applied to possible Cpg-bound structures of the intermediate (with substrate positioning taken from Ref. [4]). It was found that only a 5-coordinate, trigonal bipyramidal (TBP) geometry, with the Fe=O oriented along the ~C_3 axis (Fig. 2(c)), reproduced the 3 peak pattern and the Cl^- to Br^- intensity shift (a mass effect, but one that shifts Fe motion into lower energy modes).

We then used the O_2 activation reaction coordinate calculation we developed for αKG-dependent NHFe enzymes in Ref. [5] to define the structure of the Fe^{IV}=O intermediate generated with the native substrate L-Thr bound. This had a similar structure to that of the Cpg-bound intermediate defined above (TBP geometry) with the Fe-oxo vector oriented perpendicular to the C-H bond of the substrate. The NHFe^{IV}=O enzyme intermediates have an S=2 ground state, and we have shown that an S=2 Fe^{IV}=O species has two frontier molecular orbitals (FMOs; low-lying unoccupied orbitals with significant oxo character) available for reactivity: a σ-FMO oriented along the Fe-O bond and a π-FMO perpendicular to the bond. The perpendicular orientation of the Fe=O vector relative to the H-substrate bond requires that the π-FMO is active for H-atom abstraction. Interacting with the π-FMO, the substrate H-atom is able to be transferred to the oxo group with a barrier consistent

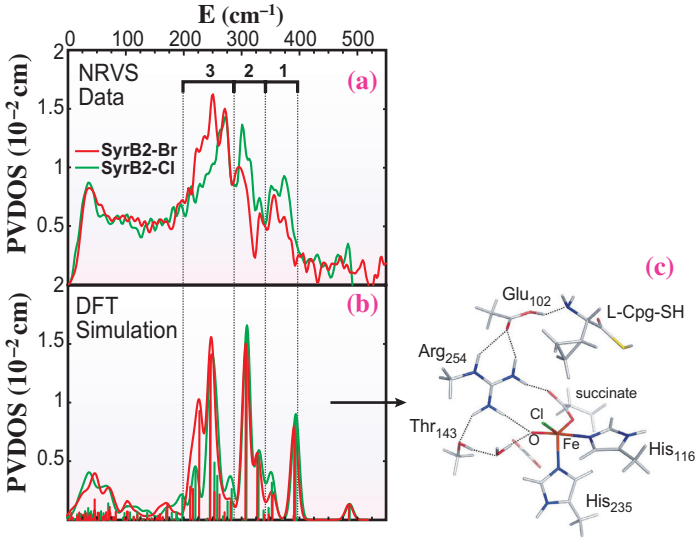

Fig. 2. NRVS data **(a)** and DFT simulations **(b)** for the SyrB2-Cl and SyrB2-Br intermediates. **(c)** DFT-derived structure of the SyrB2-Cl intermediate. [3]

with the experimental value (Fig. 3) [3]. The resulting Cl-Fe^{III}-OH species has the OH^- positioned away from the substrate carbon radical but the chloride is well-oriented towards the substrate radical for Cl• rebound and halogenation. Employing the same reaction coordinate as above with the alternative substrate L-Nva can lead to a H-bonding interaction with the O_2 intermediate along the reaction coordinate. This again results in a TBP intermediate but with its Fe=O vector oriented toward the substrate C-H bond.

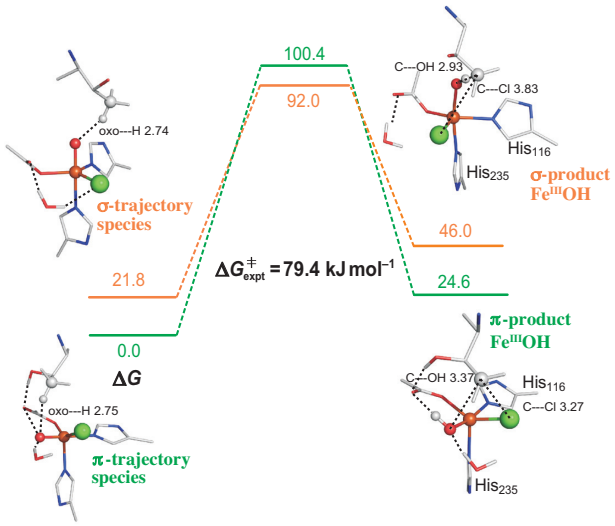

Fig. 3. Computational evaluation of the SyrB2 H-atom abstraction reaction coordinates for the π-trajectory (green text and energy levels) and the σ-trajectory (orange text and energy levels). Reactant energies are on the left, transition state energies are in the center, and product energies are on the right. The π-trajectory has a higher barrier than the σ-trajectory, consistent with experiment [2], with the π product well-oriented for halogenation while the σ product is oriented for rebound hydroxylation. [3]

This performs an H-atom abstraction using the σ-FMO of the Fe^{IV}=O that is oriented along the Fe-O bond (Fig. 3). This calculated reactivity was found to have a lower barrier than the π-pathway reactivity, consistent with experiment. For the σ attack, the resultant Fe^{III}-OH species has its OH^- group, rather than its Cl^-, aligned toward the substrate radical, thus favoring OH• over Cl• rebound, consistent with the observed hydroxylation reactivity for L-Nva (Fig. 1).

Thus, our combined NRVS and DFT methodology allowed the first elucidation of the structure of an Fe^{IV}=O intermediate in a NHFe enzyme and provided insight into the orientation dependence of the FMOs that govern halogenation *versus* hydroxylation reactivity in this class of enzymes.

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