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, biomediadation, 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-FeIV=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 FeIV=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 FeIV=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 FeIV=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 57Fe 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.

The SyrB2 FeIV=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 ~C3 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 O2 activation reaction coordinate calculation we developed for αKG-dependent NHFe enzymes in Ref. [5] to define the structure of the FeIV=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 NHFeIV=O enzyme intermediates have an S=2 ground state, and we have shown that an S=2 FeIV=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...
with the experimental value (Fig. 3) [3]. The resulting Cl-FeIIIOH 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 O₂ 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. This performs an H-atom abstraction using the σ-FMO of the FeIV=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 FeIIIOH 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 FeIV=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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