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Non-Heme Fe(IV)-Oxo Intermediates

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Abstract

High-valent non-heme iron-oxo intermediates have been proposed for decades as the key intermediates in numerous biological oxidation reactions. In the last three years, the first direct characterization of such intermediates has been provided by studies of several α KG-dependent oxygenases that catalyze either hydroxylation or halogenation of their substrates. In each case, the Fe(IV)-oxo intermediate is implicated in cleavage of the aliphatic C-H bond to initiate hydroxylation or halogenation. The observation of non-heme Fe(IV)-oxo intermediates and Fe(II)-containing product(s) complexes with almost identical spectroscopic parameters in the reactions of two distantly related α KG-dependent hydroxylases suggests that members of this sub-family follow a conserved mechanism for substrate hydroxylation. In contrast, for the α KG-dependent non-heme-iron halogenase, CytC3, two distinct Fe(IV) complexes form and decay together, suggesting that they are in rapid equilibrium. The existence of two distinct conformers of the Fe site may be the key factor accounting for the divergence of the halogenase reaction from the more usual hydroxylation pathway after C-H cleavage. Distinct transformations catalyzed by other mononuclear non-heme enzymes are likely also to involve initial C-H-cleavage by Fe(IV)-oxo complexes, followed by diverging reactivities of the resulting Fe(III)-hydroxo/substrate radical intermediates.

Keywords

oxygen activation; non-heme iron enzymes; Fe(IV)-oxo intermediates; C-H activation

Introduction

A large, functionally and mechanistically diverse family of enzymes utilize similar, mononuclear non-heme Fe(II) centers to couple the activation of oxygen to the oxidation of their substrates.¹⁻³ In most cases, oxygen is inserted into an unactivated C-H bond of the substrate (hydroxylation), but many other outcomes, including halogenation, desaturation, cyclization, epoxidation, and decarboxylation, are known.^{3,4} Each of these reactions is a

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two-electron oxidation. The remaining two reducing equivalents required for the four-electron reduction of oxygen are often provided by a co-substrate. The reducing co-substrates used by various family members include α -ketoglutarate (in the α KG-dependent enzymes³), tetrahydrobiopterin (in the pterin-dependent aromatic amino acid hydroxylases⁵), reduced nicotinamides (in the Rieske dioxygenases and (*S*)-2-hydroxypropylphosphonic acid epoxidase¹), and ascorbic acid (in 1-aminocyclopropane 1-carboxylic acid oxidase⁶). A few of the enzymes oxidize their substrates by four electrons and thus do not require a reducing co-substrate. This subset includes the extradiol dioxygenases,¹ isopenicillin *N* synthase (IPNS),⁷ and two enzymes, 4-hydroxymandelate synthase and (4-hydroxyphenyl)pyruvate dioxygenase (HPPD), which effect distinct four-electron oxidations of their common substrate.⁸ The latter two reactions are mechanistically similar to those catalyzed by the α KG-dependent enzymes, because both involve oxidative decarboxylation of an α -keto-acid moiety to provide two electrons.

This remarkable array of oxidative transformations is made possible by the tuning of a largely conserved mononuclear non-heme-iron cofactor unit, which is coordinated by as few as two protein ligands and thus has as many as four sites available to coordinate substrates. In the most common coordination sphere, three protein ligands of a (His)₂-(Asp/Glu) motif, known as the “facial triad” because they occupy one face of an octahedron, leave three remaining sites on the opposite face for substrate coordination.⁹ Reaction mechanisms proposed for these enzymes have invoked several intermediates following the addition of oxygen to the Fe(II) center.^{1,2} Two types of intermediates have been proposed: Fe-coordinated (su)peroxo complexes with an intact O-O bond, [Fe-O₂]^{2+/3+}, and high-valent Fe(IV)-oxo intermediates (or even Fe(V)-oxo for the Rieske dioxygenases), [Fe=O]^{4+/5+}, that occur after cleavage of the O-O bond. In particular, the high-valent Fe-oxo intermediates have been suggested to initiate substrate oxidation. In most cases, activation of the substrate involves abstraction of the H-atom of the target C-H bond by the Fe(IV)-oxo intermediate to yield a substrate radical and a Fe(III)-OH complex (Scheme 1). The so-called oxygen rebound, which was originally proposed for heme enzymes¹⁰ and formally involves recombination of a coordinated hydroxyl radical equivalent with the substrate radical, yields the hydroxylated product and a coordinatively unsaturated Fe(II) center. In addition to substrate hydroxylation, many other outcomes following H-atom abstraction by the Fe(IV)-oxo are documented. These include transfer (formally as the radical) of a ligand of the Fe-center to the substrate radical. Examples include transfer of a halogen atom in the α KG-dependent halogenases⁴ and transfer of a thiol group in IPNS.⁷

Alternative reactivities that do not involve radical recombination with a ligand include desaturation and cyclization of the substrate. Formally, these reactions involve abstraction of a second H-atom by the Fe(III)-OH complex to yield the desaturated or cyclized product and a Fe(II)-OH₂ complex. However, other pathways are possible, making delineation of the mechanisms of these alternative outcomes a high priority for future studies.

Other substrate oxidations by high-valent Fe-oxo intermediates that do not involve H-atom abstraction include electrophilic attack on the aromatic ring of the substrate by the pterin-dependent hydroxylases,⁵ and *cis*-dihydroxylation of an aromatic substrate by the Rieske dioxygenases.^{1,2}

Significant insight into the geometric and electronic structures of high-valent non-heme Fe-oxo complexes and their reactivity was obtained in parallel from elegant studies of inorganic complexes^{11–15} (see ¹ for a recent review), but these studies will not be reviewed here due to the brevity of this article.

The first non-heme Fe(IV)-oxo intermediate

A powerful approach to study the mechanism of a metalloenzyme-catalyzed reaction is the direct detection of intermediates and their detailed characterization by a combination of kinetic and spectroscopic methods. By this approach, one monitors changes of the geometric and/or electronic structure of the metal center during the reaction. This methodology had been used successfully in the 1990s to study O₂ activation by the non-heme diiron proteins methane monooxygenase and the R2 subunit of class I ribonucleotide reductase, but was only recently applied to the mononuclear non-heme-iron enzymes. The first direct detection of an intermediate in the reaction of a mononuclear non-heme iron enzyme with dioxygen was reported for HPPD.¹⁶ A transient absorption feature at 490 nm that forms with a second-order rate constant of 140 mM⁻¹ s⁻¹ and decays with a first-order rate-constant of 7.8 s⁻¹ was noted. More detailed spectroscopic characterization of the associated intermediate has not yet been reported. Shortly after this work, we reported the detection and characterization of two transient states in the reaction of taurine:αKG dioxygenase (TauD). The αKG-dependent oxygenases are the largest and functionally most diverse subgroup of mononuclear non-heme-iron enzymes.³ They catalyze many important reactions, including steps in the biosyntheses of antibiotics¹⁷ and collagen,¹⁸ the sensing of oxygen,^{19–23} the repair of alkylated DNA,^{24,25} and the regulation of transcription by demethylation of histones.^{26–28} A chemical mechanism was initially proposed more than 20 years ago by Hanauske-Abel and Günzler specifically for the enzyme prolyl-4-hydroxylase (P4H),²⁹ but its success in accommodating ensuing experimental data for many other αKG-dependent hydroxylases led to its becoming adopted as a consensus mechanism for the sub-family (Scheme 2).^{1–3,30}

Two iron-based intermediates were detected in TauD by stopped-flow (SF) absorption and freeze-quench (FQ) Mössbauer spectroscopies. The first intermediate, termed **J**, forms with second-order kinetics (first order in [O₂] and [enzyme]; $k = 130 \text{ mM}^{-1} \text{ s}^{-1}$). It is characterized by an absorption feature maximizing at ~318 nm and a new Mössbauer quadrupole doublet with unusual parameters (isomer shift, δ , of 0.30 mm/s and quadrupole splitting, ΔE_Q , of -0.88 mm/s).³¹ It has a nearly axial $S = 2$ ground state with a positive zero-field splitting parameter, D , of 10.5 cm⁻¹.^{31,32} The large substrate deuterium kinetic isotope effect (²H-KIE) on decay of **J** ($k_H/k_D \approx 50$) implied that it is the hydrogen-abstracting intermediate,^{33,34} which the Hanauske-Abel and Günzler mechanism predicted to be an Fe(IV)-oxo complex.^{1,2,29} The presence of the Fe(IV)-oxo group in **J** was confirmed by resonance Raman spectroscopy, which revealed a band at 821 cm⁻¹ that shifted to 787 cm⁻¹ upon use of ¹⁸O₂,³⁵ and X-ray absorption spectroscopy, which demonstrated a short (1.62 Å) interaction between the Fe and one of its ligands.³⁶ A recent comparison of experimentally determined spectroscopic parameters to those predicted by DFT calculations for several model structures suggested that **J** has either a trigonal bipyramidal or octahedral coordination environment.³⁷ The second accumulating state is an Fe(II)-containing TauD•product(s) complex (**V** in Scheme 2).³⁸

A consensus mechanism for the αKG-dependent dioxygenases

The TauD work proved (1) that a non-heme Fe(IV)-oxo complex could be trapped and characterized despite its anticipated high reactivity, and (2) that the lifetime of the Fe(IV)-oxo intermediate could be extended to a remarkable degree by deuterium substitution of the target C-H bond, due to the large ²H-KIE. We next applied this insight to a prolyl-4-hydroxylase (P4H), because the hydroxylation of proline residues has high biological significance (e.g., in collagen biosynthesis and oxygen sensing). The monomeric P4H from *Paramecium bursaria Chlorella virus 1*, which was known to modify peptide substrates containing a (Pro-Ala-Pro-Lys)_n-motif, was selected.³⁹ The combined stopped-flow absorption and freeze-quench Mössbauer data for the reaction of the P4H•Fe(II)•αKG•(Pro-

Ala-Pro-Lys)₃ complex with O₂ demonstrated the accumulation of two kinetically competent intermediates.⁴⁰ The first intermediate is a high-spin Fe(IV) complex, which exhibits a large substrate ²H-KIE on its decay ($k_H/k_D = \approx 210/3.4 \approx 60$), suggesting that it abstracts hydrogen from the substrate. The second accumulating state contains a high-spin Fe(II) center and is presumably an Fe(II)•product(s) complex. The spectroscopic properties of these two intermediates are strikingly similar to those of the TauD intermediates (Figure 1), suggesting that the distantly related αKG-dependent dioxygenases employ the same chemical mechanism and supporting the prevailing view of a conserved mechanism for the hydroxylase sub-family.

Alternative reactivities of αKG-dependent dioxygenases

Studies by Stubbe and co-workers on thymine hydroxylase shed light on alternative reactivities that can occur in the Fe(II)/αKG reaction manifold. This enzyme catalyzes three oxidations of the methyl group of thymine to the hydroxymethyl, aldehyde, and carboxylic acid in three separate O₂ activation events, of which each involves cleavage of a C-H bond (Scheme 3A). The authors demonstrated that the enzyme can catalyze epoxidation of an olefin, conversion of an alkyne to a ketene, and successive *S*-oxidations of a thioether to sulfoxide and then sulfone (Scheme 3B).^{41–43} These substrates lack C-H bonds at the position that would normally be targeted by the Fe(IV)-oxo complex, and the alternative transformations that ensue parallel those previously seen for high-valent heme-iron enzyme intermediates and inorganic model complexes. It is therefore tempting to speculate that the alternative oxidations are also effected by the Fe(IV)-oxo intermediate, but it is still conceivable that fundamentally different mechanisms might be operant (e.g., interception of an earlier intermediate, such as an Fe(III)-superoxide complex like **I** in Scheme 2).

αKG-dependent Halogenases

The recent discovery of a new class of halogenating enzymes that carry out chlorination of unactivated carbon centers in the biosyntheses of several natural products of non-ribosomal peptide origin established yet another reactivity for the Fe(II)/αKG dependent oxygenases.^{44–47} Aliphatic halogenases chlorinate the terminal methyl groups of amino acids tethered via thioester linkage to the phosphopantetheine cofactor of peptidyl-carrier proteins (PCPs). They require iron, αKG, oxygen, and chloride for their activity. Initial insight into their catalytic mechanism was derived from the crystal structure of SyrB2, which chlorinates the γ-methyl group of L-threonine in syringomycin biosynthesis.⁴⁸ The Fe center is coordinated by two protein-derived histidines, bidentate αKG, water, and chloride. The carboxylate of the “facial triad” that normally coordinates the Fe(II) center is replaced by an alanine in the protein primary structure, presenting a coordination site for the chloride ligand. Based on this observation, a Cl-Fe(IV)-oxo intermediate was proposed as the C-H-cleaving intermediates, and chlorination was proposed to proceed via “chlorine rebound” rather than “oxygen rebound” (Scheme 4). This hypothesis was tested experimentally for the non-heme-iron halogenase, CytC3 from soil *Streptomyces*.⁴⁹ CytC3 chlorinates the γ-methyl group of L-2-aminobutyric acid (L-Aba) tethered to the PCP-domain CytC2, L-Aba-*S*-CytC2 (Scheme 5) in the biosynthesis of cytostreptin.⁴⁷ Evidence for accumulation of two transient states of the catalytic cycle was obtained in the reaction of the CytC3•Fe(II)•αKG•Cl[−]•L-Aba-*S*-CytC2 complex with oxygen-saturated buffer. The first intermediate absorbs at 318 nm. Decay of A₃₁₈ is markedly slowed by use of deuterated substrate, L-4,4,4-*d*₃-Aba-*S*-CytC2, demonstrating a ²H-KIE on decay of the intermediate and implicating it as the C-H-cleaving complex. Mössbauer spectra revealed the presence of two high-spin Fe(IV) intermediates. Their proportions are constant with reaction time, suggesting that they are in rapid equilibrium. These results contrast with the αKG-dependent hydroxylases, for which the C-H-cleaving state comprises only one high-spin Fe(IV)-oxo complex. We speculated that the two intermediates are distinct conformers of the Cl-Fe(IV)-

oxo complex and that the occurrence of two Fe(IV) complexes prior to C-H cleavage may also indicate the presence of two Fe(III)-OH complexes after H-atom abstraction. The presence of two conformers might be the key factor allowing halogenation in preference to the conventional hydroxylation outcome. Ongoing efforts are directed towards (i) elucidating the structures of the two conformers by employing other spectroscopic methods (e.g. X-ray absorption, resonance Raman, magnetic circular dichroism) and (ii) defining the factors that affect the ratio of the two Fe(IV) complexes by perturbing the reaction conditions or by using modified substrates.

Exclusive halogenation (rather than hydroxylation) was also observed in an inorganic non-heme iron complex, in which the Fe center is coordinated to chloride and hydroxide.⁵⁰ The reaction is thought to proceed via hydrogen atom abstraction followed by rebound of the coordinated ligand. It was proposed that the preference for chlorination reflects the lower reduction potential of chlorine radical ($\text{Cl}^\bullet + \text{e}^- \rightarrow \text{Cl}^-$, 1.36 V) relative to hydroxyl radical ($\text{HO}^\bullet + \text{e}^- \rightarrow \text{HO}^-$, 2.02 V).⁵⁰

The second intermediate observed during the reaction of the CytC3 halogenase is an Fe(II) complex that is spectroscopically distinct from the reactant. In analogy to the hydroxylases, it is presumably a product(s) complex. The accumulation of two intermediate states in the CytC3 system, the C-H-cleaving Fe(IV)-oxo and the Fe(II)-product(s) complex, underscores the mechanistic similarity of the α KG-dependent hydroxylases and halogenases and suggests that halogenation activity may have evolved from hydroxylation by iron ligand replacement (among other less apparent adaptations). Figure 1 further emphasizes these striking similarities by showing the kinetics of formation and decay of the Fe(IV)-oxo complexes in TauD, P4H and CytC3 with both protium- and deuterium-containing substrates (left panel) and their Mössbauer spectra (center and right panels).

Mechanistic Diversity of Presumptive Fe(IV)-Oxo Intermediates in β -Lactam Biosyntheses

Nature has exploited the versatility of mononuclear non-heme iron enzymes in the biosynthetic pathways of a wide variety of β -lactam antibiotics.¹⁷ Isopenicillin *N* synthase (IPNS) is one of the more well-understood mononuclear non-heme-iron enzyme due to the extensive studies by Baldwin and co-workers. IPNS catalyzes the four electron oxidation of δ -(L- α -amino adipoyl)-L-cysteinyl-D-valine (ACV) tripeptide to isopenicillin *N*.⁵¹ Elegant biochemical and crystallographic studies provided convincing evidence that the reaction proceeds via two successive two-electron oxidations (Scheme 6).^{7,52} The first oxidation is thought to involve cleavage of the $\text{C}_{\text{Cys},\beta}$ -H bond by a formally Fe(III)-superoxo intermediate, followed by formation of the β -lactam ring, possibly by attack of the valine amidate on the thioaldehyde. This view is supported by several lines of evidence. First, the crystal structure of the IPNS•Fe(II)•ACV complex with NO as surrogate for oxygen revealed the O-atom of NO to be in close proximity to the target $\text{C}_{\text{Cys},\beta}$ -H bond.⁵³ Second, with the substrate analogue δ -(L- α -amino adipoyl)-L-cysteinyl-D- α -hydroxyvaleryl ester (Scheme 7A), $\text{C}_{\text{Cys},\beta}$ -H-cleavage but not formation of the β -lactam ring was observed.⁵⁴ Third, with ACV containing C_β -deuterated cysteine as the substrate, a kinetic isotope effect on $k_{\text{cat}}/K_{\text{M}}$ (selection effect) of 1.4 was measured.⁵²

The second oxidation is proposed to involve $\text{C}_{\text{Val},\beta}$ -H-cleavage by an Fe(IV)-oxo intermediate. Recombination of the resultant substrate radical with the coordinated sulfur (formally as the thiyl radical) results in formation of the thiazolidine ring of the substrate (Scheme 6). This sequence of events, i.e. cleavage of the $\text{C}_{\text{Val},\beta}$ -H bond by the Fe(IV)-oxo intermediate followed by transfer of a coordinated ligand other than the hydroxide, is analogous to that envisaged for the halogenases.

Although none of the proposed intermediates has been directly detected, there is convincing evidence for the intermediacy of a β -valinyl radical from studies of substrate analogues (Scheme 7). For example, formation of the [6.2.0] (Scheme 7B) ring system in the reaction of IPNS with the cyclopropane-containing substrate provides evidence in support of generation of a cyclopropylcarbonyl radical, lending credence to the proposed H-atom abstraction by the Fe(IV)-oxo intermediate.⁵⁵

Substrate analogues in which the D-valine residue is modified provided further insight into the reactivity of the presumptive Fe(IV)-oxo intermediate in IPNS. Of particular interest are conversion of the thioether of the D-S-methylcysteine-containing analogue to the sulfone,⁵⁶ and oxidation of the olefinic D-allylglycine- and D-vinylglycine-containing substrates to yield desaturated and oxygenated bicyclic products (Scheme 7C–E).^{57,58} Isotopic labeling studies demonstrated that the O-atom incorporated into the hydroxylated bicyclo-[5.2.0]-product of the D-allylglycine-containing substrate is derived from dioxygen (Scheme 7D).⁵⁹

Clavaminate synthase (CAS) from *Streptomyces clavuligerus* performs three two-electron oxidation reactions in the biosynthesis of clavulanic acid: hydroxylation, oxidative ring closure and dehydrogenation (Scheme 8).^{60–65} The hydroxylation reaction is believed to proceed via the canonical mechanism involving H-atom abstraction by the Fe(IV)-oxo intermediate, followed by hydroxyl radical rebound. It has been proposed that a different mode of reactivity of the Fe(III)-OH/substrate radical state yields the second and third reactions.^{17,66} For the dehydrogenation reaction, the Fe(III)-OH intermediate could abstract a second H-atom from the substrate radical, yielding a hexacoordinate Fe(II)-OH₂ complex and the olefinic product.⁶⁷ Similarly, the cyclization reaction could proceed by successive H-atom abstractions by the Fe(IV)-oxo and Fe(III)-OH intermediates (the latter from the hydroxyl group of the substrate) and a radical coupling step.¹⁷ It has been proposed that subtleties in the structures of the CAS•intermediate complexes favor the proposed second H-abstraction steps over other possible pathways for decay of the Fe(III)-OH/substrate radical states (e.g., hydroxyl radical rebound).

Deacetoxycephalosporin C synthase (DAOCS) from *Streptomyces clavuligerus* is an Fe(II)-containing α KG-dependant oxidase that catalyses expansion of the 5-membered thiazolidine ring of penicillin N to the 6-membered dihydrothiazine ring of cephalosporins (Scheme 9).⁶⁸ It is believed that ring expansion is initiated by abstraction of hydrogen from the C2-methyl substituent by a ferryl species. This primary alkyl substrate radical then converts to the more stable tertiary radical in the ring expansion. The intermediacy of an episulfide species, with radical character localized on the sulfur, was proposed for this transformation.⁶⁹ Subsequent abstraction of the second hydrogen from C3 of the substrate, perhaps carried out by an Fe(III)-OH species, results in the formation of the endocyclic double bond and generation of the cephalosporin nucleus.^{70–73} The delicate balance of desaturation and hydroxylation pathways observed in CAS can also be observed in DAOCS upon use of the [3-²H] penicillin N substrate, in which the hydrogen target of the second abstraction is substituted by deuterium.⁷³ In this case, hydroxylation of the six-member ring occurs, presumably by recombination of the more stable tertiary radical with the hydroxyl radical from the Fe(III)-OH intermediate.

Carbapenem synthase (CarC) from *Pectobacterium carotovorum* is a bifunctional α KG-dependent enzyme that epimerizes the unactivated C5 position of its (3*S*, 5*S*)-carbapenam-3-carboxylate substrate and installs a double bond between C2 and C3, resulting in the formation of the carbapenem binucleus (Scheme 10).¹⁷ Isotopic labeling studies by Townsend and coworkers showed that the C5-bound hydrogen is exchanged during the CarC-catalyzed epimerization.⁷⁴ The epimerization consumes α KG, despite the fact that the substrate is not oxidized in the transformation.⁷⁵ The resulting (3*S*, 5*R*)-carbapenam-3-

carboxylate is converted to (5*R*)-carbapenam-3-carboxylate by the same enzyme.⁷⁵ Computational studies suggested that the reaction is initiated by abstraction of the C5-hydrogen atom, presumably by the Fe(IV)-oxo intermediate generated by decarboxylation of α KG.⁷⁶ The Fe(III)-OH intermediate may serve as the source of the hydrogen atom to form the (3*S*, 5*R*)-carbapenam-3-carboxylate intermediate and regenerate the Fe(IV)-oxo complex.¹⁷ The Fe(IV)-oxo intermediate may then abstract the H-atom from C3, forming a stabilized, captodative C3 radical. Subsequent desaturation across the C2-C3 linkage via H-atom abstraction from C2 by the Fe(III)-OH species would be directly analogous to the mechanisms proposed for DAOCS and CAS.¹⁷

Summary and Outlook

The first examples of non-heme Fe(IV)-oxo enzyme intermediates have recently been detected in several α KG-dependent oxygenases. In each case, a large ²H-KIE on decay of the intermediate has established that it is the C-H-cleaving complex. The spectroscopic parameters of the Fe(IV)-oxo complexes observed in the hydroxylases TauD and P4H are almost identical, suggesting a conserved mechanism for substrate hydroxylation. The presence of two Fe(IV)-oxo complexes, which are apparently in rapid equilibrium and are presumably different conformers, in CytC3 may be relevant to the divergent reactivity of the halogenases. Ongoing efforts are aimed at elucidating the molecular structure of the Fe(IV) complexes by a combination of spectroscopic and computational methods. We anticipate that further insight into the factors that determine the outcome of the oxidations catalyzed by high-valent Fe-oxo intermediates may be obtained from studies of other mononuclear non-heme enzymes (e.g. enzymes involved in the biosyntheses of β -lactam antibiotics) for which alternative reaction pathways have been proposed.

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ABBREVIATIONS

TauD	taurine: α -ketoglutarate dioxygenase
P4H	prolyl-4-hydroxylase
TH	thymine hydroxylase
IPNS	isopenicillin N synthase
CAS	clavamate synthase
DAOCS	deacetoxy cephalosporin C synthase
CarC	carbapenam synthase
SF	stopped-flow
FQ	freeze-quench
²H-KIE	deuterium kinetic isotope effect
αKG	α -ketoglutarate
L-Aba	L-2-aminobutyric acid

NRPS	non-ribosomal peptide synthetase
HPPD	(4-hydroxyphenyl)pyruvate dioxygenase
AA	δ -(L- α -aminoadipoyl)
PAH	proclavamate amidino hydrolase

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Figure 1. Comparison of the spectroscopic features of the Fe(IV)-oxo intermediates from TauD (top), P4H (middle), and CytC3 (bottom) **A:** Comparison of the kinetics of the Fe(IV)-oxo intermediates monitored by SF-absorption spectroscopy using unlabeled (red) and selectively deuterated substrates (blue). **B:** 4.2-K/53-mT (left) and 4.2-K/8-T (right) Mössbauer spectra of the Fe(IV)-oxo intermediates.

Scheme 1.

Reactions proposed to be mediated by high-valent Fe-oxo intermediates

Scheme 2. General mechanism of α KG-dependent dioxygenases

Scheme 3. Reactions catalyzed by the α KG-dependent hydroxylase TH

Scheme 4.
Hydroxylation vs halogenation rebound reactions

Scheme 5.
Reaction catalyzed by the halogenase CytC3

Scheme 6.
Proposed mechanism of IPNS

Scheme 7.

Alternative reactions of the proposed Fe(IV)-oxo intermediate in IPNS

Scheme 8.
Reaction catalyzed by CAS

Scheme 9.
Reaction catalyzed by DAOCS

Scheme 10.
Reaction catalyzed by CarC