Mononuclear non-heme Fe(II)- and 2-oxoglutarate (2OG)-dependent oxygenases comprise a large family of enzymes that utilize an Fe(IV)-oxo intermediate to initiate diverse oxidative transformations with important biological roles. Here, four of the major types of Fe(II)/2OG-dependent reactions are detailed: hydroxylation, halogenation, ring formation, and desaturation. In addition, an atypical epimerization reaction is described. Studies identifying several key intermediates in catalysis are concisely summarized, and the proposed mechanisms are explained. In addition, a variety of other transformations catalyzed by selected family members are briefly described to further highlight the chemical versatility of these enzymes.

The activation of molecular oxygen for incorporation into organic substrates requires the reaction of O₂ in its triplet spin state with an organic substrate in a singlet spin state. Thermodynamically, this reaction is favorable, but it is kinetically slow because of the spin mismatch. To overcome the kinetic barrier for incorporating oxygen into a biological substrate, organisms utilize enzymes with transition metal centers (typically iron or copper) (1–5) or organic cofactors such as flavins (6). One extensive family of enzymes that activates O₂ for incorporation into a diverse range of primary substrates utilizes mononuclear non-heme iron and the co-substrate 2-oxoglutarate (2OG, also known as α-ketoglutarate) to form the desired product(s), succinate, and CO₂ (7–9). These Fe(II)/2OG-dependent oxygenases are widely distributed throughout the kingdoms of life, with more than 60 such enzymes in humans alone (10). The Fe(II)/2OG-dependent oxygenases catalyze a variety of oxidative transformations including hydroxylation, halogenation, ring closure, desaturation, epimerization, ring expansion, and epoxidation reactions. Because of their rich oxidative transformation capabilities, these enzymes play important roles in many biological processes (9) including the post-translational modification of collagen (11), fatty acid metabolism (12, 13), oxygen sensing (14, 15), DNA and RNA repair (16, 17), demethylation reactions related to epigenetic regulation (18, 19), and the biosynthesis of many antibiotics and other secondary metabolites (20, 21).

Crystallographic studies on numerous members of the Fe(II)/2OG-dependent oxygenase superfamily have revealed two conserved structural features shared among its members (22). First, the Fe(II) is ligated by two His residues and (with the exception of the halogenases) a carboxylate from either a Glu or an Asp residue; this metal-binding motif is termed the 2-His-1-carboxylate facial triad (23). Second, the 2-His-1-carboxylate motif is located within a double-stranded β-helix (DSBH) fold, also known as the jelly-roll, cupin, or jumonji C fold (24). The jelly roll is composed of eight antiparallel β-strands forming a β-sandwich structure made up of two four-stranded antiparallel β-sheets (24, 25). It has been proposed that the β-strands from the DSBH fold assist in providing selectivity for binding the primary substrate in addition to supporting the active site (24).

Because these enzymes catalyze a wide array of oxidative transformations that play critical roles in biochemical processes, the Fe(II)/2OG-dependent oxygenases have been subjected to extensive mechanistic studies. Here, we focus on experimental results obtained with a few selected representatives of this enzyme family (Fig. 1) to highlight the range of reaction mechanisms they catalyze. The archetype hydroxylase is taurine:2OG dioxygenase (also known as taurine hydroxylase or TauD) that converts taurine (2-aminoethanesulfonic acid) to an intermediate that spontaneously decomposes to aminoacetohydroxamate (26, 27). Chlorination is illustrated by SyrB2, acting on L-Thr that is covalently tethered via thioester linkage to a phosphopantetheine group on SyrB1 (28). Clavamine synthase (CAS) is the paradigm for cyclization chemistry, transforming proclavaminic acid to dihydroclavaminic acid, but this trifunctional enzyme also catalyzes the hydroxylation of deoxyxuanidinoproclavaminic acid and desaturation of dihydroclavaminic acid (20). Carbapenem synthase (CarC) is used to describe both desaturation and epimerization reactions (29, 30). Ring expansion by deacetoxycephalosporin C synthase (DAOCS) (31, 32), epoxidation by hyoscyamine 6β-hydroxylase (H6H) (33), and endoperoxide formation by FtmOx1 acting on fumitremorgin B (34) are also briefly described.

Hydroxylation: The Consensus Mechanism

The most well studied and established reactivity of the Fe(II)/2OG oxygenases is substrate hydroxylation, typically at an unactivated carbon center. This chemistry is used to modify selected structural proteins, derivatize proteins involved in the hypoxic response, repair alkylation damage to DNA, remove methylation marks involved in epigenetic regulation, degrade environmental substances, and synthesize a wide variety of cellular metabolites (9). In cases where the carbon undergoing hydroxylation is bound to a heteroatom, the initially formed product may spontaneously degrade further as illustrated below and by the reactions of demethylases.
FIGURE 1. Examples of the diverse reactions catalyzed by Fe(II)/2OG oxygenases. The four major types of oxidative reactions are highlighted using representative enzymes as emphasized in the text, with several other types of Fe(II)/2OG-dependent chemistry only briefly discussed. A, the reaction of TauD (green highlight), a representative hydroxylase; the initial product of taurine hydroxylation is unstable and spontaneously decomposes as shown. B, the reaction catalyzed by SyrB2 (gray highlight), a model halogenase. The primary substrate of this enzyme is L-Thr in thioester linkage to a phosphopantetheine group bound to the SyrB1 protein. C, the trifunctional enzyme CAS; in addition to its oxidative ring-forming reaction (highlighted in blue), this enzyme also catalyzes hydroxylation and desaturation reactions. D, reactions of CarC, illustrating both desaturation (highlighted in pink) and epimerization (highlighted in purple). E, ring expansion reaction catalyzed by DAOCS. R = 8-(L-α-aminopropyl). F, sequential hydroxylation and epoxidation reactions of H6H. G, formation of an endoperoxide by FtmOx1. See text for definition of the abbreviations.
In a remarkably prescient proposal published in 1982, Hanauske-Abel and Günzler (35) theorized a mechanism involving a metallocenter bound on one face by three protein side chains and used to form a high valent iron:oxygen atom intermediate. Since then a wealth of experimental evidence has allowed refinement of this model, yielding the consensus mechanism shown by the green cycle in Fig. 2. Catalysis initiates with Fe(II) coordinated to the 2-His-1-carboxylate facial triad (23), with three additional coordination sites occupied by water molecules (Fig. 2A). 2OG binds to the Fe(II) center in a bidentate
configuration, with its keto group opposite the Asp/Glu and its carboxylate opposite one of the His residues, thus displacing two metal-bound water molecules (Fig. 2A). Upon binding of the primary substrate to the enzyme active site (not, however, to the metal ion), the third metal-bound water is displaced (Fig. 2C). This substrate-triggered process creates a site for binding an O₂ molecule, generating an Fe(III)-peroxo intermediate (Fig. 2D). The distal oxygen atom of the Fe(III)-peroxo species attacks C2 of 2OG to yield a peroxohemiketal bicyclic intermediate (Fig. 2E). This species initiates the oxidative decarboxylation of 2OG to release CO₂ and yield, perhaps via an Fe(II)-peracid intermediate (36) (not shown), an Fe(IV)-oxo species (known as the ferryl intermediate) with bound succinate (Fig. 2F). The ferryl species abstracts a hydrogen atom from the primary substrate to generate Fe(III)-OH and a substrate radical (Fig. 2G). In the conventional mechanism, hydroxyl radical rebound yields the hydroxylated product (Fig. 2H), and this product dissociates to complete the catalytic cycle (7, 37, 38). An alternative pathway (the yellow arc of Fig. 2) depicts deprotonation of the Fe(III)-OH to yield an Fe(III)-oxo intermediate (Fig. 2I), followed by the formation of an Fe(II)-alkoxo (Fig. 2J) and dissociation to products (39).

An extensive set of structural and spectroscopic studies provides experimental support for the identity of most key intermediates in the hydroxylase mechanism proposed above. Crystal structures directly reveal the facial triads and Fe(II) coordination environments of various family members in the states corresponding to those shown in Fig. 2, A–C. For example, the structure of anaerobic Escherichia coli TauD with 2OG and taurine exhibits the three amino acid ligands, the bidentate binding of 2OG, and an open Fe(II) coordination site facing C1 of the sulfonate substrate (40). The UV-visible spectrum of TauD is colorless, whereas the enzyme with bound 2OG exhibits a metal-to-ligand charge-transfer band at 530 nm (τ = 0.30 mm s⁻¹) and taurine exhibits the three amino acid ligands, the bidentate binding of 2OG, and an open Fe(II) coordination site facing C1 of the sulfonate substrate (40). The stopped-flow UV-visible studies of TauD are consistent with rapid conversion of the ferryl species to a high-spin Fe(II) intermediate identified as the Fe(II)-product complex (44). In contrast to the ferryl intermediate, this complex shows no distinct absorption in its spectrum. Comparison of the rate constant for formation of this species with the steady-state kinetics parameters for TauD demonstrates that the release of product is the rate-determining step of the reaction (44).

An extended version of the catalytic cycle was proposed on the basis of time-resolved, cryogenic, continuous-flow resonance Raman spectroscopic studies of TauD (39). That work resulted in the tentative identification of two additional intermediates following the ferryl intermediate. One species exhibits an ¹⁸O/¹⁸O vibrational mode at 578/555 cm⁻¹ and is assigned to an Fe(III)-oxo species, requiring transfer of the Fe(III)-OH proton to a nearby base (39). This ferric species, in turn, is followed by one giving rise to a ¹⁸O/¹⁸O vibrational mode at 815/787 cm⁻¹ and provisionally assigned to an Fe(II)-alkoxo species (39). These two species have not been observed by others studying TauD, raising the possibility that they may only be detected at ~−35 °C when using cryosolvent. Experimental studies with other members of the enzyme family have thus far not provided evidence for these species; notably, however, computational studies of the 2OG-dependent DNA repair enzyme AlkB are compatible with the formation of such intermediates (49).

**Halogenation**

A subset of the Fe(II)/2OG-dependent oxygenase family is able to install a halogen atom, Cl⁻ or Br⁻, on unactivated carbon centers. These enzymes are found in the biosynthetic pathways of various natural products possessing antifungal, antimicrobial, anticancer, antiviral, or anti-inflammatory properties (50–52). Most, but not all (53), of these enzymes halogenate substrates that are tethered to a phosphopantetheine moiety in the thiolation domain of a carrier protein (28, 54–57). One example, the first of the Fe(II)/2OG halogenases to be reported, is SyrR2 from Pseudomonas syringae B301D, which chlorinates l-Thr tethered to SyrR1 (Fig. 1B). Sequence analyses and structural studies reveal a significant difference in the active sites of similar Mössbauer spectroscopy studies of prolyl-4-hydroxylase also indicated the formation of a high-spin intermediate (45). Fe K-edge extended x-ray absorption fine structure spectroscopy reveals an Fe-O distance of 1.62 Å, consistent with Fe(IV)-oxo scattering (46). Furthermore, cryogenic continuous-flow resonance Raman spectroscopy shows an Fe-O stretching mode at 825 cm⁻¹ when using unlabeled O₂ and at 788 cm⁻¹ when using labeled ¹⁸O₂, again indicating a ferryl species (47). Evidence that the ferryl intermediate is responsible for the cleavage of the unactivated C-H bond by abstraction of the hydrogen atom came from single turnover stopped-flow and rapid freeze-quench Mössbauer studies using ²H-labeled taurine substrate (48). Use of the ²H-labeled taurine substrate resulted in a large kinetic isotope effect of ~50, demonstrating that the ferryl intermediate is responsible for the H-atom abstraction from C1 of the substrate (48). Similarly, a large ²H kinetic isotope effect of ~60 was measured for prolyl-4-hydroxylase when using a ²H-substituted substrate (45). The stopped-flow UV-visible studies of TauD are consistent with rapid conversion of the ferryl species to a high-spin Fe(II) intermediate identified as the Fe(II)-product complex (44). In contrast to the ferryl intermediate, this complex shows no distinct absorption in its spectrum. Comparison of the rate constant for formation of this species with the steady-state kinetics parameters for TauD demonstrates that the release of product is the rate-determining step of the reaction (44).

Analysis of short-lived reaction intermediates that occur after oxygen addition requires the use of transient methods. Even with such approaches, the presumed Fe(III)-superoxo, bicyclic, and Fe(III)-OH species (Fig. 2, D, E, and G) are kinetically inaccessible and have not been observed. Stopped-flow UV-visible spectroscopy provides evidence for the ferryl intermediate, absorbing at 320 nm, which forms with a second-order rate constant of 1.5 × 10⁶ M⁻¹ s⁻¹ and decomposes with a first-order rate constant of 13 s⁻¹ at 5 °C (44). Rapid freeze-quench Mössbauer spectroscopy along with cryoreduction followed by EPR spectroscopy provides evidence for a high-spin (s = 2) intermediate having a quadrupole doublet with an isomer shift of 0.30 mm s⁻¹ and a quadrupole splitting parameter of 0.88 mm s⁻¹ consistent with an Fe(IV) oxidation state (44).
these enzymes when compared with other family members; the Fe(II) is coordinated by two His residues and a chloride ion, with the typical carboxylate ligand (Asp or Glu) being replaced by an Ala residue (58, 59).

The catalytic mechanism proposed for the Fe(II)/2OG-dependent halogenases (58) closely parallels that of the hydroxylases. The early steps in the reaction sequences are identical, except that chloride replaces the Asp/Glu ligand to the metal (Fig. 3A, left), until generating the ferryl intermediate, now called a haloferryl species. The haloferryl species abstracts a hydrogen atom from the primary substrate to form a substrate radical plus a cis-halohydroxo-ferric species, and the halogen
atom reacts with the substrate radical to yield the halogenated product and complete the catalytic cycle (Fig. 3A, right) (38, 58, 60).

Support for the proposed halogenase intermediates is derived from studies analogous to those used for characterizing the key intermediates in the hydroxylation reaction mechanism. Stopped-flow UV-visible kinetics and freeze-quench Mössbauer spectroscopy provide evidence for high-spin \( \text{Fe}^{2+} \) species with absorption at 320 nm (61). Significantly, extended x-ray absorption fine structure studies provide evidence for the formation of the haloferryl intermediate, with Fe-O and Fe-halogen distances of 1.66 and 2.43 Å, respectively (62).

Similar to other \( \text{Fe}(II)/2\text{OG} \)-dependent oxygenases, the halogenases also exhibit substrate triggering in which the binding of substrates accelerates the reaction with molecular oxygen (7, 63). For example, the rate of SyrB2’s reactivity with \( \text{O}_2 \) is enhanced 8,000-fold in the presence of the tethered \( \text{L}-\text{Thr} \) substrate when compared with the rate with the free amino acid (64). In addition, the halogen also plays a key role in the activation of \( \text{O}_2 \) by the \( \text{Fe}(II) \) cofactor in another halogenase, HtcB (65). In contrast to other halogenases and oxygenases in general, where the binding of the primary substrate induces a change in the geometry of the active site from 6-coordinate to 5-coordinate resulting from water molecule displacement, HtcB remains 6-coordinate in the presence of \( 2\text{OG} \) and its primary substrate. The change in coordination from six to five is only observed when the chloride atom binds to the active site (65).

One of the most intriguing questions about the mechanism of the halogenases is the selectivity for halogenation rather than hydroxylation. For SyrB2, the positioning of the primary substrate in relation to the metal center is critical for determining the relative extents of halogenation and hydroxylation (66). Tethered substrates were investigated using modified side chains, including \( \text{L}-2\)-aminobutyric acid (a 4-carbon compound like \( \text{L}-\text{Thr} \), but lacking the hydroxyl moiety on C3) and \( \text{L}-\text{norvaline} \) (a 5-carbon species with an extra methylene group and also lacking hydroxylation at C3). The halogenase shows barely detectable hydroxylase activity with its native substrate, approximately equivalent halogenase and hydroxylase activities with the \( \text{L}-2\)-aminobutyric acid adduct, and primarily hydroxylase activity toward the tethered \( \text{L}-\text{norvaline} \) at position C5 with minor halogenation activity at C4 (66). Density functional theory applied to SyrB2 with its native and non-native tethered substrates shows that the phosphopantetheine moiety of the carrier protein also has an influence on the positioning of the substrate in the active site, ultimately dictating the halogenation versus hydroxylation activity (67).

Of additional interest, SyrB2 exhibits unprecedented reactivity involving the installation of functional groups other than halogens or hydroxyl groups at unactivated C-H centers. In particular, SyrB2 performs azidation and nitrilation of tethered \( \text{L}-2\)-aminobutyrate substrate, using azide and nitrite, respectively (68). On the basis of kinetic and spectroscopic results, a ferryl intermediate is proposed to be responsible for these transformations, similar to the halogenation and hydroxylation transformations (68).

**Ring Formation**

A third mechanism associated with the \( \text{Fe}(II)/2\text{OG} \)-dependent oxygenases is oxidative ring cyclization, as epitomized by \( \text{CAS} \) from *Streptomyces clavuligerus* (Fig. 1C). This oxygenase shares the common metal-binding 2-His-1-Glu motif within the DSBH fold (69). The crystal structure of CAS in complex with \( 2\text{OG} \) reveals that it binds to the active site in a bidentate mode, displacing two of the metal-bound waters analogous to other characterized oxygenases (69). UV-visible absorption, CD, low-temperature MCD, and variable-temperature/variable-field MCD allowed Solomon and co-workers (70) to electronically characterize the enzyme and demonstrate the 6-coordinate to 5-coordinate transition upon binding of the primary substrate. On the basis of spectroscopic studies, kinetic analyses, and structural characterization, the oxidative ring-forming transformation is proposed to proceed through a ferryl intermediate (69, 70).

A still unresolved mechanistic question about the oxidative transformation is the sequence of steps taking place after generating the ferryl intermediate. Hydrogen atom abstraction from either of the two sites to be coupled, the C4’ carbon or the C3 hydroxide of proclavaminic acid (Fig. 3B), is chemically reasonable. For example, abstraction of a hydrogen atom from the proS position of the C4’ carbon would yield a substrate radical species and \( \text{Fe}(III)-\text{OH} \). In one scenario, the \( \text{Fe}(III)-\text{OH} \) could abstract a hydrogen atom from the substrate hydroxyl group (Fig. 3B, red arrows in path a), allowing for direct cyclization; however, the ferric species is only a weak oxidant and may not be capable of catalyzing this reaction. In a more likely option, the carbon-centered radical could transfer an electron to the metallocenter (Fig. 3B, cyan arrows in path b), with the resulting carbocation reacting with the substrate hydroxyl group (69, 71). Doubt is cast on both of these options by the structure of the CAS/2OG-proclavaminic acid complex, which provides a lengthy 5.4 Å distance between Fe and the C4’ carbon (69).
alternative mechanism (Fig. 3B, purple arrows in path c) was proposed by Borowski et al. (72) involving hydrogen atom abstraction from the substrate hydroxyl group, located only 4.2 Å from the metallocenter. The authors applied classical molecular dynamics simulations, potential mean force computations, and B3LYP calculations on a CAS:Fe(IV)-oxo-succinate-proclavaminic acid complex, leading to the suggestion of radical cleavage of the substrate to 3-aminopropanal and a C2 radical species, with subsequent formation of an azomethine ylide and dipolar cycloaddition to yield the cyclic product (72). Additional studies are needed to clarify the cyclization mechanism for this and other Fe(II)/2OG-dependent oxygenases (including the epoxidation reaction described under “Other Reactions,” below).

Desaturation

Desaturation reactions are catalyzed by Fe(II)/2OG-dependent oxygenases involved in a variety of flavonoid, gibberellin, and antibiotic reaction pathways (9). To illustrate a desaturation reaction, we highlight CarC from Pectobacterium carotovorum (formerly Erwinia carotovora) that converts (3S,5R)-carbapenam to (5R)-carbapenem (Fig. 1D, right). This enzyme also catalyzes the preceding epimerization reaction as described in the following section. Some studies of CarC have considered the two reactions as being coupled (30, 73), in which case a tyrosyl radical formed during the epimerization reaction could be used to generate a carbon-centered radical on the substrate. Alternatively, however, a ferryl intermediate could directly abstract a hydrogen atom of (3S,5R)-carbapenam in an uncoupled reaction.

Several potential pathways for 1,2-dehydrogenation, all initiated by a ferryl intermediate, were enumerated recently by Bollinger et al. (74), three of which are described here (Fig. 3C). In one proposal (Fig. 3C, cyan arrows in path a), the Fe(III)-OH species (resulting from the hydrogen atom transfer) abstracts a second hydrogen atom from the substrate to yield Fe(II)-H₂O, with the two radicals coupling to form the desaturation product. Unfortunately, this mechanism again suffers from the weak oxidant capacity of Fe(III)-OH. A second mechanism invokes hydroxyl radical rebound, elimination of hydroxide anion, and tautomerization to form the product (Fig. 3C, red arrows in path b). Finally, one can consider electron transfer from the carbon-centered radical to the metallocenter with subsequent rearrangement of the cation to form product (Fig. 3C, purple arrows in path c). The latter two reactions both incorporate the adjacent N atom into the mechanism. Of interest, substrates undergoing 1,2-dehydrogenation by Fe(II)/2OG-dependent oxygenases (including CAS, Fig. 1C) (20) all possess a heteroatom adjacent to the aliphatic C–C bond of interest. Additional experimental efforts are needed to further dissect these mechanisms.

Epimerization

CarC catalyzes the epimerization of (3S,5S)-carbapenam to (3S,5R)-carbapenam (Fig. 1D, left) in addition to the desaturation reaction discussed above (20). Epimerization, unusual among the Fe(II)/2OG oxygenases, is redox-neutral (75), and this unprecedented reactivity has led to intensive studies to investigate the mechanism. For example, the crystal structure of CarC in complex with the substrate analogue (S)-N-acetylproline along with analyses from computational studies led to several possible mechanisms for the epimerization of the C5 position involving a C5-radical intermediate and an external H-atom donor (30, 73, 76). Analysis of the initial crystal structure identified Tyr-67, located in a loop region near the active site and 5.7 Å away from the metal center, as a possible protein-based H-atom donor (73). It was suggested that the ferryl intermediate abstracts a hydrogen atom from C5 to form a substrate radical and the Fe(III)-OH species, with the C5 radical then accepting a hydrogen atom from the nearby tyrosine residue to form to (3S,5R)-carbapenam (30).

The epimerization mechanism of CarC was subsequently modified and elaborated by Chang et al. (77) (Fig. 3D), who overcame major obstacles including protein and substrate instability. They obtained the structure of the CarC:Fe(II)-2OG:(3S,5S)-carbapenam quaternary complex that provided essential geometric characteristics of the substrate in relation to its orientation to the iron center. It was found that C5 is 4.4 Å from the iron center and 4.8 Å from the hydroxyl group of Tyr-165, the external H-atom donor. Mössbauer spectroscopy of CarC confirmed the formation of a high-spin (s = 2) ferryl intermediate that, in contrast to the ferryl complexes of other oxygenases, which decay to Fe(II), converts to a high-spin Fe(III) species (77). Stopped-flow UV-visible absorption spectroscopy demonstrated accumulation of a tyrosyl radical species concurrent with ferryl species decay. Thus, the ferryl intermediate of CarC is proposed to abstract a hydrogen atom from C5 of (3S,5S)-carbapenam to form a substrate radical. Tyr-165 located on the opposite face of the iron center donates a hydrogen atom to the substrate radical to yield the desired epimer product and the tyrosyl radical (77).

Other Reactions

To further showcase the versatility in reaction mechanisms catalyzed by Fe(II)/2OG-dependent oxygenases (9), we highlight three more examples. DAOCS from S. clavuligerus catalyzes the oxidative ring expansion of penicillin N to yield deacetoxycephalosporin C (Fig. 1E). A DAOCS:2OG-substrate ternary complex is proposed to react with O₂ to form a ferryl intermediate that initiates the reaction (32). One hydrogen atom is abstracted from the C2 β-methyl group of the substrate to produce a methylene radical, which undergoes rearrangement leading to ring expansion with the radical repositioned to the ternary carbon (C3), and then desaturation completes the reaction, perhaps by hydrogen atom transfer to the Fe(III)-OH (20, 78). H6H is an enzyme isolated from Hyoscyamus niger (henbane) where it participates in the synthesis of scopolamine, a hallucinogenic tropane alkaloid. The enzyme catalyzes both a hydroxylation and an epoxidation reaction (Fig. 1F), where the latter step does not require intermediary formation of the alkene (33, 79, 80). Finally, FtmOX1 from Aspergillus fumigatus catalyzes the conversion of furmitremorgin B to verruculogen by creating an endoperoxide bond between two isoprene groups (Fig. 1G), a reaction that requires two molecules of O₂ (34). It was proposed that this transformation proceeds via a ferryl intermediate with hydrogen abstraction from the α-position of...
the alkenyl bond of the N-prenyl moiety to produce a substrate radical (34). Molecular oxygen would then attack the substrate radical to generate a radical species that would undergo cyclization followed by hydrogen abstraction to form the verruculogen product (34).

Conclusions and Perspectives

The Fe(II)/2OG-dependent oxygenase superfamily includes a remarkable set of enzymes with the ability to activate molecular oxygen for use in a wealth of important oxidative transformations. All of these reactions are likely to proceed through a common ferryl intermediate, which is also thought to be formed in mechanistically and structurally related enzymes. For example, variations of the mechanisms described above are utilized by 4-hydroxyphenylpyruvate dioxygenase and 4-hydroxyxylantimicrobial synthases that use the same substrate to form homogentisate and 4-hydroxymandelate, respectively (81). These enzymes possess a vicinal oxygen chelate fold that is unrelated to the DSBH fold, do not require 2OG, and carry out oxidative decarboxylation of the 2-oxo acid moiety of the substrate to generate the ferryl intermediate that is used in subsequent chemistry (81). Furthermore, three Fe(II)-dependent oxygenases possess the DSBH fold, but act independent of 2OG; isopenicillin N synthase converts the tripeptide (S,L-aminoadipoyl)-L-cysteinyl-D-valine to isopenicillin N (20, 82), 1-amincyclopropane-1-carboxylate oxygenase catalyzes the synthesis of ethylene (83), and the epoxide-forming enzyme HppE catalyzes the formation of fosfomycin from (2S)-hydroxypropylphosphonate (84, 85). New members of the Fe(II)/2OG-dependent oxygenase superfamily are certain to be discovered, and it is likely they too will utilize a ferryl intermediate to catalyze their novel oxidative transformations.

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