**Structural and mechanistic studies on 2-oxoglutarate-dependent oxygenases and related enzymes**

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Mononuclear nonheme-Fe(II)-dependent oxygenases comprise an extended family of oxidising enzymes, of which the 2-oxoglutarate-dependent oxygenases and related enzymes are the largest known subgroup. Recent crystallographic and mechanistic studies have helped to define the overall fold of the 2-oxoglutarate-dependent enzymes and have led to the identification of coordination chemistry closely related to that of other nonheme-Fe(II)-dependent oxygenases, suggesting related mechanisms for dioxygen activation that involve iron-mediated electron transfer.

**Abbreviations**

2OG 2-oxoglutarate
3,4-PCD protocatechuate-3,4-dioxygenase
4-HPPD 4-hydroxyphenylpyruvate dioxygenase
ACCO 1-amino-1-cyclopropanecarboxylic acid oxidase
ACV L-8-(a-aminoacyl)l-cysteinyld-valine
BphC 2,3-dihydroxybiphenyl 1,2 dioxygenase
CAS clavaminate synthase
DAOCS deacetoxycephalosporin C synthase
IPNS isopenicillin N synthase
NDO naphthalene dioxygenase
PDB Protein Data Bank
SLO-1 soybean lipoxigenase
TfdA (2,4-dichlorophenoxy)acetate dioxygenase

**Introduction**

The stereoselective oxidation of an unactivated alkane carbon–hydrogen bond is probably the most difficult common functional group interconversion in chemistry. In nature, such reactions are most often carried out by metal-dependent oxygenases or oxidases. The best characterised of these enzymes are the cytochrome P450 monoxygenases, for which detailed structural and mechanistic information is available (see [1,2]). It is now clear that oxygenases/oxidases that use no cofactor other than iron constitute a ‘superfamily’ of redox enzymes. This extended family includes both diiron-containing enzymes, for example, methane monoxygenase and ribonucleotide reductase, and monoiron-containing enzymes. The latter enzymes include those dependent on Fe(III), for example, lipoxigenase and the intradiol cleaving catechol dioxygenases, and those dependent on Fe(II), for example, the extradiol cleaving catechol dioxygenases, tyrosine/phenylalanine hydroxylases, benzene/naphthalene dioxygenases and the 2-oxoglutarate (2OG)-dependent oxygenases [3–6].

The 2OG-dependent oxygenases have emerged as the largest known family of nonheme oxidising enzymes [7,8] (Figure 1a). Their occurrence is ubiquitous, having been identified in many organisms ranging from prokaryotes to eukaryotes. Recent evidence also indicates that a 2OG-dependent oxygenase, prolyl 4-hydroxylase, is expressed by the *P. bursaria Chlorella* virus-1 [9]. Oxidative reactions catalysed by 2OG-dependent dioxygenases are steps in the biosynthesis of a variety of metabolites, including materials of medicinal or agrochemical importance, such as plant ‘hormones’ (e.g. gibberellins) and antibiotics (e.g. cephalosporins and the β-lactamase inhibitor clavulanic acid).

In mammals, the best-characterised 2OG-dependent oxygenase is prolyl-4-hydroxylase, which catalyses the post-translational hydroxylation of proline residues in collagen. Mammalian prolyl-4-hydroxylase is an αβ3 tetramer, in which the oxygenase activity is associated with the α subunit and the β subunit has an identical sequence to that of protein disulfide isomerase [10,11]. Two members of the 2OG-dependent oxygenase family, deacetoxycephalosporin C synthase (DAOCS) and proline-4-hydroxylase, are used currently in genetically engineered fermentation processes [12,13].

All studied 2OG-dependent oxygenases have an absolute requirement for Fe(II) and catalyse a variety of two-electron oxidations, including hydroxylation, desaturation and oxidative ring closure reactions [7,8]. In almost all cases, the oxidation of the ‘prime’ substrate is coupled to the conversion of 2OG into succinate and CO₂. One of the oxygens of the dioxygen molecule is incorporated into succinate. In the case of desaturation reactions, the other dioxygen-derived oxygen is presumably converted to water. In hydroxylation reactions, the partial incorporation of oxygen from dioxygen into the alcohol product occurs with significant levels of exchange of oxygen from water being observed [14,15].

Two enzymes, isopenicillin N synthase (IPNS) and 1-amino-1-cyclopropanecarboxylic acid oxidase (ACCO), have evolved from the same structural platform as the 2OG-dependent oxygenases, but do not use 2OG as a cosubstrate (Figure 1b,c). IPNS catalyses the four-electron oxidation of a tripeptide to give the penicillin nucleus and has been extensively studied [16,17]. ACCO catalyses the ultimate step in the biosynthesis of the plant signalling molecule ethylene from ACC, using ascorbate as a cosubstrate and CO₂ as an activator [7,8].

The first crystal structure to be reported for a member of the 2OG-dependent oxygenase and related enzyme family was that of IPNS [18]. Structures of two more typical
Reactions catalysed by 2OG-dependent oxygenases and related enzymes. (a) General scheme for hydroxylation by a 2OG dioxygenase. A water molecule is shown before and after reaction to emphasise the exchange of oxygen with dioxygen. (b) The IPNS reaction. (c) The ACCO reaction. (d) The DAOCS reaction. (e) The trifunctional role of CAS in clavulanic acid biosynthesis. Names of the oxygenase enzymes are in red. PAH, proclavaminate amidohydrolase.

Oxygenases, DAOCS [19••] and clavaminate synthase (CAS) [20; Z Zhang et al., unpublished data], have been recently determined (Figure 1d,e). DAOCS catalyses the ring expansion of penicillin N to DAOC, the committal step in cephalosporin biosynthesis. CAS catalyses three reactions during clavulanic acid biosynthesis, encompassing hydroxylation, oxidative ring closure processes and desaturation, making it an excellent case study for
investigating the structural factors directing the chemoselectivity and regioselectivity of 2OG-dependent oxygenases [14,21,22]. This article reviews recent studies on 2OG-dependent oxygenases, making a brief comparison with other mononuclear nonheme-Fe-dependent oxidising enzymes. Note that comparisons between mononuclear and dinuclear iron enzymes are beyond the scope of this review, but the distinction is somewhat arbitrary. Attention is also drawn to other recent reviews on the chemistry of nonheme oxygenases [3–6], including the 2OG-dependent oxygenases [7,8].

Three-dimensional topology and active site coordination chemistry of 2-oxoglutarate-dependent oxygenases and related enzymes

The structure of IPNS revealed a β-strand core folded into a distorted jelly-roll motif [18] (Figure 2). Sequence comparisons suggest that many 2OG-dependent oxygenases will have a similar fold to IPNS, a prediction substantiated by the crystal structure of DAOCS. Sequence analyses of CAS and other 2OG-dependent oxygenases [22] revealed little overall similarity with the DAOCS/IPNS subfamily, leading to the proposal that convergent evolution to a common mechanism and active site chemistry occurred within the wider family of 2OG-dependent and related oxygenases. The presence of a jelly-roll topology in the CAS structure, as in IPNS and DAOCS, in which certain 2OG-binding and iron-binding residues are located on analogous strands of the core, suggests the occurrence of divergent evolution within the family of 2OG-dependent oxygenases. Sequence analyses might have been misled by inserts between β strands of the jelly-roll motif in CAS. It seems possible that all of the 2OG-dependent oxygenases, including mammalian enzymes (e.g. the prolyl hydroxylases [10,11]), will contain a similar β-barrel core. The recently reported structure of 4-hydroxyphenylpyruvate dioxygenase (4-HPPD), which oxidises 4-HPP to homogentisate [23••], however, reveals that nature has evolved more than one platform for nonheme oxygenases to catalyse the oxidative decarboxylation of 2-oxoacids (see below).

In the crystal structure of IPNS complexed to Mn(II) [substituting for Fe(II)], the active site metal was ligated by two water molecules and the sidechains of Gln330, Asp216, His214 and His270 [18]. The presence of two histidines and a carboxylate ligand was anticipated by spectroscopic and sequence analysis studies [3]. Ligation by Gln330, the penultimate residue in the C terminus, was unexpected and this residue is not essential for catalysis [24,25]. Crystal structures of DAOCS–Fe(II) [19••] and CAS–Fe(II) complexes reveal that Fe(II) is coordinated by the two histidine residues of an analogous HXD/E…H motif, but CAS is unusual in that it uses a glutamate, rather than an aspartate, residue as its carboxylate ligand. There is no evidence for a fourth protein iron ligand in either DAOCS or CAS.

The HXD/E…H iron-binding motif is highly conserved in 2OG-dependent and related oxygenases [4]. Mutagenesis, chemical modification and/or spectroscopic studies are all consistent with the presence of this motif in other 2OG-dependent oxygenases, including prollyl-4-hydroxylase [11], proline hydroxylase [26], (2,4-dichlorophenoxy)acetic acid dioxygenase (TfdA) [27], CAS [28,29••] and ACCO [30••].
Partial mechanisms for (a) DAOCS ([19**,58] and references therein) and (b) IPNS ([18,52] and references therein). Dioxygen and dioxygen-derived species are in red, 2OG and derived species are in green, iron is in orange. (a) Oxygen is shown bound as a peroxide radical, but a superoxide/peroxide is also possible. See text for discussion. I shows the putative radical intermediate in DAOCS catalysis. II shows a possible intermediate in the exchange of oxygen from water to dioxygen (there is evidence that exchange can also occur after reaction of the ferryl [15]). The position from which the ferryl reacts is uncertain.
Furthermore, as pointed out by Hegg and Que [4], a ‘facial 2-His-1-carboxylate’ triad of iron-ligating residues is present in all enzymes containing mononuclear nonheme Fe sites for which structures have been reported. These include Fe(II)-dependent enzymes, such as IPNS [18], DAOCS [19••], CAS [20,28,29••], LigAB protocatechuate 4,5-dioxygenase [31••], catechol-2,3-dioxygenase [32], 2,3-dihydroxybiphenyl 1,2-dioxigenase (BphC) [33,34], naphthalene dioxygenase (NDO) [35••], phenylalanine hydroxylase [36–38], tyrosine hydroxylase [39,40] and 4-HPPD [23••], and Fe(III)-dependent enzymes, such as protocatechuate-3,4-dioxygenase (3,4-PGD) [41–44], soybean lipoxygenase (SLO-1) [45,46] and rabbit 15-lipooxygenase [47]. Also note that the conserved triad has evolved on structural platforms with different overall topologies, for example, those of 2OG-dependent oxygenases (and IPNS and ACCO), those of the class I/II extradiol dioxygenases and HPPD, those of the class III extradiol dioxygenases [28] and those of the tyrosine/phenylalanine hydroxylases. The secondary structure elements in which the triad residues are located can be either β strands, α helices or loops, but they seem generally to be located in relatively ‘rigid’ parts of the structure, consistent with the ligation of these residues to the metal during the catalytic cycle.

The presence of such an iron-binding motif is not limited to iron-dependent enzymes; it also occurs in Mn(II)-dependent extradiol oxygenases and in Zn(II)-dependent hydroxylase enzymes such as thermolysin. The latter contain an HEXXH…E motif, in which the Zn(II) ligands are provided by the sidechains of the two histidines and the C-terminal glutamate of the motif [48]. A link between the iron and zinc enzymes has also been provided by the discovery that peptide deformylase from Escherichia coli, originally thought to be a zinc-dependent hydroxylase, instead uses Fe(II) [49,50]. The iron is coordinated by two histidines and a cysteine residue, and undergoes inactivation under aerobic conditions, implying that an iron-mediated reaction with dioxygen has occurred. Fe(II) is also present in the structure of galactose-1-phosphate uridylyltransferase [51], in which it is ligated in a tetrahedral fashion by two histidines and two cysteine residues; however, the iron is believed to play a structural, rather than a catalytic, role.

In the case of Fe(II) enzymes, there is the ‘problem’ of the reaction with dioxygen leading to the generation of reactive oxidising species. This may explain (in part) why cysteine residues are normally not used as ligands in Fe(II) nonheme enzymes. Indeed, the oxidation of an iron-linked thiol is elegantly exploited in a biosynthetic sense by IPNS [52]. In a remarkable example of post-translational modification, it seems that the autocatalytic oxidation of cysteine thiols occurs in the case of nitrile hydratase, an industrially used Fe(III)-dependent enzyme involved in the hydrolysis of acrylonitrile. As isolated from Rhodococcus sp. N-771, the enzyme is in an inactive form as a result of nitrosylation of the active site iron and is activated by photolysis. Its active site iron is ligated by the sidechains of three cysteines (C109, C112 and C114) and by the mainchain nitrogen atoms of Ser113 and Cys114. The thiol sidechains of Cys112 and Cys114 are reported to be oxidised to sulfenic and sulfenic acids, assisting NO binding. It seems possible that oxidation of the sidechains of Cys112 and Cys114 occurs by reaction with dioxygen in an Fe-mediated process [53••,54•].

### Binding of substrates and mechanistic proposals for 2-oxoglutarate-dependent oxygenases

Kinetic studies of 2OG-dependent oxygenases indicate an ordered sequential mechanism, with the binding of 2OG followed by that of the prime substrate, then dioxygen [10,55]. Product release then occurs in the order bicarbonate (or CO2), succinate and prime product, with the order of release of the last two dependent on their relative concentration. The evidence for any reaction steps subsequent to the formation of an enzyme–Fe–2OG complex is limited, although it is widely believed that an Fe(IV)=O species effects oxidation of the prime substrate [56,57]. Crystallographic and spectroscopic analyses of 2OG-dependent oxygenases are starting to define the structures of the intermediates involved in the reaction pathway.

The crystal structure of a complex of DAOCS bound to Fe(II) and 2OG demonstrates that the 2OG cofactor is ligated to the metal in a bidentate fashion via its 1-carboxylate and 2-oxo groups [19••,20]. A water molecule, trans to His279, remains ligated to the metal. The terminal carboxylate of 2OG is bound to a conserved RXS motif in DAOCS. Spectroscopic studies of complexes of CAS with Fe(II) and 2OG imply a similar iron coordination arrangement in solution [28,29••]. Related studies of Cu(I) complexes with TfdA implied monodentate ligation of the 2OG [24]; however, it seems possible that this reflects the substitution of a catalytically inactive metal in these studies. Spectroscopic studies demonstrate that, on binding the monocyclic β-lactam substrate, the CAS–Fe(II)–2OG complex changes from six-coordinate octahedral to five-coordinate square pyramidal geometry [28,29••]. Crystallographic analyses of two CAS–Fe(II)–2OG–substrate complexes also revealed that the occupancy of the iron-ligated water is significantly reduced on substrate binding. These analyses all suggest that dioxygen binding is triggered by the binding of the prime substrate to the enzyme–Fe–2OG complex, as implied from kinetic studies. Possible mechanisms for the formation of the ferryl species involve electron transfer from the iron to the dioxygen, simultaneously permitting the formation of an iron-bound superoxide/peroxide species and the activation of the keto group of 2OG for nucleophilic attack to form a peroxy species that can collapse to give the reactive ferryl species and CO2/succinate or an iron-ligated anhydride (Figure 3).

The position of dioxygen binding to the iron in 2OG-dependent oxygenases is not yet unequivocally established. It is reasonable to propose that dioxygen can bind into the position occupied by the water molecule. So
far, it has not been possible to obtain a structure of DAOCS complexed with its prime (penicillin N) substrate. Modelling studies indicate that it can be bound in the DAOCS active site in such a way as to project its pro-S methyl group towards the iron centre [58]; however, if the proposed intermediate ferryl species is generated in the position opposite His183 of DAOCS, it is directed away from the substrate and it is possible that the ferryl reacts from the position opposite His243. This might be achieved by binding dioxygen to a different form of the complex compared with that observed crystallographically or via rearrangement of a pentacoordinate ferryl species subsequent to the release of CO2. If such a speculation is correct, it may provide a partial explanation for the observation that, during hydroxylation processes catalysed by 2OG-dependent oxygenases, incorporation of oxygen atoms from both dioxygen and water is observed, as water may bind to the pentacoordinate species.

Following studies of prolyl-4-hydroxylase, the oxygen exchange process has been studied in DAOCS and CAS, with the level of incorporation of oxygen from labelled dioxygen or water being substrate dependent [11,12]. Together with kinetic isotope effects, results using an unnatural substrate (exoemthylene cephalosporin C) with DAOCS have been taken to imply that the exchange process can occur either prior to or subsequent to the reaction of the ferryl species.

During hydroxylation processes, the reaction appears to be highly stereoslective, favouring a mechanism involving the direct insertion of a ferryl-derived dioxygen into a C–H bond, rather than involving radical or Fe–C bonded intermediates. When substrate analogues are used, including cyclopropyl radical traps, it seems that the reaction pathway can be biased towards processes involving radical intermediates [5,16]. Note that the natural reaction for DAOCS is itself an exception, with the ring expansion process probably proceeding via a 3β-methyl radical on penicillin N generated by a ferryl-mediated expansion abstraction process. Biasing the reaction pathway of 2OG-dependent oxygenases to deliberately generate radicals may lead to inhibition. Indeed, 5-oxaproline-containing peptides have recently been shown to cause mechanism-based inhibition of prolyl-4-hydroxylase, most probably via a radical process [59].

**Mechanism of isopenicillin N synthase**

Mechanistic studies on IPNS are more advanced than those on the 2OG-dependent oxygenases. On binding L-3-β-(α-aminoacidopropyl)-L-cysteinyl-d-valine (ACV) to the IPNS active site, one of the two water molecules (that trans to Asp216) ligated to the iron is displaced and the amide sidechain of Gln330 is displaced by the thiol of ACV [5,52]. The ACV valine carboxylate is in position to form electrostatic interactions with the same RXS motif that is responsible for binding the terminal carboxylate of 2OG in DAOCS. The ACV valine βC–H, which must be cleaved during penicillin formation, was observed to be directed away from the iron centre, implying that rotation of the valine Cα–Cβ bond occurs during catalysis. Reaction of the IPNS–Fe(II)–ACV crystals with the dioxygen analogue NO led to the binding of NO to the iron trans to Asp216, indicating that dioxygen binds similarly. The oxygen of the NO was observed to be equidistant between the NH of the cysteinyl valine peptide bond and the cysteinyln pro-S β hydrogen, both of which are removed during IPN formation. It was proposed that, excepting the thiol hydrogen, iron–dioxygen-derived species are responsible for removal of all the requisite hydrogens from ACV during formation of the penicillin nucleus.

**Figure 4**

Comparison of the DAOCS coordination chemistry with other mononuclear iron oxygenases and thermolysin. Proposed metal coordination chemistry for (a) DAOCS–Fe–2OG–O2, based upon the crystal structure of DAOCS–Fe(II)–2OG [19*], (b) ACCO based upon spectroscopic studies [30*], (c) 4-HPPD based upon the crystal structure of 4-HPPD–Fe–acetate [35*], (d) the extradiol dioxygenase catechol 2,3-dioxygenase based on the enzyme–Fe(II)–acetone crystal structure ([32]; see also studies on other extradiol dioxygenases [31**,33,34]) and (e) thermolysin [48]. See also other reviews [4,5]. The proposed (but unproven) position of dioxygen binding in (a–d) is indicated.
**ACC oxidase**

ACCO contains characteristic sequence motifs of 2OG-dependent oxygenases, including the RXS motif, which (from the structural work on DAOCS) binds to the terminal carboxylate of 2OG. It is unique in having a requirement for ascorbate as a cosubstrate and CO₂ as an activator, characteristics that make it interesting to study from a mechanistic viewpoint. Mutagenesis and spectroscopic studies have implied that ACCO also employs an HXD...H motif to bind its active site iron [60,61]. Mechanistic proposals have included schemes involving the generation of an Fe(IV)=O intermediate by the initial reaction of ascorbate with dioxygen. Recent spectroscopic studies on ACCO have revealed that ACCO–Fe(II)–ACC–NO complexes are readily formed, but analogous complexes with ascorbate substituting for ACC cannot be formed. The results demonstrate that ACC is bound to iron in a bidentate manner via both its carboxylate and amino groups. Consequently, a new outline mechanism for ACCO has been proposed: initial binding of ACC to ACCO–Fe(II) lowers the redox potential of the metal centre, activating it for dioxygen binding and superoxide formation, in analogy with the mechanism for 2OG-dependent oxygenases. Abstraction of a hydrogen atom from the ACC amino group can lead to fragmentation to form ethylene, CO₂ and HCN. The role of the ascorbate in this mechanism is to reduce the peroxide intermediate to prevent formation of reactive oxidising species. Indeed, ACCO from tomato is susceptible to such damage, undergoing partial fragmentation. Alternative mechanistic scenarios, such as the intermediacy of a Fe(IV)=O species, are also possible and the mechanism of CO₂ activation remains obscure.

**Relationships of the 2-oxoglutarate-dependent oxygenases with other nonheme oxygenases**

**Catechol dioxygenases**

Both from mechanistic and structural viewpoints, the catechol dioxygenases are probably the best characterised of the mononuclear iron oxygenases. They catalyse steps in the bacterial metabolism of aromatic compounds, catalysing the ring fission of catechol, protocatechuate and gentisate. On the basis of the position of aromatic ring cleavage and the requirement for Fe(III) or Mn(II)/Fe(II), they can be classified into two groups. The intradiol dioxygenases cleave the C–C bond between the hydroxyl groups and require Fe(III), whereas the extradiol enzymes cleave the C–C bond adjacent to the hydroxyl groups and require Mn(II) or Fe(II).

There is an important mechanistic difference between the Fe(III)-dependent intradiol dioxygenases and the other oxygenases described here. Very detailed spectroscopic and structural studies of 3,4-PCD indicate that the iron retains high spin during catalysis and thus does not bind dioxygen directly. Instead, it has been proposed that binding of 3,4-PCD to iron activates the substrate for reaction with dioxygen [43,44]. In contrast, the Fe(II)-using extradiol dioxygenases appear to employ a mechanism that is more closely related to that of the 2OG-dependent oxygenases. Thus, in both cases, current proposals involve activation of dioxygen by binding directly to the iron, forming superoxide/peroxide intermediates. Evidence has been provided for lactone and semiquinone intermediates [62–64] and a Crigee rearrangement has been proposed as a key step — an analogous rearrangement is a possibility in 2OG-dependent catalysis.

**4-HPPD**

A compelling mechanistic link between the 2OG-dependent and catechol dioxygenases is provided by 4-HPPD. This enzyme catalyses the conversion of the 2-oxoacid 4-hydroxyphenyl pyruvate to homogentisate in a process involving oxidative rearrangement and decarboxylation. It also catalyses the oxidation of other amino-acid-derived 2-oxoacids, including the conversion of α-ketoisocaproate to β-hydroxyisovalerate [65]. Thus, it has parallels to both 2OG-dependent oxygenases and IPNS catalysis in that they catalyse an oxidative decarboxylation process and the four-electron oxidation of a single substrate, respectively; however, the crystal structure of 4-HPPD clearly places it in the structural family of extradiol dioxygenases (class III) [23**]. The active site iron was once again bound by the 2-His-1-carboxylate (glutamate) motif (Figure 4). On the basis of the crystal structure, the proposed binding model for the enzyme substrate was strikingly similar to that observed for the 2OG-dependent oxygenases. Dioxygen was proposed to bind opposite Glu322 [23**].

**Lipoxygenases**

Lipoxygenases catalyse the two-electron oxidation of fatty acids containing a 1,4-diene to give hydroperoxides. They contain a single Fe(III) ligated by three histidines and the carboxylate of the C-terminal residue Ile839. The sidechain of asparagine is part of a hydrogen-bonding network around the iron, but probably does not directly interact with it. As isolated, lipoxygenases contain Fe(II) and are activated by conversion to high-spin Fe(III) [45–47]. Their mechanism may involve the generation of a free radical of the 1,4-diene substrate, but the process by which this generated and whether or not an Fe–dioxygen complex is involved in both the hydrogen abstraction and peroxide formation steps are uncertain [45–47].

**Phenylalanine hydroxylase and tyrosine hydroxylase**

Two subgroups of mononuclear-Fe-using oxygenases utilise additional cofactors. Phenylalanine hydroxylase and tyrosine hydroxylase are closely related Fe(II)- and pterin-dependent oxygenases of medicinal importance [36–40]. Structures have been reported for both tyrosine and phenylalanine hydroxylases. The active site iron is coordinated by a 2-His-1-carboxylate motif and by the pterin cofactor. It seems unlikely that the aromatic substrates coordinate the iron, but it is possible that metal ligation by the phenolic products is a mechanism for product inhibition (see [66] for a review).
Napthalene dioxygenase

The interesting crystal structure of napthalene dioxygenase, one of a family of enzymes catalysing the cis-hydroxylation of aromatic rings [35**], has been recently reported. All members of the subfamily contain a Rieske [2Fe–S] cluster. The cluster transfers electrons to the non-heme Fe(II), which catalyses addition of dioxygen to the substrate. The crystal structure reveals that the Rieske cluster and Fe(II) centre are connected via hydrogen bonds between a single residue (Asp205), suggesting a route for electron transfer. The Fe(II) is coordinated by a water molecule, the sidechains of His208 and His213 and, in a bidentate manner, by the carboxylate of Asp362.

Conclusions

The factors that govern the selectivity of different proteins for binding different metals in vivo are not yet clearly established. Nature uses a variety of strategies, including the control of relative metal/protein concentrations and the strength of binding in a particular environment. Binding constants minimally reflect a combination of geometric (Fe may prefer an octahedral coordination, compared with Zn, which has a preference for tetrahedral) and electronic (choice of ligand) factors. The definition of general principles for metal selectivity in biological systems, together with an understanding of both the role of metals in dioxygen activation and the control of the reactivity of reactive oxidising species in an oxidising environment, represents a challenge for structural and bioinorganic chemists.

Recent years have seen structural and spectroscopic studies going a considerable way towards defining the overall fold and coordination chemistry of mononuclear nonheme iron oxygenases. Extensive studies on certain members of the family, for example, the catechol dioxygenases and IPNS, have led to detailed mechanistic proposals. There is still considerable uncertainty regarding the mechanisms of most members of the family, however, including the ubiquitous 2OG-using enzymes. The future will see the test of existing proposals, with the objective of generating knowledge in order to exploit these fascinating enzymes in synthesis, in agrochemistry and as therapeutic targets.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


The authors reveal the first structure of a 2-oxoglutarate-(2OG)-dependent oxygenase. Bidentate coordination of 2OG to iron is observed.


New mechanistic proposals for ACC oxidase.


This paper provides the first structure of a class II extradiol dioxygenase, defining them as a distinct structural family from the class II enzymes.


This remarkable crystal structure of naphthalene dioxygenase, which contains Rieske and mononuclear iron centres, provides a structural framework for mechanistic studies.


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See annotation to [54*].


Together with [53**], this paper reveals the remarkable post-translational modifications at the active site of nitrite hydratase.


