The 3-His Motif: A New Subset of Non-Heme Mono-Iron Enzymes

Seino A. K. Jongkees and Guy N. L. Jameson

Chemistry Department, Otago University, PO Box 56, Dunedin (e-mail: gjameson@chemistry.otago.ac.nz)

The chemistry of iron has undergone something of a renaissance in the last ten years. This increase in activity has been heavily influenced by biochemical investigations into non-heme iron enzymes and it stands out as a good example of healthy interaction between the fields of chemistry and biochemistry. On the chemical side, higher oxidation states[1-3] (up to VI) have been accessed through macrocyclic ligands other than heme groups and new reactivity has been unearthed. Iron tends to like to hold on to at least five of its d-electrons and, therefore, any states higher than Fe(III) usually have been treated as novelties. Recently, however, principally through work by Que and Wieghardt, the landscape of iron chemistry has shifted considerably. Karl Wieghardt[1] has reported the formation of an Fe(VI) complex other than the well known FeO_4^{2-}. Meanwhile, Lawrence Que[2] has produced a series of Fe(IV) complexes with different macrocyclic ligands that allowed their spectroscopic characterization and their chemistry to be probed. This should enable the formation of such species in biochemical systems to be examined more closely.

In particular, the Fe(II)-dependent non-heme mono-iron enzymes have provided fertile ground in the search for interesting chemistry. Taurine/α-ketoglutarate dioxygenase (TauD) has provided the first example[4] of a non-heme iron-oxo species in biology and other members of the family have been extensively studied. Nearly all of these enzymes display characteristic coordination of the Fe(II) centre: two histidines and a carboxylate. Recently, however, two enzymes have been discovered which use three histidines[5,6] – a coordination configuration favoured by zinc (in for example the carbonic anhydrases).[7] These may well be only the first of a new subclass of non-heme mono-iron enzymes and one in particular, cysteine dioxygenase, is the subject of this article.

Cysteine dioxygenase (CDO) is an enzyme from the cupin superfamily (Fig. 1A), which catalyses the addition of both atoms of molecular O_2 onto the thiol sulfur of cysteine side chains, generating cysteine sulfinic acid (see Scheme 1). It achieves this using a single Fe(II) cofactor which is four-coordinate in its resting state. Three of the ligands for this metal site are from protein histidine side chains, with water occupying the final position.[8] Further modifying this unique metal environment is an equally rare post-translationally modified pair of side chains, a cysteine Sγ cross-linked to the aromatic Cϵ of tyrosine. This is in contrast to the active sites of other members of the same family, which carry out an array of reactions such as oxygenative bond cleavage, bond formations and hydroxylations using an octahedral Fe(II) with a two histidine and one carboxylate facial triad of ligands. It might be significant that most previously described dioxygenas-

es catalyse the cleavage of aromatic rather than aliphatic substrates.

There appear to be, in fact, very few enzymes with Fe(II) that do *not* use a two histidine carboxylate motif. Examples include acetylacetone dioxygenase (Dke1) (which coordinates Fe(II) through three histidines),[6] SyrB2 halogenase (which coordinates through two histidines),[9] and a chloride, and apocarotenoid-15,15'-oxygenase (which coordinates through four histidines).[10] Importantly, however, Dke1 is the only other known enzyme to use three histidines. It catalyses the cleavage of acetylacetone and adds one oxygen to each product from molecular O_2 generating acetate and methyl-glyoxal.

The CDO resting state has been shown to be tetrahedral in an X-ray crystal structure analysis, coordinated by three histidine ligands with a well-refined solvent water molecule filling a fourth site as shown in Fig. 1B. This water is hydrogen bonded to a further solvent water molecule and the post-translationally modified tyrosine. It is important to note that such a tetrahedral coordination of Fe(II) is unique in this protein superfamily. From the structure of human CDO co-crystallised with cysteine, the substrate can be seen to coordinate to the metal through the thiol sulfur and amino terminus nitrogen,[11] thus expanding the coordination sphere to distorted octahedral. However, this appears to be in disagreement with the results of extended X-ray absorption fine structure (EXAFS) analyses, which showed all coordination likely to be through O or N atoms. Furthermore, sulfur ligands were specifically *excluded* because they gave a poorer fit to the observed data.[12] These EXAFS studies were carried out on *oxidised* protein with the iron centre in the Fe(III) state and this could readily account for the different coordination environments reported. Coordination of the substrate oxygen may occur in an end-on fashion following the coordination of the cysteine. Affinity of the metal for oxygen increases dramatically after this first substrate binding step, likely serving to protect the enzyme from oxidative damage when no substrate is present.

The post-translational modification 4.2 Å from the metal cofactor, linking a cysteine side-chain to a tyrosine side-chain Cϵ shows co-planarity of Cβ and Sγ of cysteine with the aromatic ring of the tyrosine, suggesting that this has some partial double bond character.[8] This type of modification has been observed only in the enzyme galactose oxidase[13] until now. This enzyme catalyses the reduction of a range of primary alcohols to aldehydes using a mono-copper centre, for which the cross-linked tyrosine is a ligand through the phenolic oxygen. The extended aromaticity of this ligand is thought to stabilize a free radical likely formed in the galactose oxidase mecha-
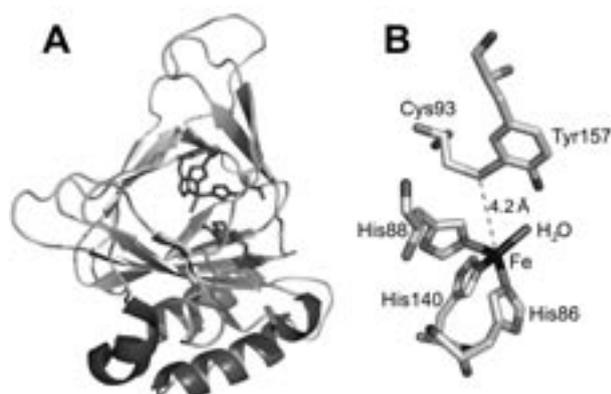
Fig. 1. (A) *Rattus norvegicus* cysteine dioxygenase structure (CDO, PDB 2B5H) clearly showing the active site present within the *β*-barrel of the cupin fold, and (B) the active site showing the unique geometry of the Fe(II) in the resting state and the proximal post-translationally modified thioether. Diagrams produced with PyMOL, see: http://pymol.sourceforge.net.

nism. Electron donation by this cross link also serves to reduce the bond dissociation energy of the tyrosine -OH, giving it unique electronic properties. In CDO this post-translational modification is thought to have two roles.[11] Structurally, this modification may aid in placement of the tyrosine to participate in the enzyme catalytic activity. Electronically, it has been suggested that it prevents formation of HO• by the enzyme. The modification is not universally conserved as it is not present in some prokaryotic forms, and so it cannot have a crucial role in preventing free radical formation *in vivo*. The electronic properties would, however, help to stabilise the tyrosine radical proposed in the mechanism based upon the human CDO structure.[11] It has been suggested that this modifica-

tion takes place by a single turnover of the enzyme using intramolecular substrates. Thus, electron transfer to the metal centre generates a phenyl radical on the tyrosine that is attacked by the protein cysteine thiol. Following electron transfer and proton loss this generates the mature cross-link.[8]

The CDO-catalysed reaction is the first step in the catabolic pathway of cysteine which yields pyruvate and sulfate as eventual end products (Scheme 1). The product of the CDO catalysed-reaction is also converted into taurine through two further reactions. Sulfur from methionine ultimately also passes through this same pathway after conversion into cystathionine, which is cleaved to yield cysteine and α-ketobutyrate.[14] A block in cysteine metabolism at CDO is thought to be involved in several disease states, including rheumatoid arthritis, liver disease, Parkinson disease, Alzheimer disease, motor neuron disease, and systemic lupus erythematosus. In these cases, blood levels of cysteine are elevated while taurine and inorganic sulfate are depleted.[8] This has several adverse
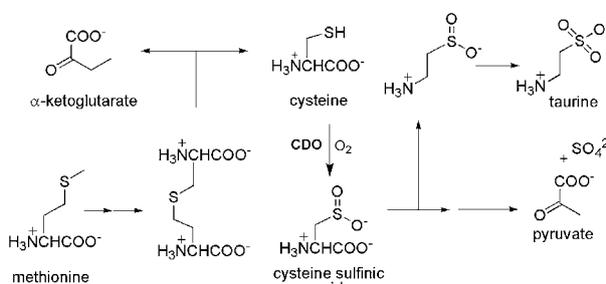
Scheme 1. Metabolic pathway for decomposition of sulfur-containing amino acids to pyruvate, and sulfate and production of taurine. Central is the CDO-catalysed conversion: cysteine to cysteine sulfinic acid using molecular oxygen.
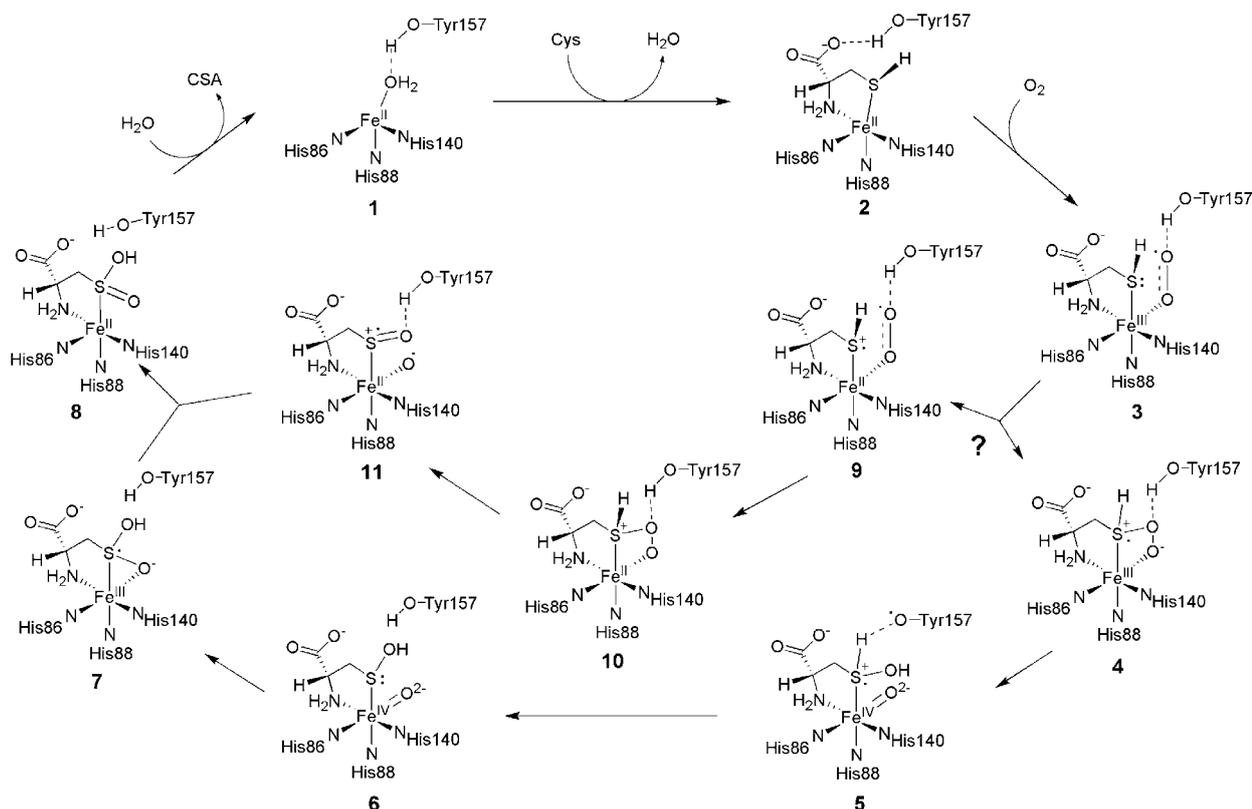
Scheme 2. The mechanisms proposed for CDO. It is not yet clear which pathway is followed after intermediate 3; the point of bifurcation is denoted by the question mark - see text for discussion.

effects. High levels of cysteine are toxic in rats,[15] and it is thought to be neuroexcitotoxic and can form toxic species also with other compounds.[16] Low levels of sulfate prevent sulfation out of proteins, and this is important in the modulation of many protein-protein interactions.[17] Finally, low levels of taurine have been associated with many abnormalities, mostly involving neuron and photoreceptor function.[18] CDO thus catalyses a reaction that is biologically very important.

X-ray crystallographic data from native forms of CDO from mouse and rat, and human CDO in complex with its substrate L-cysteine, have become available in the last few years. Based upon these, several mechanisms for the CDO catalysed reaction have been proposed. These differ in the order of S=O and S–O bond formation, the oxidation states of the iron, and the coordination/binding of the substrate. Scheme 2 summarises some of the more pertinent features of these mechanisms. The tetrahedral resting state **1** binds cysteine followed by oxygen to give the iron(III) superoxide **3**, which has an increased coordination number and is possibly octahedral. Ye *et al.*[11] have suggested that this is followed by attack of the distal oxygen on the thiol sulfur to produce the peroxo intermediate **4** that homolytically cleaves to form an Fe(IV)-oxo species **5**. This Fe(IV) species can then further oxidise the sulfur in two one-electron steps, *via* **6** and **7**, to produce the iron bound cysteine sulfinic acid **8** that can then be released. In contrast, Stipanuk[8] and Phillips[5] and their groups suggest that the thiol radical cation **9** is first produced through reduction of the iron. Radical recombination then produces the cyclic peroxo species **10**. Cleavage of the peroxide O-O bond in **10** results in the metal-bound sulfoxy-cation **11**, which can react with the activated iron oxygen atom, again to produce the metal bound **8**.

Unfortunately, these mechanisms at present lack spectroscopic or kinetic support, and are formulated purely from a study of the active site environment of the native and substrate-bound enzyme structures. Hence experimental testing of this proposal will be extremely valuable to understanding this exciting and unique enzyme system. The biological significance of understanding this system has already been made clear and due to the author's interests in neurodegenerative diseases[19] kinetic and spectroscopic investigations into the mechanism are being carried out in Dunedin.

References

1. Berry, J. F.; Bill, E.; Bothe, E.; George, S. D.; *et al. Science* **2006**, *312*, 1937-1941.
2. Que, L. Jr. *Acc. Chem. Res.* **2007**, *40*, 493-500.
3. Tiago de Oliveira, F.; Chanda, A.; Banerjee, D.; Shan, X.; Mondal, S.; *et al. Science* **2007**, *315*, 835-838.
4. Bollinger, J. M., Jr.; Price, J. C.; Hoffart, L. M.; Barr, E. W.; Krebs, C. *Eur. J. Inorg. Chem.* **2005**, 4245-4254; Price, J. C.; Barr, E. W.; Hoffart, L. M.; Krebs, C.; Bollinger, J. M., Jr. *Biochemistry* **2005**, *44*, 8138-8147; Price, J. C.; Barr, E. W.; Tirupati, B.; Bollinger, J. M., Jr.; Krebs, C. *Biochemistry* **2003**, *42*, 7497-7508.
5. McCoy, J. G.; Bailey, L. J.; Bitto, E.; Bingman, C. A.; *et al. Proc. Natl. Acad. Sci. USA* **2006**, *103*, 3084-3089.
6. Straganz, G. D.; Nidetzky, B. *ChemBioChem* **2006**, *7*, 1536-1548.
7. Duda, D. M.; McKenna, R. *Handbook of Metalloproteins* **2004**, *3*, 249-263.
8. Simmons, C. R.; Liu, Q.; Huang, Q.; Hao, Q.; *et al. J. Biol. Chem.* **2006**, *281*, 18723-18733.
9. Blasiak, L. C.; Vaillancourt, F. H.; Walsh, C. T.; Drennan, C. L. *Nature* **2006**, *440*, 368-371.
10. Kloer, D. P.; Ruch, S.; Al-Babili, S.; Beyer, P.; Schulz, G. E. *Science* **2005**, *308*, 267-269.
11. Ye, S.; Wu, X. a.; Wei, L.; Tang, D.; Sun, P.; *et al. J. Biol. Chem.* **2007**, *282*, 3391-3402.
12. Chai, S. C.; Bruyere, J. R.; Maroney, M. J. *J. Biol. Chem.* **2006**, *281*, 15774-15779.
13. Ito, N.; Phillips, S. E. V.; Stevens, C.; Ogel, Z. B.; *et al. Nature* **1991**, *350*, 87-90; Osako, T.; Ueno, Y.; Tachi, Y.; Itoh, S. *Inorg. Chem.* **2004**, *43*, 6516-6518.
14. Stipanuk, M. H. *Ann. Rev. Nutr.* **2004**, *24*, 539-577.
15. Karlsen, R. L.; Grofova, I.; Malthe-Sorenssen, D.; Fonnum, F. *Brain Res.* **1981**, *208*, 167-80.
16. Jameson, G. N. L.; Zhang, J.; Jameson, R. F.; Linert, W. *Org. Biomol. Chem.* **2004**, *2*, 777-782; Zhang, F.; Dryhurst, G. *J. Med. Chem.* **1994**, *37*, 1084-98.
17. Moore, K. L. *J. Biol. Chem.* **2003**, *278*, 24243-24246.
18. Huxtable, R. J. *Physiol. Rev.* **1992**, *72*, 101-63.
19. Jameson, G. N. L.; Linert, W. In *Free Radicals in Brain Pathophysiology*; Poli, G.; Cadenas, E.; Packer, L. (Eds.); Marcel Dekker Inc.: New York, 2000, 247-272; Linert, W.; Jameson, G. N. L.; Jameson, R. F.; Jellinger, K. A. In *Neurodegenerative Diseases and Metal Ions*; Sigel, A.; Sigel, H.; Sigel, R. K. O. (Eds.), Wiley & Sons Ltd.: Chichester, England, 2006, 281-305.

News Bits

A Podcast to Ponder

Looking for something to listen to on the way to work or while out walking the dog? Radiolab is an hour radio programme on New York's Public Radio, WNYC. You can download podcasts for free from *www.wnyc.org/shows/radiolab*

It has two excellent hosts and they talk to some interesting people about various scientific topics in an easy to understand and entertaining way.

A wide range of topics is covered including emergence (which covers everything from bees to Google) to shows on morality, placebo effect and another on space. Currently there are three seasons of five shows available online. Definitely worth checking out.

Scientific Paper Organiser

An application for organising scientific papers on your computer won the 2007 Apple Design Award for Best Scientific Computing solution. Papers 1.0 was borne out of the developers' frustration with organising their downloaded copies of scientific articles. It is written by two Dutch scientists, who did their PhDs together at the Netherlands Cancer Institute.

The application has an easy to use interface and lets the user import already downloaded pdfs as well as integrating with the PubMed search engine with full text, open access. It has a host of other clever, useful features that you can read about at *http://mekentosj.com/papers*