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Review

Post-translational self-hydroxylation: A probe for oxygen activation mechanisms in non-heme iron enzymes ☆

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Abstract

Recent years have seen considerable evolution in our understanding of the mechanisms of oxygen activation by non-heme iron enzymes, with high-valent iron-oxo intermediates coming to the forefront as formidably potent oxidants. In the absence of substrate, the generation of vividly colored chromophores deriving from the self-hydroxylation of a nearby aromatic amino acid for a number of these enzymes has afforded an opportunity to discern the conditions under which O_2 activation occurs to generate a high-valent iron intermediate, and has provided a basis for a rigorous mechanistic examination of the oxygenation process. Here, we summarize the current evidence for self-hydroxylation processes in both mononuclear non-heme iron enzymes and in mutant forms of ribonucleotide reductase, and place it within the context of our developing understanding of the oxidative transformations accomplished by non-heme iron centers.

Keywords: Non-heme iron; Oxygen activation; Post-translational modification; Self-hydroxylation; Ribonucleotide reductase; α-Ketoglutarate dependent enzymes

Non-heme iron centers are utilized throughout living systems to activate dioxygen to catalyze a remarkably diverse array of transformations [1–3]. The potential oxidizing power of this class of enzymes is strikingly illustrated by methane monooxygenase (MMOH), which is capable of breaking the high energy C—H bond (104 kcal/mol) in methane to yield methanol [4,5]. This potent reactivity stems from the generation of high-valent iron-oxo intermediates through pathways that involve either a dinuclear or mononuclear iron center (Scheme 1).

For mononuclear non-heme iron enzymes, two distinct pathways are proposed for the generation of the high-valent intermediate (cf. Scheme 1). In the first, associated with the Rieske dioxygenases, binding of O_2 and injection of an electron from a nearby iron–sulfur cluster lead to an iron(III)-peroxo species, and subsequent cleavage of the O—O bond presumably generates an iron(V)-oxo species [2]. This scheme is mechanistically analogous to the generally accepted process in the heme-containing cytochrome P450 enzymes [6,7]. In the second pathway, the iron center utilizes an external cofactor, such as α-ketoglutarate (α-KG) or pterin, to supply two electrons for O_2 reduction. This process generates an iron(IV)-oxo species [8–10], which has recently been shown to be responsible for substrate oxidation in the α-KG dependent enzyme TauD [11].

Diiron non-heme enzymes also share a common oxygen activation process (cf. Scheme 1), which begins with O_2

☆ *Abbreviations:* α-KG, α-ketoglutarate; AlkB, alkylated DNA repair enzyme; ENDOR, electron nuclear double resonance; EPR, electron paramagnetic resonance; ESI-MS, electrospray ionization mass spectrometry; HPPD, 4-hydroxyphenylpyruvate dioxygenase; HppE, (*S*)-2-hydroxypropylphosphonic acid epoxidase, MALDI-TOF, matrix assisted laser desorption ionization-time of flight; MMOH, hydroxylase component of methane monooxygenase; PheH, phenylalanine hydroxylase; PMI, phosphomannose isomerase; R2, the R2 subunit of ribonucleotide reductase; TauD, taurine/α-KG dioxygenase; TfdA, 2,4-dichlorophenoxyacetate/α-KG dioxygenase; TyrH, tyrosine hydroxylase.

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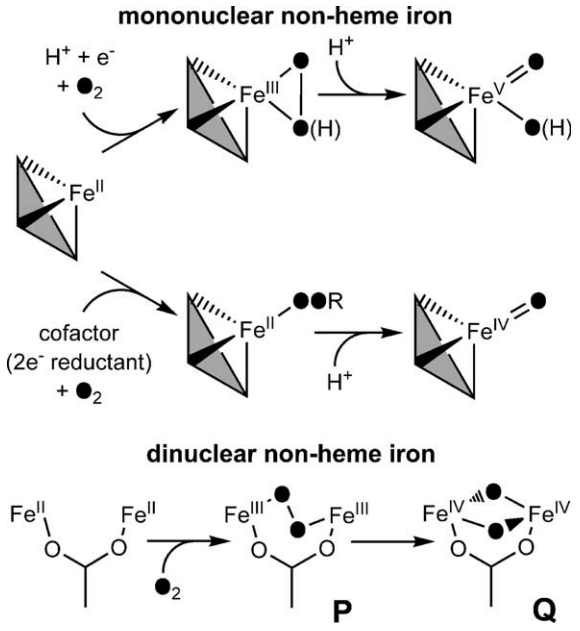

Scheme 1. General pathways for the activation of dioxygen to yield high-valent iron-oxo intermediates in both mononuclear and dinuclear non-heme iron enzymes.

binding to the diiron(II) center, forming a μ-1,2-peroxo diiron(III) complex, termed intermediate **P** (or H_{peroxo}) in the MMOH catalytic cycle. The O—O bond then is cleaved to yield an oxo-bridged diiron(IV) moiety termed **Q** [1,5]. Oxidative transformation of the substrate may be effected by either of these species [12]. Moreover, the situation can be further complicated by one-electron steps, which are required for the assembly of the diiron-tyrosyl radical center of the R2 protein of ribonucleotide reductase from *Escherichia coli* [13].

O_2 activation in the non-heme iron enzyme family is a well-regulated process, as the substrate is typically required to prime the active-site iron for the binding and reduction of oxygen to generate high-valent iron-oxo intermediates [1,14]. Nevertheless, this process occasionally appears to break down, with unexpected results. Under certain circumstances, O_2 (or its reduced counterparts) can be activated in the absence of substrate in the active site, in which case the high-valent intermediate produced must find an alternative reductive outlet, typically at the expense of further catalytic activity, in a process termed oxidative inactivation. One reductive pathway, which has attracted much recent attention, involves the oxidation of an aromatic amino acid located near the active site to yield a hydroxylated amino acid residue. In some instances, these hydroxylated amino acids remain coordinated to the iron center, generating brightly colored complexes that are amenable to analysis by a variety of techniques, as shown in Table 1. Herein, we describe the oxidative self-hydroxylation of several non-heme iron enzymes, as well as the spectroscopic evidence used to characterize these modified enzymes.

Ribonucleotide reductase

Chronologically, a mutant form of ribonucleotide reductase R2 (RNR R2, from *E. coli*) was the first example of a self-hydroxylation process recognized in non-heme iron enzymes and was thus subjected to a detailed structural and spectroscopic analysis. The oxygen activation

Table 1
UV/vis absorption maxima and spectroscopic methods used to characterize non-heme iron enzymes that self-hydroxylate an aromatic amino acid residue

Residue	Oxidative modification	Enzyme	λ_{max} (nm)	ε_{max} (M^{-1} cm^{-1})	Spectroscopic methods
Phe	*m*-OH-Phe	Y122F/E238A R2	515	Not reported	XRD
		W48F/D84E R2	550	1000–2100	XRD, rR
		TyrH-7,8-dihydrobiopterin[a]	—	—	XRD
	Phenoxy radical	Y122H R2	—	—	ENDOR
	Tyrosinate	HPPD	595	2600	rR
		Y325F PheH	—	—	ESI-MS
Tyr	DOPA	F208Y R2	720	2500	XRD, rR
		Y122F/F208Y R2	ca. 700	Not reported	—
		W48F/F208Y R2	675	Not reported	Mössbauer
		TauD-α-KG	550	700	rR
		TauD-succinate	720	380	rR
		PMI	680	2100	rR
		HppE	680	450	rR
Trp	Hydroxyindole	TfdA-α-KG	580	1000	rR
		AlkB-α-KG	590	960	ESI-MS
		Y325F PheH	—	—	ESI-MS

Abbreviations: α-KG, α-ketoglutarate; AlkB, alkylated DNA repair enzyme; DOPA, dihydroxyphenylalanine; ENDOR, electron nuclear double resonance; ESI-MS, electrospray ionization mass spectrometry; HPPD, 4-hydroxyphenylpyruvate dioxygenase; HppE, (*S*)-2-hydroxypropylphosphonic acid epoxidase; PheH, phenylalanine hydroxylase; PMI, phosphomannose isomerase; R2, the R2 subunit of ribonucleotide reductase; rR, resonance Raman; TauD, taurine/α-KG dioxygenase; TfdA, 2,4-dichlorophenoxyacetate/α-KG dioxygenase; TyrH, tyrosine hydroxylase; XRD, X-ray diffraction.

[a] The observed self-hydroxylation [54] is an artifact of the crystallography conditions and is not catalytically relevant [55].