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A Review on Biologically Active Oxo-Bridged Diiron (III, III) Centers

Dr. Suranjana Chatterjee

Assistant Professor, Department of Chemistry, Ananda Mohan College, Kolkata 700009

Abstract:
Particular metal ions played vital role in functioning of enzymes. In this following discussion we concentrate over oxo bridged diiron III,III centers. Fe–O–Fe is playing important role in many enzymes like hemerythrin, ribonucleotide reductase, methane monooxygenase etc. Presence of metal ions in active centers may facilitate the electron transfer process.

Keywords: Oxo-bridge, Di-iron, Hemerythrin, ribonucleotide reductase, methane monooxygenase.

Introduction

The omnipresence of Fe-O-Fe unit in iron chemistry has been abundantly established through chemical and biological studies over the years. Its ubiquitous involvement in the active sites of many metalloenzyme systems including hemerythrin (Hr), ribonucleotide reductase (RNR), methane monoxygenase (MMO), purple acid phosphatase (PAP), etc has been amply demonstrated. All these enzymes have di- or polynuclear iron cores in their active sites(Table 1).
The great stability of Fe^{III}-O-Fe^{III} unit is arising out of the superexchange of the two d^5 high spin iron(III) centers linked by an oxo-bridge. But the high spin iron(II) and iron(III) are probably less strongly bound to O^{2-}, since both the oxidation states having two antibonding electrons are directed towards the formal bond axes. This would impart weaker Fe^{II}-O-Fe^{III} and Fe^{II}-O-Fe^{II} bonds, both of which should be rapidly broken by aquation. In fact, the oxo-bridge in the mixed-valent system Fe^{II}-O-Fe^{III} is not much common and in general putative outside a protein environment.

Table 1: Diiron oxo proteins: their abbreviations and biological functions.

Protein	Biological function	Occurrence	Ref
Hemerythrin (Hr)	Oxygen transport by dioxygen reversible binding	Marine invertebrates	1
Ribonucleotide reductase (RNR)	Reduction of ribo- to deoxyribonucleotides by tyrosyl radical generation	Animals, viruses, bacteriophases, prokaryotes	2
Purple acid phosphatases (PAP)	Hydrolysis of phosphate ester	Mammals, plants	2a, 3
Methane mono oxygenase (MMO)	Oxidation of methane to methanol	Methanotropic bacteria	4
Ferritin	Iron storage	Most life forms	5

Toluene-4-monoxygenase	Toluene-4-hydroxylation	*Pseudomonas sp.*	6
Stearoyl-acyl carrier protein Δ^9-desaturate	Desaturation of alkane to alkene	Mammals, plants	7
Ruberythrin	NADH peroxidation	Anaerobic bacteria	8

The lack of information on reactivity of Fe-O-Fe unit is most probably due to the great stability of the μ-oxo diferric unit that translates to inertness under a variety of conditions and in fact, one likely reason for large number of the synthetic complexes is that the Fe–O–Fe unit is difficult to avoid in ferric chemistry. A second reason may be the gross instability of the mixed-valent and diferrous units that translates to a lack of selectivity.

Hemerythrin (Hr)

Hemerythrin, among the non-home diiron proteins, is the only example of dioxygen carrier which is found in a limited number of marine organisms. Because of its low abundance, Hr may be a dead end of evolution and is even called a 'Cinderella' in the family of the dioxygen carriers. The chemistry of this metalloprotein, however, was extensively studied and provided an important structural, spectroscopic and mechanistic benchmark for understanding other more complicated diiron systems. The two iron atoms in Hr are bridged by one oxygen atom and two bidentate carboxylate groups from glutamate and aspartate amino acid residues. The $[Fe_2O_2(O_2CR)_2]^{2+}$ core structure is consistent with magnetic and spectroscopic data on met forms of the protein, indicating two strongly antiferromagnetically coupled ($J \approx -134\ cm^{-1}$) high-spin Fe^{III} centers.

Magnetic susceptibility, Mössbauer, MCD (magnetic circular dichroism), EXAFS, ^1H NMR and low-resolution (3.9 Å) X-ray data on deoxyHr suggest the presence of a $[Fe_2(OH)(O_2CR)_2]^+$ core with one five coordinated (Fe_A) and one six-coordinated (Fe_B) high spin Fe^{III} center and weak antiferromagnetic coupling ($J \approx -15\ cm^{-1}$). Similar studies, including resonance Raman spectroscopic experiments, indicate that oxyHr is best formulated as a hydroperoxy derivative of the (μ-oxo)bis(μ-carboxylato)diiron(III) core, with hydroperoxide ligand filling the sixth coordination site of Fe_A. Thus the reversible dioxygen binding reaction of Hr may be viewed as oxidative addition and reductive elimination of O_2 to the diiron center.

Since dioxygen binding to *P. gouldi* Hr is pH-independent, internal proton transfer from bridging hydroxide ligand in deoxyHr to the hydroperoxide group in oxyHr is postulated (Figure 1).[17] Moreover, resonance Raman studies of oxyHr, including O and ^2H isotope substitution experiments, suggest that the proton on the hydroperoxide ligand is hydrogen bonded to the μ-oxo group. This hydrogen bond may be a key component that facilitates the kinetics of oxygen binding reactions. Protonation of the bridging oxygen atom results in elongation of Fe-O bridge bonds by ca. 0.17 Å. The corresponding reduction in bridge bond order eliminates the structural *trans* influence on the pyrazole ring nitrogen atom *trans* to the bridging oxygen atom, and substantially diminishes the antiferromagnetic exchange interaction.

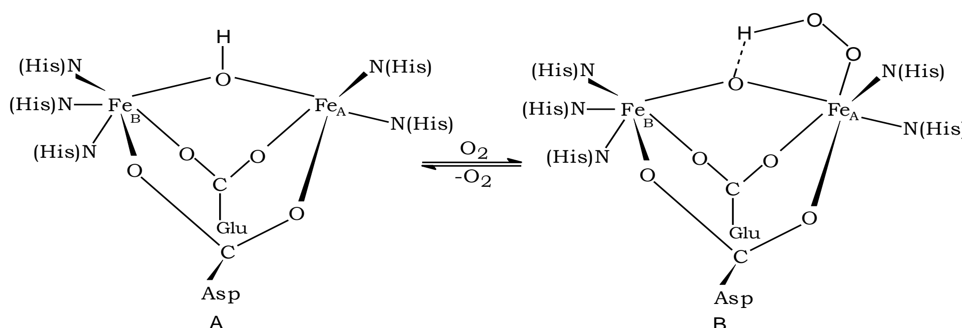

Figure 1. Proposed model for reversible oxygen binding to Hr, A = deoxyHr and B = oxyHr.

Another point of interest about Hr is the existence of various redox states of Hr-deoxy ($Fe^{II}Fe^{II}$), oxy ($Fe^{III}Fe^{III}$), functionally inactive met form ($Fe^{III}Fe^{III}$), several mixed-valent semimet ($Fe^{II}Fe^{III}$) forms are known. Numerous kinetic studies of electron transfer to, from, and among these forms have been reported.[10]
Although the main features of the functional center in Hr are reasonably known and well understood, the protein continues to be a focus for physical and chemical studies because it is the prototype for other oxo-bridged diiron proteins about which much less is known.

Ribonucleotide Reductase (RNR)

RNR is a key enzyme for all living organisms as it catalyses the formation of deoxyribonucleotide di- or triphosphates, the committed first step in DNA synthesis. Three types of RNR have been identified, each possessing a different metal cofactor: one requires adenosyl–cobalamin (vitamin B_{12}); the second, iron and and last, manganese. The iron enzyme has been found in animals, certain bacteria and virus affected mammalian cells. The enzyme forms *Escherichia coli* is the best characterised and available data on other iron containing systems are generally consistent with the results obtained on the *E-coli* enzyme.
RNR from *E-coli* contains two homodimeric proteins that can be separated during purifications. Each polypeptide chain of small protein named protein R_2 contains a stable radical located on tyrosine-122 as well as a binuclear iron center in which Fe(III) irons are antiferromagnetically coupled by a μ-oxo bridge. Protein R_2 has been recrystallised and a refined 3D structure is available. Results obtained from different spectroscopic technique[11] support an oxo-bridged diiron formulation (Figure 2).
The tyrosine radical is found buried within the protein[12] and known to react with a large number of radical scavengers (hydroxyurea, hydroxylamine, etc.), antioxidants and reductants. A few reports have shown that electrons could be transferred to the deeply buried diiron(III) center in the protein *via* a long range electron transfer chain resulting in the formation of a reduced R2 is a key step during enzymatic activation of protein R2 since the tyrosyl radical can only be generated during the reoxidation of Fe(II) center by O_2. There is no evidence that the dinuclear center plays a role in catalysis; instead it has been proposed that the dinuclear iron center is involved in the general and stabilization of the tyrosine radical[11].

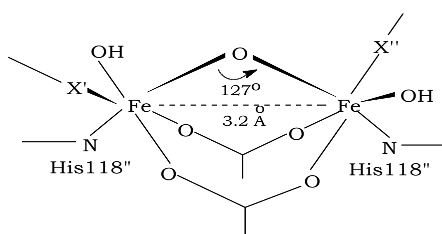

Figure 2. Postulated structure[27] of diiron center in RNR. N presents histidine nitrogen atom; OH, a hydroxide anion; X, an assigned ligand. Ligands from different subunits are designated by superscripts ' and ''.

Purple acid phosphatases (PAP)

Purple acid phosphatases (PAP) are transition metal containing glycoproteins and have been isolated from a variety of microbial, plant and animal sources. The most extensively characterised PAP's, uteroferrin and beef spleen PAP contain two iron atoms per molecule [13]. They occur in purple (λ_{max} = 550 – 575 nm), catalytically inactive diiron(III) form and an enzymatically active mixed-valence pink (λ_{max} = 505 – 515 nm) diiron(II,III) form. Treatment of the isolated enzymes with mild reductants affects a blue shift in the maximum absorbance from 550 to 510 nm (purple to pink).

PAP's catalyze the dephosphorylation of phosphoproteins and nucleotides and dephosporylation of phosphoproteins and nucleotides and are assayed by their ability to hydrolyze p-nitrophenylphosphate to p-nitrophenol and inorganic phosphate.

The magnetic and ESR properties of mixed-valent form of the enzymes obtained from beef spleen and porcine uterine fluid (uteroferrin) resemble those of semimetHr. As a consequence it is suggested that $[Fe_2O]^{3+}$ core might be present in these enzymes[14]. X-ray absorption studies of PAP reveal an Fe...Fe distance of 3.00 Å, consistent with a bridged binuclear iron center, Fe–$O_{tyrosine}$ linkages of 1.8–1.9 Å, which prohibit positive identification of Fe–$O_{\mu\text{-oxo}}$ linkages, and an Fe...P (of phosphate) distance of 3.06 Å assigned to a monodentate phosphate ligand. From this information, the structures shown in Figure 3 have been proposed for the binuclear iron center in PAP[14].

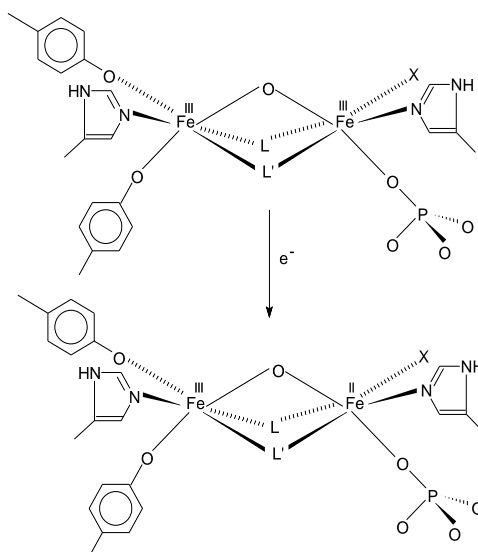

Figure 3. Proposed structures for the oxidised (purple) (top) and reduced (pink) (bottom) forms of PAP. L' and X are unidentified.

Methane Monooxygenase (MMO)

Methanotrophs are bacteria capable of utilizing methane as their sole energy and carbon source. These organisms oxidised methane to CO_2 *via* CH_3OH, HCHO and H_2CO^- [15]. The first and most demanding step of this reaction sequence, the conversion of CH_4, is catalysed by the enzyme methane monooxygenase (MMO).

MMO's are capable of inserting a single oxygen atom from dioxygen into the C–H bond of a variety of hydrocarbons in addition to the *in vivo* substrate methane[15]. Two types of MMO's have been isolated: a membrane bound copper containing enzyme and a soluble non-heme iron containing protein (the focus of this discussion) known as sMMO.

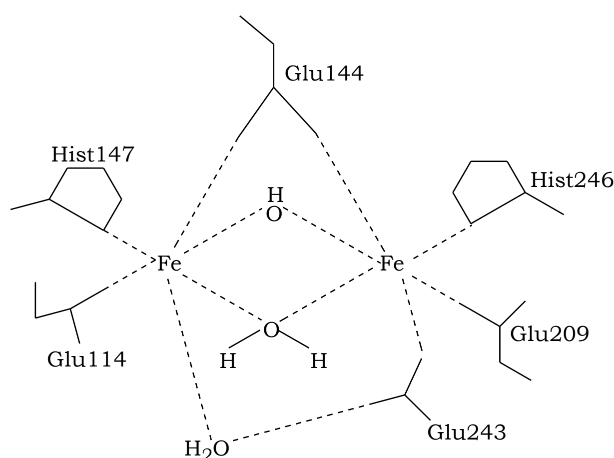

Figure 4. Proposed structure of sMMO

Non-heme iron containing MMO was first isolated from *Methylococcus capsulatus* and it consists of three protein components A–C. The active site of the enzyme appears to consist of a binuclear iron center capable of accusing the redox states $[Fe^{III}Fe^{III}]$, $[Fe^{III}Fe^{II}]$, and $[Fe^{II}Fe^{II}]$[16].

Crystal structure data available for sMMO from a number of different investigations[4] have served as a valuable starting point of many mechanistic studies. EPR spectral data[16] of the component A on comparison with those similar studies on Hr, RNR and PAP strongly suggest that MMO's contain a dinuclear oxo- or hydroxo- bridged iron center. Hence EPR silent oxidised form of component A presumably corresponds to an antiferromagnetically coupled Fe(III)–Fe(III) pair. Considering all points the proposed structure (Figure 4) of $(sMMO)_{ox}$ by Kurtz[17] is shown above.

The recognition of these iron-oxo proteins as a separate class has stimulated efforts on the part of inorganic chemists to prepare and characteristic model compounds that replicate the physical properties and functions of the polymetallic protein cores. As a consequence, a variety of oxo-bridged di-, tri-, tetra-, hexa-, octal-, and undecarion aggregates have been synthesisd. Starting from early thirties to the new millennium enormous number of dinuclear iron complexes with oxo, hydroxo, alkoxo, peroxo or carboxylato bridge(s) have been synthesised and extensively studied depending on their relevance as model complexes for the mentioned metalloenzymes or intermediates in the catalytic cycles of these metalloenzymes[18]. With dioxygen as an oxidant diiron complexes have shown significant catalytic activity toward alkane oxidation. While a large number of unsupported μ-oxo bridged diiron complexes have been structurally characterized[19] to our knowledge only a few diiron complexes linked by an

unsupported μ-hydroxo bridge are known. However, mono μ-oxo-bridged dinuclear ferric complexes will be the focus of the following part of this chapter with some relevant mixed-valent forms.

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