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Functional models of non-heme diiron enzymes

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Contents

Abstract

Diiron complexes are structural models of active centers of a variety of enzymes (methane monooxygenase, ribonucleotide reductase and purple acid phosphatase). More recently, they proved to have the potential to catalyze the oxidation of alkanes, alcohols, sulfides by peroxides or molecular oxygen (in the presence of electrons) and the hydrolysis of phosphodiesters. By selection of appropriate ligands, some reactions can be made highly selective, as shown from our report of the first enantioselective oxidation catalyzed by a chiral μ-oxo diiron complex.

Keywords: Dioxygen activation; Hydrogen peroxide; Hydrolysis of phosphodiesters; Methane monooxygenase; Non-heme diiron complexes; Ribonucleotide raductose

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1. Introduction

Metalloenzymes with non-heme diiron centers in which the iron ions are bridged by an oxide (or a hydroxide) and carboxylates (glutamate or aspartate) have emerged as an important class of enzymes in the past decade [1]. Several members of that class, isolated from mammals, plants or bacteria, are now structurally characterized [2].

The first structurally characterized protein was hemerythrin (Hr), which is the O_2 carrier protein in marine invertebrates (Fig. 1). To our knowledge, this is the only example of a diiron protein involved in oxygen transport [3].

In contrast, it appears that this type of metal center has been frequently utilized by living organisms for oxygen activation and substrate oxidation [4]. Well-known examples are the iron centers in ribonucleotide reductase from *Escherichia Coli* and eukaryotes, methane monooxygenase from methanotrophic bacteria, plant desaturases and a variety of bacterial monooxygenases.

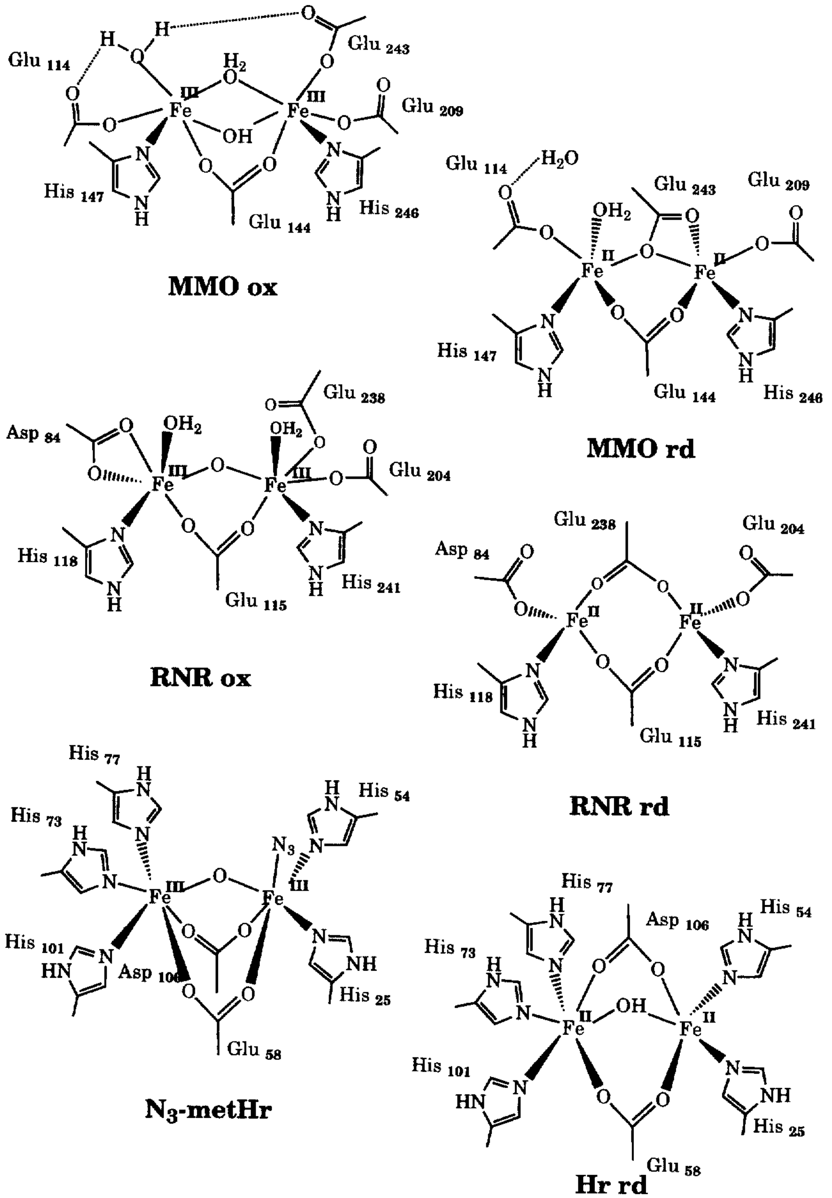
In the small protein of class I ribonucleotide reductase (RNR), the diiron center serves to oxidize an endogenous tyrosine into a tyrosyl radical [5–7]. This radical is essential for initiating the conversion of ribonucleotides to deoxyribonucleotides, the DNA precursors. The oxo-bridged diiron center, shown in Fig. 1, also has the potential to catalyze hydroxylation reactions, as shown from the transformation of a tyrosine residue, introduced in the active iron center by site directed mutagenesis, into 3,4-dihydroxyphenylalanine [8].

The hydroxylase component of the methane monooxygenase (MMO) [4,9,10] contains a hydroxo-bridged diiron center (Fig. 1) which catalyzes a remarkable reaction, the oxidation of methane to methanol by molecular oxygen. MMO is also able to oxidize a great variety of alkanes, alkenes and aromatic compounds [11].

The plant soluble stearoyl–acyl carrier protein (ACP) Δ^9-desaturases introduces a *cis* double bond at the 9,10 position of stearoyl–ACP to form oleyl–ACP [12]. They have both sequence and structural homology with ribonucleotide reductase and methane monooxygenase (Fig. 1). There are also a number of related membrane-bound desaturases found in mammals and plants, which have not been structurally characterized but are likely to contain a diiron complex in their active sites [1].

Bacterial toluene monooxygenase, phenol hydroxylase and alkene monooxygenase show significant homology to MMO [13]. Some membrane-bound hydrocarbon hydroxylases such as xylene monooxygenase or alkane hydroxylase also contain a diiron center [14,15]. It has recently been suggested from sequence analyses that diiron clusters might be present in a larger number of enzymes, thus indicating a much greater role of these clusters in O_2 activation biochemistry than previously appreciated.

Finally, diiron centers have been found in mammalian purple acid phosphatases (PAP), which catalyze the hydrolysis of phosphate esters at low pH [16]. The purple color originates from a charge transfer transition from a tyrosinate ligand to a ferric iron. The active enzyme contains one ferric ion and one ferrous ion [3,17,18]. In contrast, the plant enzyme has an Fe^{3+}–Zn^{2+} center. Only the latter has been structurally characterized (Fig. 2) [19]. However, it is very likely that the mammalian enzyme active site has a very similar metal coordination to that of the plant Fe–Zn



MMO ox

MMO rd

RNR ox

RNR rd

N$_3$-metHr

Hr rd

Fig. 1. Structural representation of different redox states of the diiron site of non-heme iron enzymes.

enzyme. This is shown from spectroscopic data and from the observation that exchange of Zn(II) for Fe(II) gives a plant enzyme with nearly identical spectroscopic and kinetic behavior to the mammalian enzyme. It is interesting to note that an Fe–Zn center has also been found in the structures of Ser/Thr-specific protein phosphatases, such as calcineurin [20], and is likely to be present in a number of

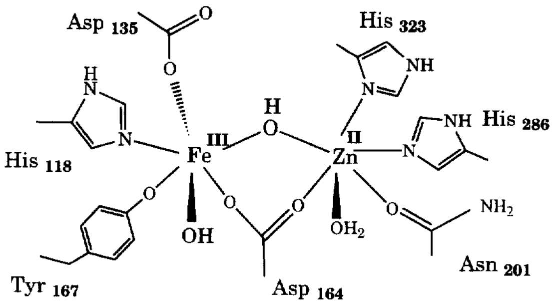


Fig. 2. Structural representation of the active site of purple acid phosphatase from kidney bean.

other phosphomonoesterases, in diadenosine tetraphosphatase, in exonucleases and nucleotidases [16].

A crucial contribution to the understanding of the spectroscopic properties and the reactivity of the biological diiron sites came from the design and characterization of structurally characterized model diiron complexes [21]. Such simple complexes display similar light absorption spectra, dominated by oxo-to-iron charge transfer [22,23], and strongly enhanced Fe–O–Fe vibration in their Raman resonance spectra [24]. They also remarkably reproduce the magnetic properties, characterized by a strong antiferromagnetic coupling between the two iron ions [4,21].

It was, however, only recently that some were able to mimic enzyme activities and to catalyze the hydroxylation of C–H bonds by molecular oxygen or hydrogen peroxide or the hydrolysis of phosphate esters. Here we briefly review this field and report some of our recent results on bipyridine- and phenanthroline–iron complexes as functional models of the dinuclear iron centers. Mononuclear non-heme iron catalysts have also been investigated [25,26] but are not discussed in this paper.

2. Mechanism of dioxygen activation and oxidation reactions catalyzed by methane monooxygenase and ribonucleotide reductase

In the recent past, the increased structural information, together with spectroscopic and kinetic studies, allowing the detection and characterization of key intermediates of the catalytic cycle, has clarified the mechanism of reductive oxygen activation by RNR and MMO enzymes (Fig. 3).

2.1. The diiron(II) intermediate

It is clear that it is the diiron(II) form that interacts with O_2 to generate the active oxidizing species responsible for the oxidative transformations performed by these enzymes [27]. In both enzymes, this first intermediate has been structurally characterized (Fig. 1) [2,28]. Even though the synthesis of reduced model complexes is quite challenging, because ligands bound to Fe(II) are kinetically labile, a few

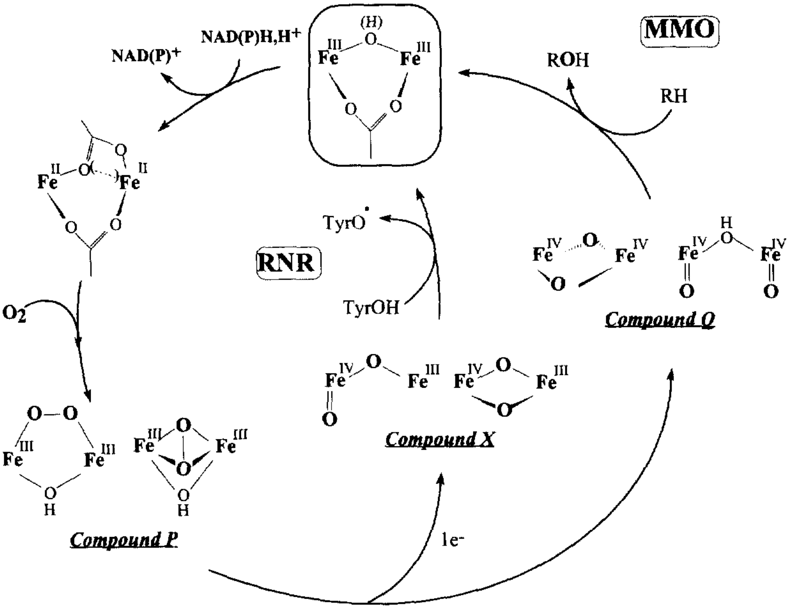


Fig. 3. Proposed catalytic cycle for MMO and RNR.

diferrous complexes representing good structural models for the reduced forms of RNR and MMO have been prepared and characterized [29–31].

Spectroscopic evidence for transient oxidizing species, rapidly freeze-quenched, has been uncovered in the reaction of O_2 with diiron(II) centers of RNR and MMO [4]. In the case of MMO, compounds **P** and **Q** have been reported; both are kinetically competent to hydroxylate methane. In the case of RNR, compound **X** is responsible for the one-electron oxidation of the tyrosine residue.

2.2. *The diiron–peroxo intermediate*

Compound **P** is the first intermediate detected in the reaction and then decays to compound **Q**. Based on chemical considerations and its Mössbauer properties, it has been proposed that compound **P** is a diiron(III)–peroxide species [32,33]. Very recently, three model complexes of compound **P**, with comparable spectroscopic properties, have been structurally characterized [34–36]; in these models O_2 is bound in a *cis* or *trans* μ-1,2-peroxo form. As in the enzyme, they were all generated during reaction of diiron(II) precursors with molecular oxygen.

That O_2 binds to the iron center through an oxidative addition is consistent with the fact that MMO can function with hydrogen peroxide as the oxidant in the absence of electrons and oxygen [37]. In this reaction, compound **P** is also likely to be an intermediate.

2.3. *The high-valent diiron intermediate*

In MMO, compound **Q** has been identified as a coupled Fe(IV)Fe(IV) species, on the basis of its Mössbauer properties [32,38]. While different structures for compound **Q** are possible, there is now some spectroscopic evidence (X-ray absorption fine structure and Mössbauer spectroscopy) that the diiron center in compound **Q** is a high-valent $Fe_2(\mu\text{-}O)_2$ diamond core [39]. In the proposed mechanism, reaction products are derived from the two-electron oxidation of the substrate by compound **Q**.

Even though no diiron(IV) model complex is available yet, some recent observations strongly support the notion that high-valent iron complexes may play a role in alkane hydroxylation mediated by non-heme iron systems. For example, a dinuclear $S=3/2$ Fe(III)Fe(IV) complex, containing an $Fe_2(\mu\text{-}O)_2$ core, has been generated during reaction of H_2O_2 with (μ-oxo)diiron(III)(tpa) [tpa = tris(2-pyridylmethyl)amine] (see Fig. 4 for synthetic ligands) [40]. This complex can carry out oxidation reactions corresponding to those associated with MMO (oxidation of ethylbenzene), desaturase (desaturation of ethylbenzene), or RNR (oxidation of 2,4-di-*tert*-butylphenol to the corresponding phenoxyl radical) [41].

In the case of RNR, only one intermediate, named compound **X**, could be detected and characterized [42]. Compound **X**, an $S=1/2$ species, contains only one oxidizing equivalent above the diferric state and is catalytically competent for oxidizing the tyrosine residue to its essential radical state. While it has first been described as a coupled system consisting of two high-spin Fe(III) plus a ligand radical, recent reanalysis of the ENDOR and Mössbauer results now strongly suggests that compound **X** carries a spin-coupled Fe(III)Fe(IV) diiron center [43,44]. Accordingly, an $S=1/2$ model Fe(III)Fe(IV) complex, containing an $Fe_2(\mu\text{-}O)_2$ core, has been obtained during reaction of H_2O_2 with a (μ-oxo)diiron(III)(6-Me-tpa) [6-Me-tpa = N-(6-methyl-2-pyridylmethyl)-N,N-bis(2-pyridylmethyl)amine] complex. Its EPR properties are strikingly similar to those of compound **X** [45].

The fact that no intermediate corresponding to compounds **P** and **Q** could be detected does not necessarily mean that they are not transiently formed. Electron transfer to the metal site may be too fast and prevent accumulation of such species [46]. Nevertheless, two-electron, and not only one-electron, oxidation has been observed in the case of RNR, suggesting the intermediate formation of a compound **Q**-like species, with two oxidizing equivalents above the diiron(III) state [8].

3. Dinuclear iron model complexes as new catalysts for the oxidation of hydrocarbons

The ability of MMO to catalyze the oxidation of methane to methanol has fascinated chemists interested in C–H activation. There has thus been a significant but still limited amount of interest in the use of dinuclear iron complexes as catalysts for alkane, alkene, arene or sulfide functionalization. These complexes may, however, have some industrial applications (oxidation of cyclohexane, for example), either for homogeneous catalytic oxidation or for depollution processes.

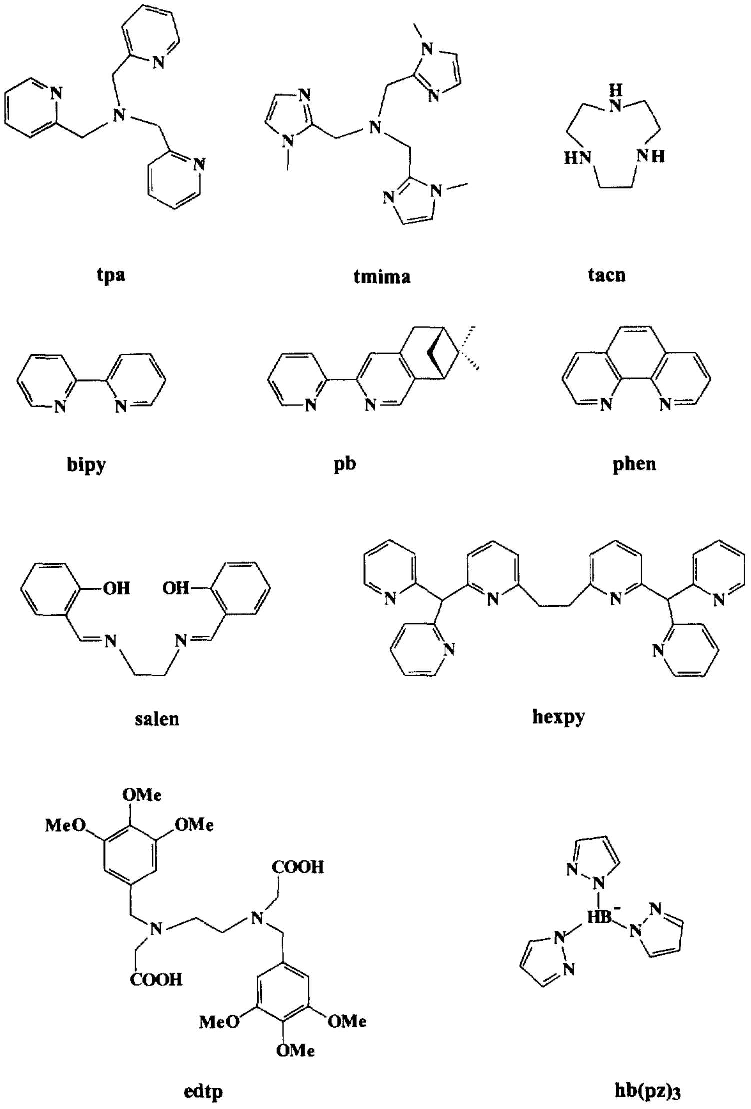


Fig. 4. Synthetic ligands for model complexes.

3.1. *Oxygen activation for oxidation catalyzed by diiron complexes*

The most biologically relevant model systems are those utilizing molecular oxygen and a source of electrons that would achieve a reductive activation of oxygen for substrate oxidation. However, this is highly challenging as it requires conditions for allowing, in a homogeneous medium, a highly oxidizing intermediate species to react with an alkane, for example, before being reduced by the excess of the reducing

Table 1
Alkane oxidation by O_2/reductant catalyzed by μ-oxo diferric complexes

Complex	Solvent	Reductant	Substrate	Catalytic activity	Ref.
$Fe_2O(OAc)_2[hb(pz)_3]_2$	CH_2Cl_2	Zn	Cyclohexane[a]	1.8 TN/30 h	[47]
$Fe_2O(hfacac)_2[hb(pz)_3]_2$	CH_2Cl_2	Zn	Cyclohexane[a]	4.7 TN/30 h	[48]
$Fe_2O(OAc)_2(bipy)_2Cl_2$	CH_3CN	Zn	Cyclohexane[b]	2.5 TN/3 days	[49]
$Fe_2O(salen)_2$	C_5H_5N	Ascorbate or mercaptoethanol	Adamantane	3–4 TN/4 h	[50]

[a] Cyclohexanol and cyclohexanone are formed.
[b] Cyclohexanone is formed.

agent. Monooxygenases have remarkably solved this problem and during enzyme catalysis electrons are not consumed for reducing the high-valent active iron complex. The very few such reported model systems were not effective enough to find interesting applications. Only a few turnovers in several hours were obtained, with zinc powder [47–49], mercaptoethanol or ascorbate as the electron source [50], as shown in Table 1.

Recently, we showed that a diiron complex could be prepared with an ethylenediamine tetraacetic acid (EDTA) derivative, named edtp, in which two carboxylato moieties have been replaced by two phenyl groups serving as substrates for the oxidation reaction (Fig. 4) [51]. When the complex was incubated in acetonitrile/water with an excess of ascorbate under aerobic conditions, a new complex was formed with an excellent yield within less than 30 min. The complex, characterized by UV–visible and EPR spectroscopies, mass spectrometry, was found to be a mononuclear iron complex resulting from the hydroxylation of one of the phenyl groups of the ligand (as demonstrated by ^1H and ^{13}C NMR spectroscopy and mass spectrometry of the isolated oxidized ligand) and binding of the new phenol group to iron (Fig. 5). The oxygen atom derives from molecular oxygen as shown from experiments carried out in the presence of $^{18}O_2$. When $H_2{}^{18}O$ was used as the solvent, no incorporation of ^{18}O in the ligand could be detected by mass spectrometry, indicating a true reductive dioxygen activation mechanism.

This reaction is a model of the hydroxylation of a tyrosine residue into 3,4-dihydroxyphenylalanine, within a mutant of ribonucleotide reductase, recently reported [8]. As in the enzyme the hydroxylated reaction product becomes a ligand of the iron center, resulting in a charge transfer band in the visible region and thus in the coloration of the complex, and in its inactivation. The same product was obtained when the model complex was treated with hydrogen peroxide in the absence of ascorbate.

3.2. ROOH-dependent oxidation catalyzed by diferric complexes

The systems based on diferric complexes, such as the one prepared in our laboratory and shown in Fig. 6, that provide the largest yields of products per unit time, during oxidation of alkanes or alcohols, involve pyridine-based ligands (tpa, bipy,

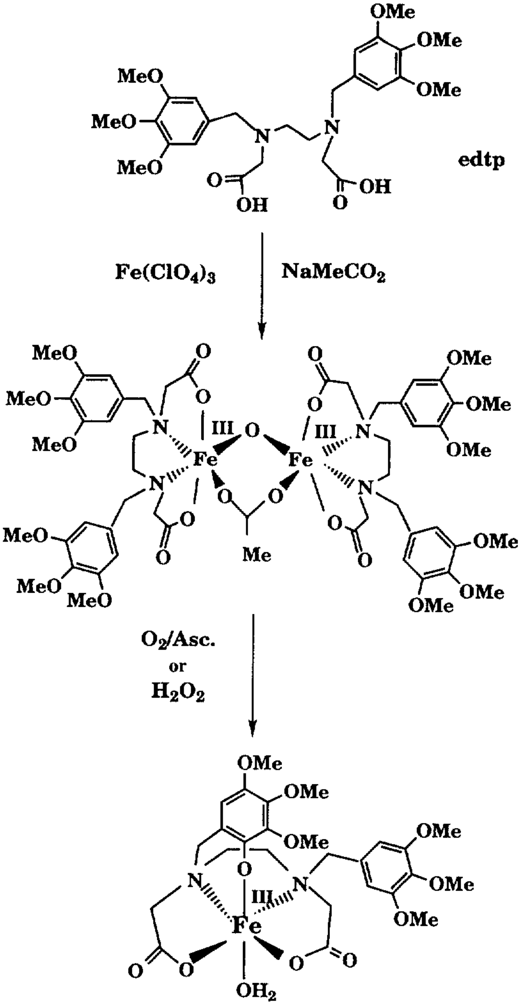


Fig. 5. Dioxygen activation for edtp oxidation by a diiron complex.

phen) and alkylhydroperoxides (ROOH), peracids or hydrogen peroxide as the oxidant (Table 2). Activity requires the presence of solvent-exchangeable sites in the complex for oxidant binding and activation [52]. The very few active systems reported so far include the $Fe_2O(tpa)_2$/ROOH combination studied by Que and coworkers [53,54], the Fe_2O(tmima)/ROOH one studied by Fish and coworkers [55,56], the $Fe_2O(bipy)_4$/ROOH system we have investigated [52,57,58], and more recently the Fe_2(hexpy)/m-CPBA system, based on hexpy, a dinucleating hexapyridine ligand [59]. The remarkable efficiency of the latter might suggest a significant advantage of a dinucleating ligand. This may generate a larger stability of the dinuclear structure during catalysis. All these reactions are characterized by: (i) a

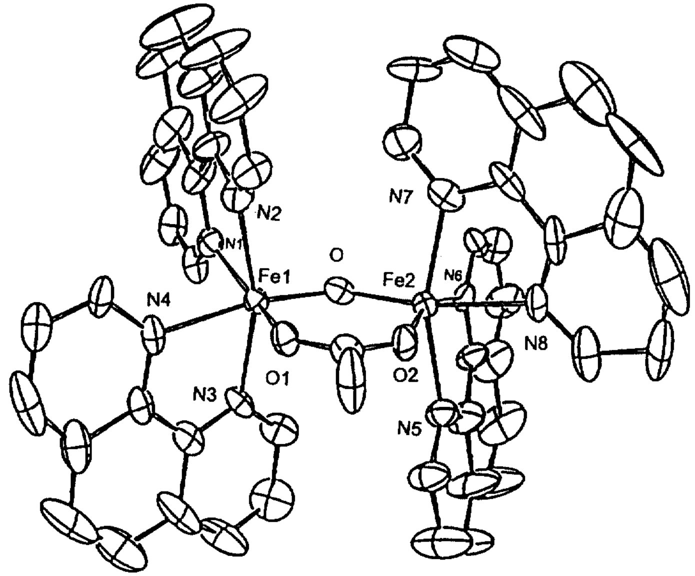


Fig. 6. X-ray structure of $Fe_2O(phen)_4(OAc)(ClO_4)_3$.

Table 2
Cyclohexane oxidation catalyzed by μ-oxo diferric complexes in acetonitrile

Complex	Oxidant	$A+K/P$[a]	A/K	TN/h[b]	Yield(%)[c]	Ref.
$Fe_2O(H_2O)_2(tmima)_2(ClO_4)_4$	$TBHP/O_2$	1.5	1	3.7	nr[d]	[56]
$Fe_2O(OAc)(tmima)_2(ClO_4)_3$	H_2O_2/O_2	–[e]	0.8–1.1	15	10(10)	[55]
$Fe_2O(H_2O)_2(tpa)_2(ClO_4)_4$	TBHP	1.5	0.5	250	16(32)	[54]
$Fe_2O(OAc)(tpa)_2(ClO_4)_4$	TBHP	1.3	0.8	80	22(47)	[53]
$Fe_2O(OAc)_2(hexpy)_2(ClO_4)_4$	m-CPBA	3.86	2.4	4300	75(90)	[58]
$Fe_2O(H_2O)(bipy)_2(ClO_4)_4$	TBHP	5.1	0.9	1000	39(44)	[52,57]
$Fe_2O(OAc)_2(bipy)_2(ClO_4)_4$	TBHP	5.5	1.2	120	34(38)	[52]
$Fe_2O(H_2O)_2(pb)_2(ClO_4)_4$	TBHP	5.5	0.77	400	43(49)	–
	H_2O_2	–[e]	1.0	200	12(12)	[67]
	H_2O_2(10eq)	–[e]	2.4	200	40(40)	[67]

Experimental conditions: 0.77 mM complex; 0.01 M oxidant; 0.77 M cyclohexane except in the m-CPBA case (400 equivalent); T = 25°C.

[a] A corresponds to cyclohexanol; K to the corresponding ketone; P to other oxidation products.

[b] TN = turnover number calculated as moles of K times 2 plus moles of A per mole of catalyst.

[c] Yield based on the oxidant: $A+2K$ moles/moles of oxidant. The yield is given in parentheses considering also the number of moles of P.

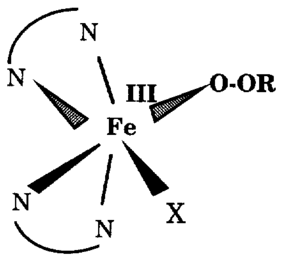
[d] nr = not reported.

[e] Only alcohol and ketone are observed.

stoichiometric formation of cyclohexanol and cyclohexanone during oxidation of cyclohexane; (ii) a large C3/C2 ratio (around 10) in the case of the oxidation of adamantane [C3/C2 is a parameter indicating the selectivity of the oxidizing system for the tertiary C–H bonds versus secondary ones]; (iii) a large kinetic isotope effect, for the formation of cyclohexanol from cyclohexane, with a k_H/k_D value of 4–7; (iv) no retention of configuration during oxidation of *cis*-dimethylcyclohexane [60].

That iron is important for activation of the peroxide through a direct interaction has been shown from identification of the first alkylperoxoiron(III) complex observed during oxidation of alkanes and alcohols by *tert*-butyl hydroperoxide (TBHP) catalyzed by $Fe_2O(bipy)_4(H_2O)_2(ClO_4)_4$, that we reported recently [61]. This complex is characterized by: (i) an intense and broad absorption band at 640 nm, which is responsible for its blue color; (ii) strong resonance-enhanced Raman features at 808 and 678 cm^{-1} which were shifted when [d_9]TBHP was used; (iii) a rhombic EPR signal with g values at around 2, characteristic of $S = 1/2$ iron species. This led us to propose the structure shown in Fig. 7 and to suggest that during the reaction the dinuclear unit decomposed into mononuclear species thus liberating coordination sites for binding both the peroxo ligand and the substrate (in the case of alcohols). We have some preliminary indications that the mononuclear iron–peroxo complex is directly involved in the oxidation of the alcohol, probably within the coordination sphere of the iron. Thus, even though the starting complex is dinuclear, the active one is probably mononuclear. Nevertheless, at the end of the reaction, the complex is mainly recovered in a dinuclear form. A similar transient mononuclear Fe–peroxo complex has been observed during oxidation reactions using $Fe_2O(tpa)_2(H_2O)_2(ClO_4)_4$ as the catalyst [62]. We suggest that a stable dinuclear complex can be an active catalyst in TBHP-dependent systems for its ability to decompose into highly reactive mononuclear intermediates, thus simultaneously liberating free coordination sites for binding oxidants and substrates.

The mechanism of alkane oxidation by TBHP catalyzed by diiron complexes was a matter of controversy. In fact, it is now quite clear that, when a large excess of TBHP is present in the reaction mixture, most of the products, alcohol, ketone and mixed dialkylperoxide, are derived from the reaction of the *tert*-butylalkoxyl radical with the substrate [57,63]. The alkoxyl radical, which is thus the active species, is formed during the homolytic decomposition of the intermediate iron–peroxo complex. The whole oxidation reaction involves a very complex chemistry in which



X= OH_2 or (H)OR'

Fig. 7. Proposed structure for the alkylperoxo–iron intermediate during oxidation by alkylperoxides catalyzed by μ-oxo diferric catalysts. N–N = bipy, phen.

several radicals and molecular oxygen participate. However, under certain conditions (limiting TBHP for example) and with certain ligands such as tpa, it has been claimed that non-radical metal-based mechanisms are also implicated, as shown from the large alcohol to ketone products ratio [64].

Even though these very first attempts to model the reactivity of biological diiron centers were rather successful, it is also noteworthy that they are fraught with some drawbacks, in particular the relative instability of the catalysts which are slowly inactivated during the reaction [58]. Moreover, until recently, H_2O_2, as the oxidant, gave rather poor results. This is essentially explained by the strong ability of non-heme iron complexes to catalyze the dismutation of hydrogen peroxide, which diverts a large amount of the oxidant away from the oxidation of substrates [65]. This was unfortunate as H_2O_2 is clearly a much better oxidant than TBHP, both for synthetic applications and for the modelization of biological monooxygenases. It is less expensive than TBHP and gives only water as a by-product, thus greatly simplifying purification of reaction products.

3.3. H_2O_2-dependent oxidation

The very first complexes with significant, even though limited, catalytic activities for H_2O_2-dependent oxidation of alkanes and sulfides were our bipy- and phen-based iron complexes [65]. Large turnover numbers could be obtained but rather low yields based on the oxidant (about 10% in the case of cyclohexane but 50–70% in the case of sulfides). Also, conversion of methane to methanol was reported [60]. One major problem of these systems was the rather fast accumulation of a degraded and inactive form of the catalyst, i.e. the $Fe(bipy)_3^{2+}$ or $Fe(phen)_3^{2+}$ and, as mentioned above, the fast dismutation of the peroxide. However, some interesting features seemed to indicate that a metal-based mechanism contributed to the reaction to a significant extent.

(i) During the reaction, an intermediate species was observed and characterized as a μ-peroxodiferric complex, as shown by the intense broad absorption band at 600 nm, reflecting a peroxo-to-iron charge transfer, the characteristic O–O vibration in the resonance Raman spectrum ($860\ cm^{-1}$) and the absence of an EPR signal, revealing that the system had retained its antiferromagnetically coupled diiron(III) structure. Furthermore, ESI mass spectrometric analysis of that intermediate confirmed the presence of a peroxo group on the μ-oxo dinuclear unit [66].

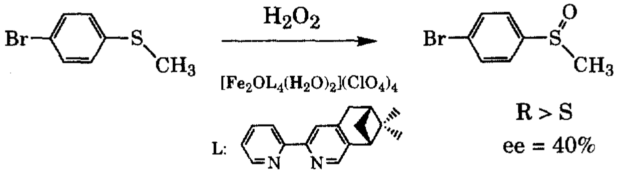
(ii) A large C3/C2 ratio (around 13) was obtained, a value much larger than that expected for OH°-dependent reactions.

(iii) A kinetic isotope effect, in the case of the formation of cyclohexanol from cyclohexane, of 2–2.5, significantly larger than that for OH° (1.0–1.7) [60].

(iv) A significant retention of configuration (50–70%) during oxidation of *trans*-dimethylcyclohexane, larger than what would be found in a pure OH°-dependent system.

In our opinion, the most straightforward way to check whether non-heme diiron systems use metal-based pathways is to demonstrate their ability to carry out catalytic asymmetric oxidations. Even though there is no report of a chiral dinuclear iron complex able to catalyze asymmetric oxidations, one should mention the case of a mononuclear chiral bleomycin–iron complex which catalyzes the stereoselective epoxidation of alkenes by H_2O_2 [67].

By selecting the ligand named pb (Fig. 4), a chiral analog of bipy, we were able to prepare a diiron complex, $Fe_2O(pb)_4(H_2O)_2(ClO_4)_4$, which showed a significant ability to catalyze asymmetric oxidation of sulfides to sulfoxides by hydrogen peroxide. Even though the observed stereoselectivity was moderate, an enantiomeric excess of 40% was obtained in the case of p-bromophenylmethyl sulfide [68]. No enantioselectivity could be observed with TBHP as the oxidant, further supporting the radical chain auto-oxidation mechanism for that system.



(1)

In contrast, $Fe_2O(bipy)_4(H_2O)_2(ClO_4)_4$ generated only a racemic mixture of the sulfoxides. Asymmetric sulfoxidation with an iron catalyst has been reported only in the case of chiral iron porphyrins, but with iodosylbenzene as an oxidant, with comparable enantioselectivities [69]. Preliminary results showed significant enantioselectivity during oxidation of 1,1-dimethylindane.

Additional properties make this new catalyst attractive for further study:

(1) the reaction was fast (20–30 turnovers per minute) for both sulfide and alkane oxidation;

(2) the yields were high (80–100% for sulfide oxidation, 40–50% for cyclohexane oxidation), especially when the oxidant was added slowly to the reaction mixture (Table 2);

(3) unexpectedly, the catalyst was highly robust, being degraded only marginally into inactive mononuclear complexes such as $Fe(pb)_3^{2+}$;

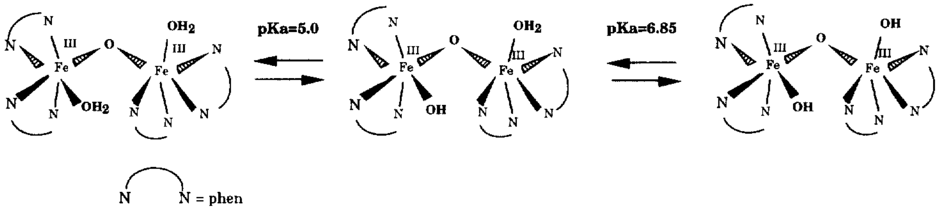
(4) the oxidation of 1,2-dimethylcyclohexane was totally stereospecific (unpublished results).

We think that these results provide the first direct evidence that oxidation reactions catalyzed by non-heme iron do not proceed exclusively through radical chain auto-oxidation. Under certain conditions, metal-based pathways in which the oxygen active species is bound to the metal, in the form of an iron–peroxo or a high-valent iron–oxo species, may be involved, thus allowing some control of the stereoselectivity of the reactions. This was proposed earlier by Groves and Van der Puy [70], and more recently by Que and coworkers [26,64] and ourselves [57,68].

4. Diiron complexes as models of purple acid phosphatases

Although the exact roles of the metal ions in purple acid phosphatases are not known, it is postulated that the dinuclear center facilitates hydrolysis of phosphodiesters by using one metal site to activate the substrate and the other as a Lewis acid to increase the pK_a of bound water, thus efficiently delivering the hydroxide nucleophile to the substrate [71]. As $Fe(OH_2)$–O–$Fe(OH_2)$ complexes were available, both with tpa or phen as ligands, it was tempting to study their acid/base properties and check whether water could be activated and utilized for hydrolysis of phosphoesters.

It has been demonstrated that successive deprotonation of Fe-bound water molecules is occuring during treatment with bases both in organic solvent [72,73] and in water [74]. During the reaction, the dinuclear structure is retained, allowing the generation of either the diiron complex with one terminal hydroxo and one terminal water or the complex with two terminal hydroxo ligands, as shown in Eq. (2):



(2)

Both have recently been structurally characterized, the first one in the tpa series [72,73], the second in the phen series (Fig. 8) [74]. With phen as the ligand, we were able to determine, in water solutions, the pK_a values of the two successive

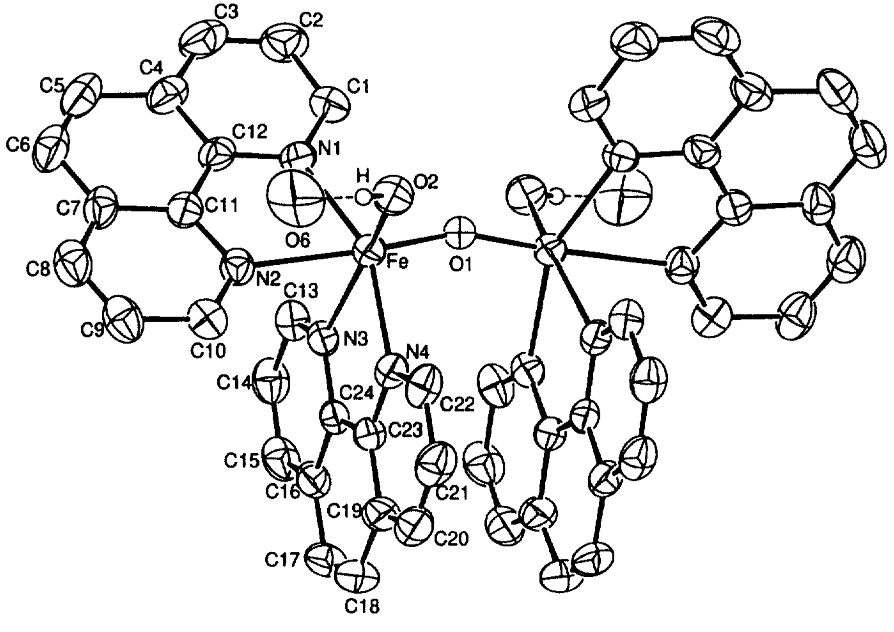


Fig. 8. X-ray structure of $Fe_2O(phen)_4(OH)_2(NO_3)_2$.

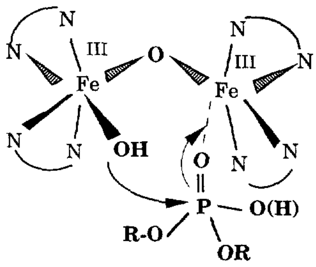


Fig. 9. Possible active intermediate during phosphodiesters hydrolysis catalyzed by diiron complexes in water. N–N = phen.

titrable protons at 5.0 and 6.85, respectively. The first pK_a value is only slightly higher than that for the deprotonation of water bound to Fe(III) in the mammalian purple acid phosphatase, showing that the model complex can stabilize a nucleophilic hydroxide under weakly acid conditions. This also likely suggests that in the enzyme the Fe(III) site serves a similar function.

We have shown that only the monohydroxo complex is able, in buffered water solution, both to bind a phosphodiester at the exchangeable Fe–OH_2 site and to use the adjacent Fe–OH nucleophilic site to hydrolyze it. Maximal activity was found for pH 6.0 [$k_{obs} = 5 \times 10^{-4}$ s^{-1} during the hydrolysis of 100 μM bis(2,4-dinitrophenyl) phosphate promoted at 50 °C by 80 μM model complex]. In separate studies, Toflund and Que showed that, in acetonitrile, the monohydroxo complex was able to convert one equivalent of the solvent into acetamide, which was found bound to the diiron complex, probably bridging the two irons. However, all these reactions are still slow when compared to hydrolytic reactions catalyzed by Zn and Co complexes [75,76] and further work is needed to circumvent this limitation.

These novel observations demonstrate the potential of diiron complexes for catalysis of hydrolytic or hydration reactions. We suggested that this could be due to the combined ability to increase the nucleophilicity of the water molecule on one Fe site and to increase the electrophilicity of the phosphoester substrate by coordination on the adjacent Fe center (Fig. 9). The diaquo and dihydroxo complexes were shown to be inefficient, as a consequence of the absence of a nucleophilic hydroxide in the former and of an exchangeable site for substrate binding on the latter.

This chemistry deserves further investigation as results are just preliminary. It might lead to new agents for hydrolytic cleavage of DNA, that may have biotechnological applications.

5. Conclusion

There is now ample evidence that structural and more recently functional models of MMO, RNR and PAP provide very useful tools to study the reactivity of these fascinating unique enzymes. A lot of challenges remain, for example the preparation of complexes modeling compound Q, the design of new catalysts able to use hydrogen peroxide or molecular oxygen for efficient and selective (including enantioselective) oxidation reactions.

A new field is also emerging, i.e. the utilization of dinuclear model complexes to achieve complex hydrolytic reactions, in particular with regard to DNA cleavage.

Abbreviations

bipy	2,2′-bipyridine
edtp	N,N′-bis(3-methoxybenzyl)ethylenediamine N,N′ diacetic acid dianion
$hb(pz)_3$	hydrotris(pyrazolyl)borate anion
hexpy	1,2-bis[2-di(2-pyridyl)methyl-6-pyridyl]ethane
hfacac	hexafluoroacetylacetone
m-CPBA	m-chloroperbenzoic acid
OAc	acetate anion
pb	(−)4,5-pinene 2,2′-bipyridine
phen	1,10-phenanthroline
salen	N,N′-ethylenebis(salicylideneamine)
tacn	triazacyclononane
tmima	tris[(1-methylimidazol-2-yl)methyl] amine
tpa	tris(2-pyridylmethyl)amine

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