μ-Oxo-bridged Diiron(III) Complexes and H_2O_2: Monooxygenase- and Catalase-like Activities

Stéphane Ménage, Jean Marc Vincent, Claude Lambeaux and Marc Fontecave*
Laboratoire d'Etudes Dynamiques de la Structure et de la Sélectivité, URA 332, Université Joseph Fourier, BP 53, 38041 Grenoble Cédex 09, France

The μ-oxo-bridged diiron(III) complex $[Fe_2O(bipy)_4(OH_2)_2][ClO_4]_4$ **1** (bipy = 2,2'-bipyridine) was found to exhibit monooxygenase-like activity, using H_2O_2 as the oxidant. The system oxidizes alkanes to alcohols and ketones quite efficiently (46 mmol of cyclohexanol + cyclohexanone per mmol complex in 10 min). In the case of adamantane, selectivity for the tertiary hydrogen was indicated by a high normalized $C_3:C_2$ ratio of 9:1. The same reaction yields and rates were obtained whether argon or dioxygen was bubbled through the solution. Dimethyl sulfide was transformed into dimethyl sulfoxide and dimethylsulfone and benzene into phenol. These results exclude O_2 as a key reactant in this system and suggest that high-valent oxoiron intermediates and hydroxyl radicals are the active species. The potential of this system is strongly limited by the instability of the catalyst and by its strong catalase-like activity. Complex **1** is actually a very efficient catalyst for hydrogen peroxide dismutation, thus transforming 50% of the excess of H_2O_2 into O_2 in 10 min.

Binuclear oxo-bridged non-haem iron complexes (containing the Fe–O–Fe unit) have recently received much attention.[1] They provide structural models for the diiron sites in proteins involved in the storage (Haemerythrin)[2] or the reductive activation (methane monooxygenase,[3] ribonucleotide reductase[4]) of dioxygen. In methane monooxygenase, the diiron centre is involved in the oxidation of alkanes such as methane,[5] while in ribonucleotide reductase it is implicated in the one-electron oxidation of a tyrosine residue near the active centre.[6] More recently, some studies have shown that well designed binuclear iron complexes were efficient catalysts for the oxidation of hydrocarbons by oxygen-atom donors, providing an original strategy in the search for new catalysts.[7–9] However, the initial work has, with only one exception,[10] utilized *tert*-butyl hydroperoxide as the oxidant.

Hydrogen peroxide is clearly a much better oxidant, in terms of synthetic applications. It is less expensive than Bu^tO_2H and gives only water as a by-product, greatly simplifying purification of the reaction products. In most biological oxidations, including those mentioned above, hydrogen peroxide can serve as an oxygen source. In this study, we show that model non-haem diiron complexes may catalyse the oxidation of organic substrates by H_2O_2. However, a great limitation arises from the strong catalytic activity of all studied complexes for H_2O_2 dismutation which competes with oxidation. This property has thus also been investigated here since the potential of diiron complexes as catalase-like systems has been very little explored.

Experimental

Synthetic Methods.—The complex $[Fe_2O(bipy)_4(OH_2)_2][ClO_4]_4$ **1** (bipy = 2,2'-bipyridine) and all the complexes $[Fe_2OL_4X_n][ClO_4]_m$ were synthesised as previously described.[9] Hydrogen peroxide (30% in aqueous solution) was titrated by iodometry. All other chemicals were obtained commercially and used as received.

Kinetics of Hydrogen Peroxide Decomposition.—All reactions were carried out at 19 °C in a 20 cm^3 reactor containing a stirring bar under air. Acetonitrile (14.85 cm^3) was added to the complex (3 μmol, 0.2 mmol dm^{-3}) and the flask was closed with a rubber septum. Hydrogen peroxide (1.35 mmol, 150 μl) was injected through the septum with a syringe. The reactor was connected to a graduated burette filled with water and dioxygen evolution was measured at time intervals during 10 min by volumetry. Observed initial rates were expressed as mol dm^{-3} s^{-1} by taking the volume of the solution (0.015 l) into account and calculated from the maximum slope of curves describing evolution of O_2 *versus* time. Plots of the initial rates *versus* the concentrations of H_2O_2 and iron complexes allowed determination of the reaction orders in both reactants. In some experiments the rates were measured at 37 °C.

Oxidation Reactions.—The complex concentration was 0.2 mmol dm^{-3} and the ratio complex:hydrogen peroxide kept at a value of 1:580. In all experiments, the total volume (15 cm^3) was kept unchanged and cyclohexane (360 μl, 3.3 mmol), dimethyl sulfide (240 μl, 3.3 mmol), benzyl alcohol (660 μl, 6.4 mmol), benzene (1.035 cm^3, 3.3 mmol) or adamantane (164 mg, 0.4 mmol) was added before adding the oxidant. After 10 min at 25 °C, the products were determined by GC analysis using acetophenone and naphthalene as internal standard for cyclohexanol, cyclohexanone, alcohols, dimethyl sulfoxide, dimethyl sulfone, adamantan-1-ol and adamantan-2-one and phenol respectively. The structures of the products were confirmed by GC–mass spectrometry and by comparison with authentic samples. All these substrates had no effect on the complex stability under these conditions. No oxidation products were formed in the absence of the complexes.

Physical Methods.—Visible spectra were recorded on a Uvikon 930 spectrophotometer, EPR spectra with a E102 Varian spectrometer at 4 K. Gas chromatography was performed on a Perkin-Elmer Autosystem instrument connected to a Shimadzu Chromatopac CR6A with a flame ionization detector and a BP 20 and an OV 17 (30 m) capillary column.

Results

Monooxygenase-like Activity of $[Fe_2OL_4X_2][ClO_4]_m$ *Complexes.*—In a previous study we have shown that a series of μ-oxo-bridged diiron(III) complexes $[Fe_2OL_4X_n][ClO_4]_m$, containing two bidentate nitrogen ligands [L = bipy, 4,4'-dimethyl-2,2'-bipyridine (dmbipy) or 1,10-phenanthroline (phen)] and one exchangeable co-ordination site (X = H_2O,

Cl^-, $MeCO_2^-$ or $CF_3CO_2^-$) per iron, were efficient catalysts for the functionalization of alkanes using Bu^tO_2H as oxidant.[9] With X = Cl^-, $CF_3CO_2^-$ or H_2O, the complexes only contained a μ-oxo bridge, whereas with X = $MeCO_2^-$ they are μ-oxo-μ-carboxylato bridged. Moreover, H_2O and $MeCO_2^-$ were found to be substitution-labile ligands while Cl^- was much less exchangeable. The efficiency of these catalysts correlated well with the lability of the Fe–X bond, with complex **1**, shown in Fig. 1, being the most active.

We have now studied the oxidation of cyclohexane by H_2O_2 in the presence of catalytic amounts of complex **1** (**1**:H_2O_2 = 1:580). The reaction mixture was continuously flushed with argon. It yielded cyclohexanol and cyclohexanone, in a 1.3:1 ratio. The turnover number (mmol product per mmol catalyst) was 46 and the alcohol + ketone yield was 8% after 10 min, with no increase during prolonged incubations. As a comparison, the yield was 40% after 10 min with Bu^tO_2H as the oxidant. A primary kinetic isotope effect k_H/k_D of 1.7 for the formation of cyclohexanol and 2.7 for cyclohexanone was measured during oxidation by H_2O_2 of a 1:1 C_6H_{12}:C_6D_{12} mixture, under standard conditions. Replacing the bipy ligand by phen or dmbipy had no major effect on the yields and rates of the reaction (data not shown). Moreover, the catalyst obtained by replacing the water ligands by an acetate bridge was slightly less active (data not shown). As shown in Table 1, this system was also able to oxidize adamantane, benzyl alcohol, benzene and dimethyl sulfide.

In all reactions gas evolution was noticed and identified as dioxygen. In the absence of the catalyst, no evolution of O_2 was observed. The fact that oxidation of adamantane gave the same yields whether the reaction mixture was vigorously bubbled with argon or with pure dioxygen strongly suggests that H_2O_2-derived O_2 was not involved in the reaction. Moreover, the normalized C_3:C_2 ratio, a measure of the preference for tertiary positions, was about 9:1 under both conditions.

Catalase-like Activity of $[Fe_2OL_4X_2][ClO_4]_m$ *Complexes.*—The H_2O_2 dismutation activity was tested by measuring the dioxygen evolution over a 10 min period in acetonitrile with a complex **1**:H_2O_2 ratio of 1:450 and a complex catalyst concentration of 0.2 mmol dm^{-3}. These conditions were found to be optimal in terms of reaction yields. The amount of residual hydrogen peroxide (measured by iodometric titration) was compared with the amounts of dioxygen formed (as calculated by volumetry) and found to satisfy the stoichiometry implied by equation (1).

$$2H_2O_2 \longrightarrow 2H_2O + O_2 \qquad (1)$$

The characteristics of this new catalase-like system are as follows: (*i*) yields were around 50% based on H_2O_2 after 10 min of reaction with about 30 and 150 initial turnovers per min at 19 and 37 °C respectively in the case of complex **1** (turnover is mmol of O_2 per mmol of complex); (*ii*) yields were dependent on the X ligand and ranged from 0 to 50% with Cl^- < $CF_3CO_2^-$ < $MeCO_2^-$ < H_2O in agreement with the exchangeability of the X ligand; (*iii*) bipyridine-containing complexes were more efficient than corresponding phenanthroline-containing ones; (*iv*) inactivation of the catalyst occurred during the reaction. Addition of a new quantity of hydrogen peroxide after 10 min of reaction led to a much decreased catalase activity. At the end of the reaction spectroscopic study of the solution showed the presence of two mononuclear complexes. One was identified as the iron(II) complex $[FeL_3]^{2+}$ characterized by its visible spectrum, and shown to be inactive during H_2O_2 dismutation. The second was a monomeric high-spin iron(III) ion characterized by an EPR signal centred at $g = 4.3$. In all probability, the complexes became inactive by breaking down into monomers. It should be mentioned that a simple iron salt such as $Fe(ClO_4)_3$ has, under standard conditions, no activity.

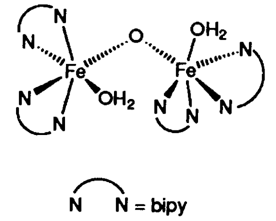


Fig. 1 Schematic representation of complex **1** and related complexes

Table 1 Substrate oxidation by H_2O_2 catalysed by complex **1**[a]

Substrate	Products (turnover)[b]	Yield[c] (%)	Total turnover[b]
Cyclohexane	Cyclohexanol (18) Cyclohexanone (14)	8	46
Adamantane	Adamantan-1-ol (6) Adamantan-2-one (1.5)	1.5	9
Benzyl alcohol	Benzaldehyde (88)	15	88
Benzene	Phenol (14)	2.5	14
Dimethyl sulfide	Dimethyl sulfoxide (120) Dimethyl sulfone (20)	27.5	160

[a] See Experimental section. [b] Turnover = mmol products per mmol catalyst after 10 min of reaction. [c] Based on starting H_2O_2. Total yield and turnover values take into account that 2 equivalents of H_2O_2 are required to make 1 equivalent of ketone or sulfone.

Kinetic Studies of H_2O_2 *Decomposition.*—The dependence of initial rates of dioxygen evolution was examined with varying concentrations of H_2O_2 and complex **1**. In the first experiment, the hydrogen peroxide concentration was kept constant ($[H_2O_2]_0$ = 0.18 mol dm^{-3}) and the concentration of the complex was varied from 0.1 to 2 mmol dm^{-3}. Plots of the initital rate *versus* catalyst concentration indicated that the reaction was first order in complex concentration. In a second experiment the catalyst concentration was kept constant at 0.2 mol dm^{-3} and the H_2O_2 concentration was varied between 10 and 300 mmol dm^{-3}. The reaction was also first order in $[H_2O_2]$. In the rate law (2) k_{obs} was calculated to be 2.2 ± 0.2 $dm^3\ mol^{-1}\ s^{-1}$.

$$\text{rate} = k_{obs}[H_2O_2][\mathbf{1}] \qquad (2)$$

A remarkable observation is that the solution turned yellow-brown to deep blue-green during the first minutes of the reaction. This change is tentatively assigned to the formation of a transient peroxide adduct of the binuclear iron complex. Rapid-scan electronic spectroscopy on a chilled sample at an early stage of the reaction demonstrated the appearance of an intense broad absorption band at around 600 nm (ε > 1 $dm^3\ mmol^{-1}\ cm^{-1}$ per Fe).[11] The absence of EPR signals revealed that the system retained its antiferromagnetically coupled diiron(III) structure. Such features were previously reported for other well characterized μ-peroxo-diiron(III) complexes.[12]

The reaction was accelerated by addition of imidazole (Fig. 2). With 2 equivalents of imidazole, based on complex concentration, a three- to four-fold increase in the initial rate was observed (inset, Fig. 2). 1-Methyl- or 1,2-dimethyl-imidazole had similar effects. However, the presence of imidazole also resulted in a much faster inactivation of the catalyst as shown by the increased amount of the iron(II) mononuclear complex. Consequently, the reaction yields were in fact slightly decreased.

Competition between Catalase and Oxygenase Activities.—Whether the same intermediate oxidizing species is responsible for both substrate and H_2O_2 oxidation (in the latter case, leading to O_2) may be investigated by monitoring the dioxygen evolution and substrate oxidation simultaneously. The yield

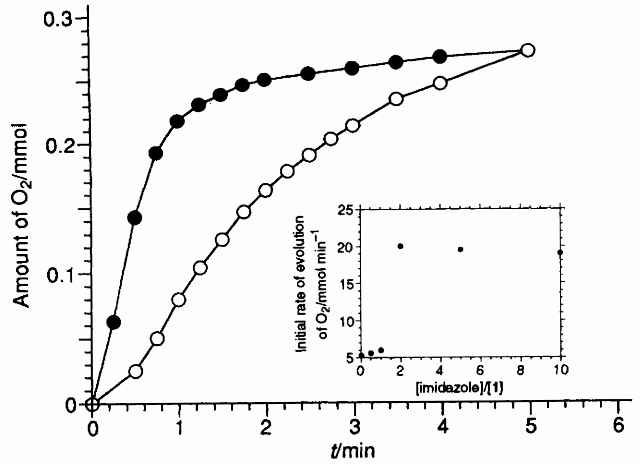


Fig. 2 Stimulatory effects of imidazole on dioxygen evolution from H_2O_2 dismutation catalysed by complex **1**. (○) Complex **1** alone; (●) **1** + 2 equivalents imidazole. Inset: imidazole concentration curve

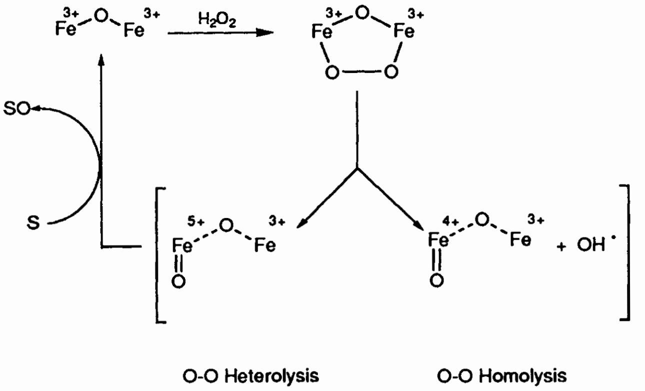
and initial rate of dioxygen generation from hydrogen peroxide were not affected significantly by the addition of cyclohexane, in good agreement with the limited reactivity of the substrate and thus the very low yields of oxidation products. On the other hand, addition of dimethyl sulfide greatly inhibited H_2O_2 dismutation and dioxygen release (turnover = 75 *versus* 220 in the absence of dimethyl sulfide). The amount of oxidation products (dimethyl sulfoxide and dimethyl sulfone: 120 and 20 mmol per mmol complex respectively after 10 min of reaction) roughly matched the missing O_2. Oxidations were totally inhibited when 0.4 mmol dm^{-3} imidazole was present in the reaction mixture with both dimethyl sulfide and cyclohexane as substrates.

Discussion

In order to evaluate correctly the potential of μ-oxo-diiron(III) complexes as catalysts for the oxidation of hydrocarbons it is important to know whether these complexes may utilize hydrogen peroxide as the oxygen source. To our knowledge only one study has been reported on such systems. However, molecular oxygen was not excluded from the medium and the reaction looked more like a metal-catalysed free-radical chain autoxidation process than a monooxygenase-like reaction.[10] The oxidations were slow and greatly inhibited by radical scavengers and anaerobiosis, alkyl hydroperoxide was identified as an intermediate species during oxidation of alkane, and $Fe(ClO_4)_3$ was more active than were binuclear complexes.

In our investigation the reactions were subjected to a dynamic argon atmosphere to avoid autoxidations by O_2 from air or from H_2O_2 dismutation. We thus exclude O_2 as a key reactant in our system and cyclohexyl hydroperoxide as the precursor of cyclohexanol and cyclohexanone. Complex **1** with one exchangeable H_2O ligand per iron, was found to be the most effective catalyst and $Fe(ClO_4)_3$ was totally inactive.

Even though no detailed investigation of the mechanism has been carried out, it is possible to suggest the following mechanism (Scheme 1). Reaction of H_2O_2 with complex **1** yields a peroxo complex. Such an intermediate has been observed transiently and is characterized by an intense and broad absorption band at around 600 nm. Further spectroscopic studies would be needed to support the proposed structure. This unstable peroxo complex might generate, by heterolytic cleavage of the O–O bond, high-valent oxoiron intermediates which would be the active oxidizing species, in good agreement with the formation of dimethyl sulfoxide and, probably more significant, dimethyl sulfone from dimethyl sulfide,[13,14] and with the high $C_3:C_2$ ratio during oxidation of adamantane.[15]



Scheme 1 Proposed mechanism for the disproportionation of H_2O_2 and substrate oxidation by complex **1**; S represents the co-substrate or a second H_2O_2 molecule

One of the possible limiting forms of such a complex is shown in Scheme 1. However, the observed hydroxylation of benzene leads us also to consider that generation of OH$^{\bullet}$ radicals by homolytic cleavage of the O–O bond might simultaneously occur.[16]

The efficiency of the complex **1**–H_2O_2 system is, by far, not as good as that of **1** and Bu^tO_2H and the reaction yields were not satisfactory. We discovered that a major drawback of binuclear complexes resides in their strong activity for dismutation of H_2O_2 in acetonitrile which makes them good candidates for catalase-like systems. The diiron(III) centre of ribonucleotide reductase has been shown to dismutate hydrogen peroxide in water at neutral pH.[17] There has been one recent report that a synthetic μ-oxo-diiron(III) complex, modelling the haemerythrin metal centre, has a good catalase-like activity.[18] The catalase-like activity of complex **1** in organic solvent was comparable to that of manganese or iron porphyrins, but its stability is much lower due to decomposition into inactive monomers during multiple turnovers.[19] Good catalase-like activity has been reported with mononuclear non-haem iron complexes, but comparison to the present system is difficult since the solvent conditions were so different.[20] The fact that production of O_2 from H_2O_2 was greatly inhibited by addition of dimethyl sulfide might suggest that the same high-valent oxoiron intermediate shown in Scheme 1 is responsible for H_2O_2 oxidation to O_2.

The observed stimulatory effects of imidazole on H_2O_2 dismutation could be explained by its general acid–base catalytic properties, which would facilitate proton transfers. It has also been reported that bases such as imidazole stimulate catalase-like activities of iron or manganese complexes by binding to the metal centre.[14] However, a direct interaction between imidazole and complex **1** is here excluded on the basis of spectroscopic investigations (data not shown).

Conclusion

While they have the potential to utilize H_2O_2 for the oxidation of alkanes and may provide a new class of catalysts, μ-oxo-bridged diiron(III) complexes might have limited use because of their strong activity for H_2O_2 dismutation, on the one hand, and of their great instability on the other. There is thus a need for further studies aimed at designing new complexes.

References

1 L. Que, jun., and A. E. True, *Prog. Inorg. Chem.*, 1990, **38**, 97; J. Sanders-Loehr, in *Iron Carriers and Iron Proteins*, ed. T. M. Loehr, VCH, New York, 1989, p. 373.

2 R. E. Stenkamp, L. C. Sieker and L. H. Jensen, *J. Am. Chem. Soc.*, 1984, **106**, 618.

3 A. C. Rosenzweig, C. A. Frederick, S. J. Lippard and P. Norlund, *Nature (London)*, 1993, **366**, 537.

4 P. Nordlund, B.-M. Sjöberg and H. Eklund, *Nature (London)*, 1990, **345**, 593.

5 J. Green and H. Dalton, *Biochem. J.*, 1986, **236**, 155; M. Shimoda, M. Ono and I. Okura, *J. Mol. Catal.*, 1989, **52**, L37.

6 M. Fontecave, P. Norlund, H. Eklund and P. Reichard, *Adv. Enzymol.*, 1992, **65**, 147.

7 J. B. Vincent, J. C. Huffman, G. Christou, Q. Li, M. A. Nanny, D. N. Hendrickson, R. H. Fong and R. H. Fish, *J. Am. Chem. Soc.*, 1988, **110**, 6898.

8 R. A. Leising, J. Kim, M. A. Pérez and L. Que, jun., *J. Am. Chem. Soc.*, 1993, **115**, 618.

9 S. Ménage, J.-M. Vincent, C. Lambeaux, G. Chottard, A. Grand and M. Fontecave, *Inorg. Chem.*, 1993, **32**, 4766 and refs. therein.

10 R. H. Fish, M. S. Konings, K. J. Oberhausen, R. H. Fong, W. M. Yu, G. Christou, J. B. Vincent, D. K. Coggin and R. M. Buchanan, *Inorg. Chem.*, 1991, **30**, 3002.

11 L. Que, jun., in *Bioinorganic Catalysis*, ed. J. Reedjik, Marcel Dekker, New York, 1993, p. 347.

12 S. Ahmad, J. D. McCallum, A. K. Shiemke, E. M. Appelmann, T. M. Loehr and J. Sanders-Loehr, *Inorg. Chem.*, 1988, **27**, 2230; B. P. Murch, F. C. Bradley and L. Que, jun., *J. Am. Chem. Soc.*, 1986, **108**, 5027; B. A. Brennan, Q. Chen, C. Juarez-Garcia, A. E. True, C. J. O'Connor and L. Que, jun., *Inorg. Chem.*, 1991, **30**, 1937; D. T. Sawyer, M. S. McDowell, L. P. Spencer and K. Tsang, *Inorg. Chem.*, 1989, **28**, 1166; N. Kitajima, H. Fukui and T. Morooka, *J. Am. Chem. Soc.*, 1990, **112**, 6402; S. Ménage, B. A. Brennan, C. Juarez-Garcia, E. Münck and L. Que, jun., *J. Am. Chem. Soc.*, 1990, **112**, 6423; Y. Hayashi, M. Suzuki, A. Uehara, Y. Mizutani and T. Kitagawa, *Chem. Lett.*, 1992, 91.

13 S. Oea, Y. Watanabe and K. Fujimori, *Tetrahedron Lett.*, 1982, **23**, 1189; F. P. Guengerich and T. L. Macdonald, *Acc. Chem. Res.*, 1984, **17**, 9.

14 B. Meunier, *Chem. Rev.*, 1992, **92**, 1411.

15 See, for example, J. T. Groves, T. E. Nemo and R. S. Myers, *J. Am. Chem. Soc.*, 1979, **101**, 1032; D. R. H. Barton, M. J. Gastiger and W. B. Motherwell, *J. Chem. Soc., Chem. Commun.*, 1983, 731.

16 C. Walling, *Acc. Chem. Res.*, 1976, **9**, 175.

17 M. Sahlin, B.-M. Sjöberg, G. Backes, T. Loehr and J. Sanders-Loehr, *Biochem. Biophys. Res. Commun.*, 1990, **167**, 813.

18 B. Mauerer, J. Crane, J. Schuler, K. Wieghardt and B. Nuber, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 289.

19 A. Robert, B. Loock, M. Momenteau and B. Meunier, *Inorg. Chem.*, 1991, **30**, 706 and refs. therein.

20 C. C. Cairns, R. A. Heckman, A. C. Melnyk, W. M. Davis and D. H. Busch, *J. Chem. Soc., Dalton Trans.*, 1987, 2505.

Received 8*th March* 1994; *Paper* 4/01378F