BioMetals **12:** 195–199, 1999.

Mini-review

Iron and activated oxygen species in biology: The basic chemistry

J.L. Pierre[1,*] & M. Fontecave[2]
[1]*Laboratoire de Chimie Biomimétique, LEDSS, UMR CNRS 5616, Université J. Fourier, BP 53 , 38041 Grenoble cedex 9, France*
[2]*Laboratoire de Chimie et Biochimie des Centres Rédox biologiques, DBMS-CB CEA Grenoble/EP 1087 CNRS/Université J. Fourier, 17 rue des Martyrs, 38054 Grenoble cedex 9, France*
[*]*Author for correspondence (E-mail: Jean-Louis.Pierre @ujf-grenoble.fr)*

Received 27 April 1999; accepted 3 May 1999

Key words: hydrogen peroxide, iron, iron proteins, superoxide, synthetic iron complexes

Abstract

This paper briefly presents a critical review concerning the chemical reactions involved when superoxide or hydrogen peroxide meet iron complexes. The data commented on are required for a correct interpretation of the chemical processes which play a paramount role in the biological activation of dioxygen and arise in normal metabolism as well as in pathological processes.

Introduction

Living organisms have selected iron to achieve a large number of biological processes (Crichton 1991). The unique suitability of iron comes from the extreme variability of the Fe^{3+}/ Fe^{2+} redox potential which can be finely tuned by well-chosen ligands. Superoxide and hydrogen peroxide are the first elements in the chain of oxygen activation, a cascade of biological one-electron transfer processes which are often iron-catalyzed (Nivière & Fontecave 1995; Pierre 1995). Hydroxyl radicals are usually involved to explain oxidative damage. An understanding of the chemistry of these processes is an essential prerequisite for a comprehensive description of the iron/oxygen-mediated biological events.

Iron and oxygen species

The successive one-electron transfers, the corresponding redox potentials and the pK values associated with the concomitant proton transfers involved in the oxygen activation chain are depicted in Figure 1.

Iron(II) complexes can act as one-electron reducing agents and iron(III) complexes as one-electron-oxidizing agents. Moreover, iron complexes can react with molecular oxygen or with its reduced species, leading to highly reactive high-valent iron-oxo species. In Figure 2 are summarized several redox reactions which can occur upon the interaction of activated oxygen species and iron complexes.

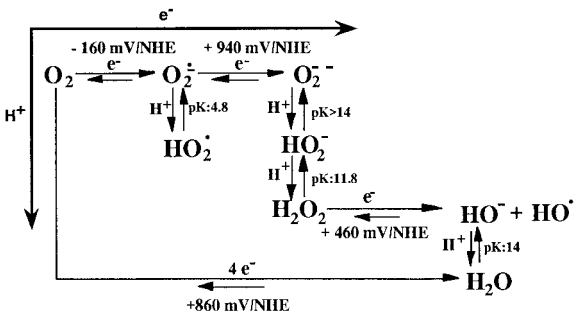

Figure 1. Chemistry of oxygen: electron and proton transfers.

It must be emphasized that the species involving iron(IV) or iron(V) oxidation states have been mostly observed with heminic complexes (biological or model compounds). Nevertheless, in the past ten years, model compounds of non heme iron enzymes, especially those containing diiron centers, have received more attention (Fontecave *et al.* 1998).

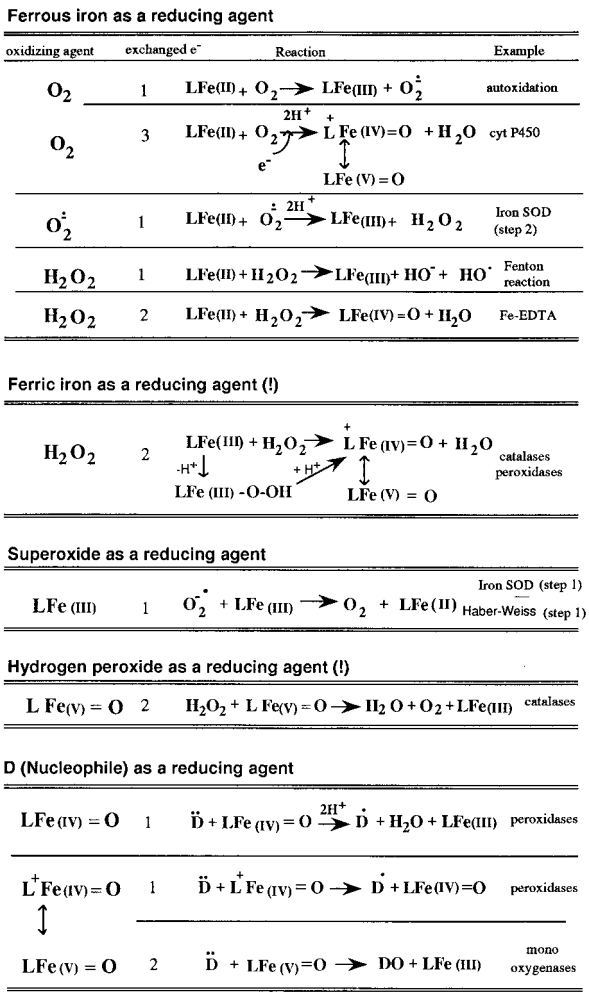

Figure 2. Redox chemistry of iron and oxygen species (L: ligand; D: Nucleophile).

Redox potentials: how to use them

Some relevant redox potentials (at physiological pH) are summarized in Figure 3. The value of −500 mV for the redox potential of transferrin is more accurate than the usual value of 400 mv (Kraiter *et al.* 1998).

A given ferric complex can catalyze the formation of hydroxyl radicals only if two conditions are simultaneously filled: (i) the complex is reducible into its ferrous state; (ii) the ferrous complex has a redox potential allowing the Fenton reaction, i.e. the transfer of one electron to H_2O_2. This is thermodynamically allowed for the complexes of Figure 3 whose redox potentials are in the range $-324\ \text{mV} < E^\circ < +460\ \text{mV}$ *under standard conditions of equilibrium* . These conditions are obviously not always fulfilled within cells and incorrect uses of redox potential leading to misinterpretations are often met in the literature. We will illustrate this with the two following cases:

Iron and superoxide

Ascorbate, glutathione, cysteine but also specific reductases may reduce the ferric complexes. Some ferric complexes can be reduced by superoxide and lead to ferrous complexes able to catalyze the Fenton reaction (the overall process is the well-known Haber-Weiss cycle) (Figure 4).

The thermodynamic condition for the reducibility of iron(III) is: $E^\circ(LFe^{3+}/LFe^{2+}) > -160$ mV, this last value corresponding to $E^\circ O_2/O_2^-$ (see Figure 3). *This is effective only if* $[\mathbf{O_2}] = [\mathbf{O_2^-}]$. However, in the living cell (in the absence of oxidative stress), the actual concentration can be estimated at $[O_2] = 3.5 \times 10^{-5}$ M and $[O_2^-] = 10^{-11}$ M. Therefore, the (LFe^{3+}/LFe^{2+}) potential required for Fe(III) reduction in a living cell becomes from the Nernst equation: $E = -160 + 0.06 \log[O_2]/[O_2^-]$, i.e. +230 mV! *There are many fewer ferric complexes that can be reduced by superoxide than predicted by the usual thermodynamic data* . The situation may be different during an oxidative stress when $[O_2^-]$ is drastically increased. The natural ratio $[O_2]/[O_2^-]$ exerts, *per se*, a protective effect against oxidative damage.

Iron release from ferrisiderophores

The redox potentials depicted in Figure 3 for ferrisiderophores (ferrioxamine B, ferrienterobactin) and for all the known ferrisiderophores seem to forbid the reduction of iron by the biological reducing agents. Nethertheless, some flavin reductases exhibit ferrisiderophore reductase activity (Coves & Fontecave 1993): a ferrous iron complexing agent is present in the medium (giving rise to an air-stable ferrous complex) and the complexation of ferrous iron drives the equilibrium to the reduction of the ferric complexes: *the system is out of equilibrium*. Apoproteins or porphyrins (which incorporate iron in the ferrous state) may play the role of the ferrous complexing agent. As shown by the Nernst equation $E = E^\circ + 0.06 \log[Fe^{3+}]/[Fe^{2+}]$ applied to the ferrisiderophore, the sequestration of Fe^{2+} by a ferrous chelator decreases $[Fe^{2+}]$ and drastically increases E, allowing the reduction by the usual biological reducing agents. Therefore, in some circumstances, Desferal (desferrioxamine B), used for the teatment of iron

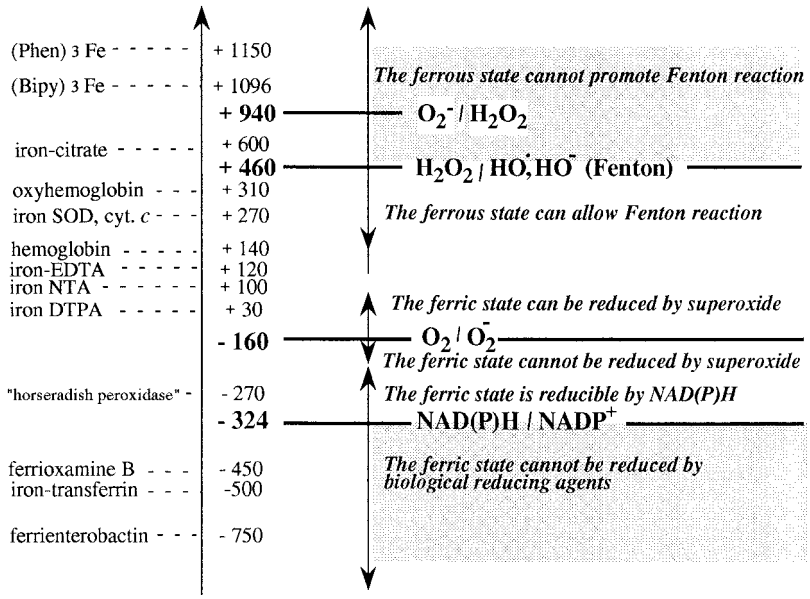

Figure 3. Fe^{3+}/Fe^{2+} redox potentials at pH: 7 (mV/NHE).

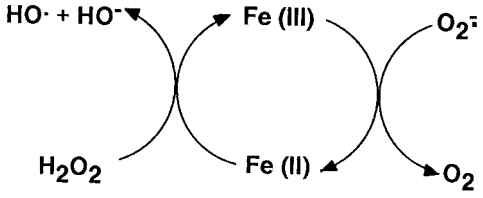

Figure 4. The Haber-Weiss cycle (left part: Fenton reaction).

overload, may act as a prooxidant (Borg & Schaich 1986)! It has been recently pointed out that, in the presence of a Fe(II) chelator with a $\beta > 10^3$, the effective redox potential of transferrin (Figure 3) was shifted positive of NADH, and therefore can be reduced by this biological reducing agent (Kraiter *et al.* 1998).

When strong ferrous chelating agents with high redox potentials (such as ferrozine or phenantroline), are used as tools to evidence iron release from a ferric complex via a reduction step, the release might be essentially due to the presence of the ferrous chelating agent: the tool itself thus greatly affects the process under study!

Ferrous complexes and hydrogen peroxide: hydroxyl radical or iron-oxo species?

As shown in Figure 2, two pathways may be envisaged leading either to hydroxyl radicals (Fenton chemistry) or to a ferryl species. The spin-trap DMPO usually allows discrimination between the two, benzoate and tBuOH do not. There is often an overestimation of hydroxyl production when benzoate or tertiobutanol are used as $HO^{\bullet}$ scavengers: both hydroxyl radicals and ferryl complexes are scavenged. Numerous papers concerning the reaction of hydrogen peroxide with ferrous complexes of EDTA, DTPA, nucleotides, citrate, ... led to fuzzy results. Recent papers (Yamazaki & Piette 1990, 1991; Luzzatto *et al.* 1995) contribute to clarify the situation. They make a distinction between free and masked hydroxyl radicals (hydroxyl in strong interaction with the metal center) and, of course, with ferryl species. The relative rates of the processes depend on L, the stoichiometry, the solvent and the pH: in acidic medium or with an excess of H_2O_2 only hydroxyl radicals are formed, while with higher $[Fe^{2+}]$, Fe(IV) = O species can be obtained at physiological pH . In hydrophobic medium, formation of the ferryl species predominates (this may suggest that in membranes, oxidative degradations are due to ferryl species). Another cause of error is observed for iron complexes with bulky hexacoordinating ligands: the reaction of hydrogen peroxide with the ferrous center may be very slow and, furthermore, the access of the iron center by the spin-trap may be difficult.

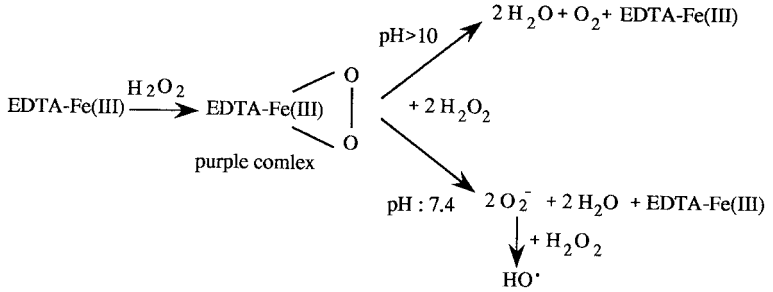

Figure 5. Reactivity of Fe(III)-EDTA with H_2O_2.

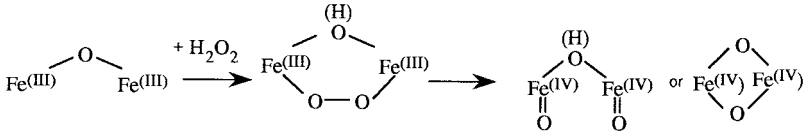

Figure 6. μ-oxo diferric complexes and hydrogen peroxide.

Ferric complexes and hydrogen peroxide

Various events are summarized in Figure 2. It is well known that iron(III) porphyrins give iron-oxo species and their reactivity has been studied to a great extent (Meunier 1992):

$$\mathrm{PFe(III)} + \mathrm{H_2O_2} \rightarrow \mathrm{PFe(V)} = \mathrm{O}$$
$$\leftrightarrow \mathrm{P^{\cdot +}Fe(IV)} = \mathrm{O} + \mathrm{H_2O}.$$

The reactions of non heme iron(III) complexes are less known. Fe(III)-EDTA has been the most studied complex (Ahmad *et al.* 1988; Gutteridge & Maidt 1990). The isolated purple adduct with H_2O_2 (pH: 9) is decomposed into water and oxygen (catalase like activity) in alkaline medium. At physiological pH, superoxide ions are obtained in the presence of an excess of hydrogen peroxide, followed by an Haber-Weiss reaction catalyzed by Fe(III)-EDTA (the process is inhibited by SOD) (Figure 5).

μ-oxo diferric complexes (ribonucleotide reductase, methane monooxygenase and their abiotic models) usually lead, during reaction with peroxide, to μ-peroxo adducts which are converted to high-valent iron (IV)-oxo species (Figure 6) (Brennan *et al.* 1991; Fontecave *et al.* 1998). These complexes are highly reactive with regard to a variety of substrate including alkanes (C-H bond oxidation).

Acknowledgements

We are grateful to Alvin L. Crumbliss (Durham, USA) for fruitful discussions.

References

Ahmad S, Mc Callum JD, Shiemke AK *et al.* 1988 Raman spectroscopic evidence for side-on binding of peroxide ion to Fe(III)-EDTA. *Inorg Chem* **27**, 2230–2233.

Brennan BA, Chen Q, Juarez-Garcia C *et al.* 1991 Models for diiron-oxo proteins: the peroxide adduct of $Fe_2(HPTB)(OH)(NO_3)_4$. *Inorg Chem* **30**, 1937–1943.

Borg DC, Schaich KM. 1986 Prooxidant action of desferrioxamine : Fenton like production of hydroxyl radicals by reduced ferrioxamine. *J Free Rad Biol Med* **2**, 237–243.

Coves J, Fontecave M. 1993 Reduction and mobilization of iron by a NAD(P)H:flavinoxidoreductase from Escherichia coli. *Eur J Biochem* **211**, 635–641.

Crichton RR. 1991 *Inorganic Biochemistry of Iron Metabolism*. New-York, London: Ellis Horwood.

Fontecave M, Menage S, Duboc-Toia C. 1998 Functional models of non-heme iron enzymes. *Coord Chem Rev* **178–180**, 1555–1572.

Gutteridge JMC, Maidt L, Poyer L. 1990 Superoxide dismutase and Fenton chemistry; reaction of ferric-EDTA complex and ferric bipyridyl complex with hydrogen peroxide without the apparent formation of iron(II). *Biochem J* **269**, 169–174.

Kraiter DC, Zak O, Aisen P, *et al.* 1998 A determination of the reduction potentials for diferric and C- and N-lobe monoferric tansferrins at endosomal pH(5.8). *Inorg Chem* **37**, 964.

Luzzatto E, Cohen H, Stockheim C, *et al.* 1995 Reactions of low valent transition metal complexes with hydrogen peroxide. Are they Fenton-like or not? The case of Fe (II)L, L = EDTA, HEDTA and TCMA. *Free Rad Res* **23**, 453–463.

Meunier B. 1992 Metalloporphyrins as versatile catalysts for oxidation reactions and oxidative DNA cleavage. *Chem Rev* **92**, 1411-1456.

Nivière V, Fontecave M. 1995 Biological source of reduced oxygen species. In: Favier AE, Cadet J, Kalyanaraman B *et al.* eds.

Analysis of Free Radicals in Biological Systems. Basel, Boston, Berlin: Birkhäuser; 11–19.

Pierre JL. 1995 Chemistry of dioxygen and its activated species. In: Favier AE, Cadet J, Kalyanaraman B, *et al.* eds. *Analysis of Free Radicals in Biological Systems*. Basel, Boston, Berlin: Birkhäuser; 1–10.

Yamazaki I, Piette LH. 1990 ESR spin-trapping studies on the reaction of Fe^{2+} ions with H_2O_2-reactive species in oxygen toxicity in biology. *J Biol Chem* **265**, 13589–13594.

Yamazaki I, Piette LH. 1991 ESR spin-trapping studies on the oxidizing species formed in the reaction of the ferrous ion with hydrogen peroxide. *J Am Chem Soc* **113**, 7588–7593.