ELSEVIER

Coordination Chemistry Reviews 233–234 (2002) 311–318

COORDINATION CHEMISTRY REVIEWS

www.elsevier.com/locate/ccr

Oxidations of iron(II)/(III) by hydrogen peroxide: from aquo to enzyme

H. Brian Dunford *

Biophysical Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

Received 11 September 2001; accepted 18 January 2002

Contents

Abstract

The mechanism of reaction of hexaquo iron(II) with hydrogen peroxide has been unresolved for 70 years. Most scientists, perhaps by default, have accepted the free radical chain mechanism of Barb et al. (Trans. Faraday Soc. 47 (1957) 462). However an earlier proposal involved formation of the ferryl ion, FeO^{2+} (J. Am. Chem. Soc. 54 (1932) 2124). Recent work has favored a mechanism involving FeO^{2+} and $FeOFe^{5+}$ species. Similarly there are differences of opinion on the mechanism of reaction of iron(III), both hexaquo and chelated, with hydrogen peroxide. These differences have fostered a recent burst of activity, with claims on one hand that hydroxyl radicals play a key role, and on the other, that there is a non-free radical mechanism. In contrast, the mechanism of reaction of the heme-containing peroxidase and catalase enzymes with hydrogen peroxide, orders of magnitude faster than reactions of iron(II)/(III), now appears to be well established. In this paper I attempt, as objectively as possible, to delineate the proposed mechanisms, discuss their possible physiological relevance, and summarize the current state of knowledge.

Keywords: Fenton chemistry; Gif chemistry; Haber–Weiss reaction; Peroxidases; Catalases

1. Introduction

This is a story about the mechanisms of oxidation of iron(II) and (III) by hydrogen peroxide, with emphasis on the supposedly simplest systems, hexaquo iron(II) and (III) at one extreme, and iron(III) in heme-containing peroxidases and catalases at the other. Since much of the relevant literature is devoted to reactions involving oxygen species in oxidation states ranging from 0 to −2, I shall also discuss reactions of hydroxyl and hydroperoxyl/superoxide radicals.

---

* Tel.: +1-780-492-0228; fax: +1-780-492-8231.
E-mail address: brian.dunford@ualberta.ca (H.B. Dunford).

0010-8545/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0010-8545(02)00024-3

2. Fenton chemistry

Oxidation of organic substrates by iron(II) and hydrogen peroxide is called Fenton chemistry, on the basis of an 1894 paper [1] on the oxidation of tartaric acid by hydrogen peroxide in the presence of iron(II). The mechanism of the reaction between iron(II) and hydrogen peroxide has been widely assumed to be the following, based upon a comprehensive paper published by Barb et al. [2].

$$\mathrm{Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + HO^-} \qquad (1)$$

$$\mathrm{Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + HO^-} \qquad (2)$$

$$\mathrm{{}^{\bullet}OH + H_2O_2 \rightarrow HO_2{}^{\bullet} + H_2O} \qquad (3)$$

$$\mathrm{HO_2{}^{\bullet} + Fe^{2+} \rightarrow Fe^{3+} + HO_2^-} \qquad (4)$$

$$\mathrm{HO_2{}^{\bullet} + Fe^{3+} \rightarrow Fe^{2+} + O_2 + H^+} \qquad (5)$$

They noted that reaction (5) might better be written as

$$\mathrm{O_2{}^{\bullet -} + Fe^{3+} \rightarrow Fe^{2+} + O_2} \qquad (5')$$

The above scheme is a chain mechanism in which iron(II) is regenerated. Rate constants for many of the above elementary reactions have been obtained by radiation chemists and are summarized in Table 1. An intensive investigation of reaction (4) showed that it is a composite reaction that can be broken down into elementary steps [3].

$$\mathrm{HO_2{}^{\bullet} + Fe^{2+} \overset{k_{4A}}{\rightarrow} Fe^3 + HO_2^-} \qquad (4A)$$

$$\mathrm{Fe^3 + HO_2^- + Fe^{2+} \underset{k_{-4B}}{\overset{k_{4B}}{\rightleftarrows}} Fe^3 + HO_2^- Fe^{2+}} \qquad (4B)$$

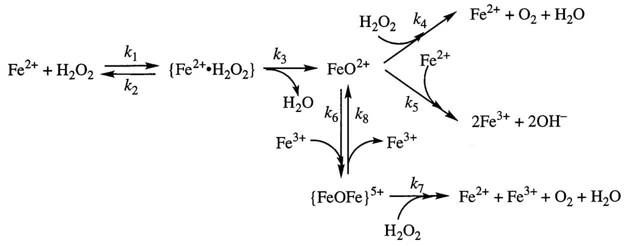
$$\mathrm{Fe^3 + {}^{\bullet}HO_2^- \overset{k_{4C}}{\rightarrow} Fe^{3+} + HO_2^-} \qquad (4C)$$

The iron-peroxide species are outer-sphere complexes. The authors obtained spectra for $\mathrm{Fe^{3+}HO_2^-}$ and $\mathrm{Fe^{3+}HO_2^-Fe^{2+}}$. They also showed a different dissociation for the binuclear iron complex:

$$\mathrm{Fe^3 + HO_2^- Fe^{2+} \overset{k_{4D}}{\rightarrow} Fe^{2+}HO_2^- + Fe^{3+}} \qquad (4D)$$

Table 1
Elementary rate constants for the reaction of hydrogen peroxide with iron(II) according to the mechanism of Barb et al. [2]

Reaction numbers	Rate constant	References
(1)	$(53.0 \pm 0.7)\ \mathrm{M^{-1}\ s^{-1}}$	[2]
(2)	$(3.3 \pm 0.6) \times 10^8\ \mathrm{M^{-1}\ s^{-1}}$	[63]
(3)	$2.7 \times 10^7\ \mathrm{M^{-1}\ s^{-1}}$	[64,65]
(4A)	$(1.20 \pm 0.05) \times 10^6\ \mathrm{M^{-1}\ s^{-1}}$	[3]
(4B)	$(6.8 \pm 0.5) \times 10^5\ \mathrm{M^{-1}\ s^{-1}}$	[3]
(4−B)	$(2.5 \pm 0.1) \times 10^4\ \mathrm{s^{-1}}$	[3]
(4C)	$(1.8 \pm 0.01) \times 10^3\ \mathrm{s^{-1}}$	[3]
(5)	Range: $< 1.2 \times 10^4$ to $3.6 \times 10^5\ \mathrm{M^{-1}\ s^{-1}}$	[24]



Scheme 1.

The rate constants for reactions (2)–(5) in Table 1 are of great utility in evaluating the mechanisms of many chemical and physiological overall reactions, but perhaps not in discussing Fenton chemistry! See below.

Over 100 years after the paper by Fenton, a comprehensive reinvestigation of the reaction of ferrous ion with hydrogen peroxide has been published by Kremer [4]. His mechanism for the iron(II) reaction is shown in Scheme 1.

This paper deserves serious study, especially by chemists who think that the mechanism of reaction ferrous ion with hydrogen peroxide was settled long ago. Kremer's mechanism appears to account for all of the salient features. With iron(II) in excess, oxygen evolution is negligible. With hydrogen peroxide in excess, reaction with $\mathrm{FeO^{2+}}$ causes oxygen evolution in the initial stages, whereas in later stages the new species $\mathrm{\{FeOFe\}^{5+}}$ is responsible. Kremer pointed out that there is an error in the analysis of Barb et al. [4]. They assumed a steady state is attained in $[\mathrm{Fe^{2+}}]$ whereas in fact $[\mathrm{Fe^{2+}}]$ goes to zero! The appreciable possibility exists that the mechanism or reaction of iron(II) with $\mathrm{H_2O_2}$ does not involve free radicals at all, and the extensive radiation chemistry data on elementary reactions of oxy radicals, while undoubtedly correct, is irrelevant to Fenton chemistry. A further discussion of iron-oxo species is given below.

3. Haber–Weiss (Haber–Willstätter) reaction

Haber and Willstätter believed that the mechanism by which enzymes reacted with substrates was via initiation of chain reactions, after which the enzyme played no direct role [5]. Thus catalase caused the decomposition of hydrogen peroxide via a free radical chain reaction set off by the enzyme. The iron of the catalase generated hydroxyl radicals, so they believed, that reacted in similar fashion to reaction (3), followed by regeneration of hydroxyl

$$\mathrm{{}^{\bullet}OH + H_2O_2 \rightarrow O_2{}^{\bullet -} + H^+ + H_2O} \qquad (6)$$

$$\mathrm{O_2{}^{\bullet -} + H^+ + H_2O_2 \rightarrow {}^{\bullet}OH + O_2 + H_2O} \qquad (7)$$

Willstätter remained firmly convinced that enzyme reactions occurred by catalysis initiated by trace inorganic elements. Sumner, of jack bean urease fame, was unable to change Willstätter's mind despite a personal visit to his laboratory. Reactions (6) and (7) are the Haber–Willstätter cycle, and reaction (7) has become known as the Haber–Weiss reaction. In a recent lecture Koppenol pointed out that one should not name a reaction that does not occur after the wrong person [6]. To be fair, Weiss was a staunch defender of reaction (7), and published a later paper with Haber devoted to the reaction of iron salts with hydrogen peroxide [7]. The evidence against reaction (7) is overwhelming.

i) Harcourt showed that when solid potassium superoxide is dissolved in water or dilute acid, equimolar amounts of oxygen and hydrogen peroxide are rapidly produced [8].

$$2K^+O_2^- + 2H_2O \xrightarrow{H^+} O_2 + H_2O_2 + 2K^+ + 2OH^- \tag{8}$$

As pointed out by Bray, $HO_2^\bullet$ would be formed as an intermediate in reaction (8) and the stoichiometry precludes any significant reaction of $HO_2^\bullet$ with H_2O_2 under the experimental conditions that were used. Bray stated: "the specific rate of the reaction $2HO_2^\bullet = H_2O_2 + O_2$ is much greater than that of ...$HO_2^\bullet + H_2O_2 = {}^\bullet OH + H_2O + O_2$" [9].

ii) George worked directly with potassium superoxide in solutions ranging from dilute alkali to dilute acid and concluded there was no reaction of superoxide with H_2O_2. The amount of oxygen evolved was independent of whether hydrogen peroxide was present or absent [10].

iii) Halliwell showed using competition kinetics that the direct Haber–Weiss reaction does not occur [11].

iv) A radiation chemistry study by Weinstein and Bielski determined the rate constant for reaction (7) to be 0.50 ± 0.09 M^{-1} s^{-1}, so small that the reaction would be ineffective as a chain carrier [12].

v) Reaction (7) was also shown not to occur in non-aqueous media [13].

Despite overwhelming evidence to the contrary that already existed at the time, the Haber–Weiss reaction was 'rediscovered' in 1970. It was claimed that superoxide generated by xanthine oxidase reacted with hydrogen peroxide to form hydroxyl radicals [14]. On this basis Koppenol predicts its next rediscovery to occur by about 2018 [6].

It is ironic that the Haber–Weiss (Haber–Willstätter) reaction became so popular in the biochemical literature, since the reaction was first proposed by chemists who refused to admit the true catalytic power of enzymes.

3.1. *Metal-ion catalyzed Haber–Weiss reaction*

After failure to gain a new acceptance of the Haber–Weiss reaction, it was reincarnated in its metal-ion catalyzed form:

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \tag{5$'$}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^\bullet OH + HO^- \tag{1}$$

$$O_2^{\bullet -} + H_2O_2 = {}^\bullet OH + O_2 + HO^- \tag{9}$$

Thus the sum of two reactions provides the equivalent of the Haber–Weiss reaction. The so-called metal-ion-catalyzed Haber–Weiss reaction is Eq. (1) and (5′) from the mechanism of Barb et al. for the ferrous-ion catalyzed decomposition of hydrogen peroxide, with reactions (2)–(4) being ignored.

3.2. *Peroxynitrite*

Peroxynitrite was discovered in 1952. It can be formed by the reaction of hydrogen peroxide and nitrous acid [15], and also by the superoxide/hydroperoxyl reaction with nitric oxide [16]. The rate constant for reaction 10 is 3.7×10^7 M^{-1} s^{-1} [17,18].

$${}^\bullet NO + O_2^{\bullet -} \rightarrow ONOO^- \tag{10}$$

The acid form of peroxynitrite (peroxynitrous acid, pK_a 6.8) decomposes slowly by homolytic cleavage of the peroxy bond [19–21].

$$ONOOH \rightarrow {}^\bullet OH + {}^\bullet NO_2 \tag{11}$$

Nitric oxide is the endothelium-derived relaxation factor (EDRF). A plausible, albeit indirect, in vivo route from superoxide to hydroxyl radical is established via reactions (10) and (11) [22,23].

3.3. *Reactive oxygen species*

All species from dioxygen to hydroxyl radicals, with alkoxyl radicals, peroxynitrite, and singlet oxygen included, are often referred to as reactive oxygen species (ROS), widely accepted to be harmful. It has become a cliche that oxygen is the enemy of life and good wine. However, both the mechanisms of oxidative physiological damage and its prevention are, with few exceptions, not understood. The harmful physiological iron complexes are unknown [6]. At one stage controversies were generating more heat than light. Harmful effects of ROS for one set of conditions can be beneficial in another.

4. Superoxide and its dismutation

Three reactions are required to describe the uncatalyzed dismutation of superoxide.

$$HO_2^{\bullet} \rightleftarrows O_2^{\bullet -} + H^+ \quad pK = 4.8 \tag{12}$$

$$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow O_2 + H_2O_2 \quad k = (8.3 \pm 0.7) \times 10^5\ M^{-1}\ s^{-1} \tag{13}$$

$$HO_2^{\bullet} + O_2^{\bullet -} \overset{H^+}{\rightarrow} O_2 + H_2O_2 \quad k = (9.7 \pm 0.6) \times 10^7\ M^{-1}\ s^{-1} \tag{14}$$

The following reaction (15) does not occur

$$O_2^{\bullet -} + O_2^{\bullet -} \xrightarrow[\times]{2H^+} O_2 + H_2O_2 \quad k = 0.00\ M^{-1}s^{-1} \tag{15}$$

The striking pH-rate profile that summarizes the above results is shown in Fig. 1 [24]. At high pH one can use potassium superoxide as a direct quantitative source of $O_2^{\bullet -}$ [25,26], but at lower pH one must rely upon radiation chemistry [27]. Bielski and Cabelli discuss in detail the reactions of superoxide and perhydroxyl radicals [28].

The landmark discovery that the blue copper–zinc containing protein erythrocuprein was a superoxide dismutase was made by McCord and Fridovich [29]. Both iron- and manganese-containing superoxide dismutases were also discovered by Fridovich's group [30,31]. In the presence of superoxide dismutase, the disproportionation of superoxide occurs by a 'ping pong' type of mechanism in which the catalytic metal ion alternates between M^{n+} and $M^{(n-1)+}$ oxidation states. The former catalyzes O_2 release (rate constant k_1) and the latter H_2O_2 (rate constant k_2). The rate of disappearance of superoxide k_{cat} ($M^{-1}\ s^{-1}$) is given by

$$-\frac{d[\text{superoxide}]_{tot}}{dt} = v = k_{cat}[M^+]_{tot}[\text{superoxide}]_{tot} \tag{16}$$

where the subscript tot refers to the total concentration of enzyme, represented by $[M^+]$, and of $O_2^{\bullet -}$ and $HO_2^{\bullet}$. The value of k_{cat} is given by a weighted mean of k_1 and k_2 [32].

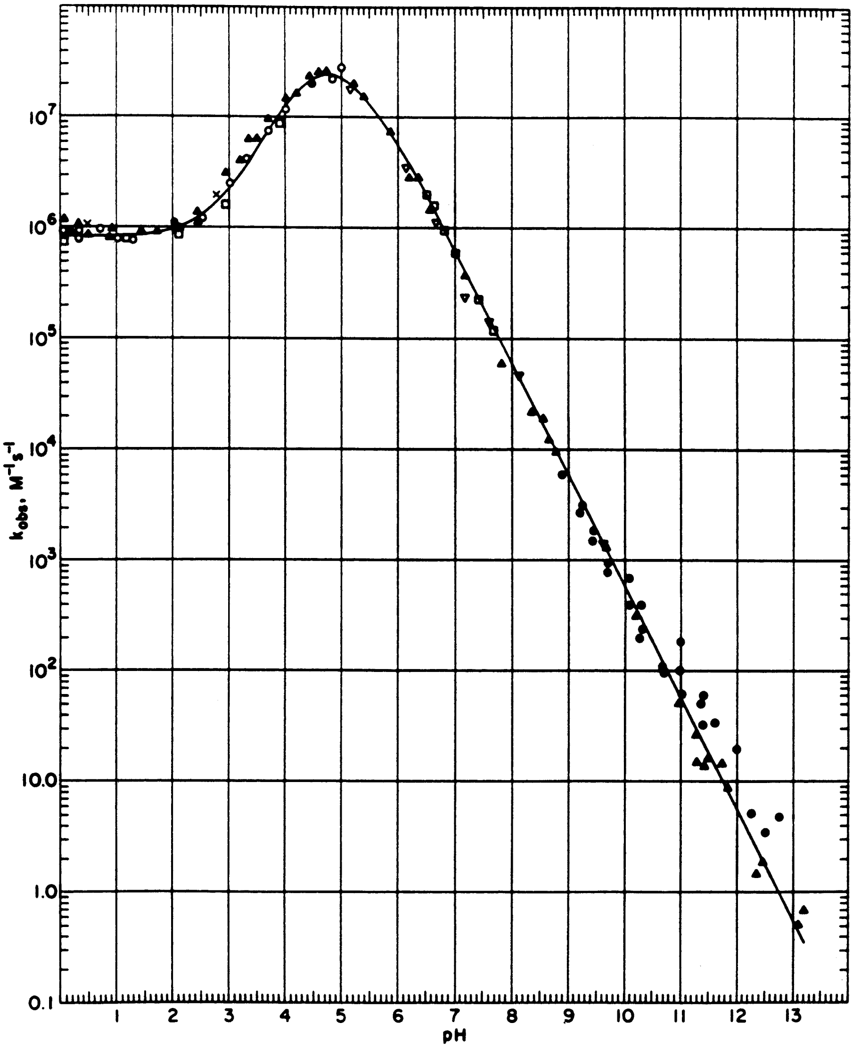


Fig. 1. Observed second-order rate constant, k_{obs}, for the decay of $HO_2/O_2^{\bullet -}$ plotted as a function of pH. Reproduced with permission from Ref. [24].

$$k_{cat} = \frac{2k_1k_2}{k_1 + k_2} \quad (17)$$

For Cu–Zn SOD k_{cat} has been reported to be constant from pH 4.8 to 9.5 and to have a value of 4.6×10^9 M^{-1} s^{-1}. Over the latter pH range, with increasing basicity, $[HO_2^•]$ is decreasing to the same extent that $[O_2^{•-}]$ is increasing.

In order to compare the second-order uncatalyzed and first-order catalyzed rates of the dismutase reaction, one must plug in arbitrary concentrations. Thus at pH 7 the uncatalyzed rate constant, taken from Fig. 1, is 5×10^5 M^{-1} s^{-1} and

$$v_{uncatalyzed} = 5 \times 10^5[\text{superoxide}]^2_{tot} \quad (18)$$

For the superoxide dismutase-catalyzed reaction

$$v_{catalyzed} = \sim 5 \times 10^9[M^+]_{tot}[\text{superoxide}]_{tot} \quad (19)$$

If catalyst concentration $[M^+]_{tot}$ is 10^{-5} M and $[\text{superoxide}]_{tot}$ is 10^{-3} M, then $v_{uncatalyzed}$ is 0.5 M s^{-1} and $v_{catalyzed}$ is 50 M s^{-1}, a 100-fold increase in rate for the catalyzed reaction. If $[M^+]_{tot}$ is 10^{-7} M and $[\text{superoxide}]_{tot}$ is 10^{-3} M, then $v_{uncatalyzed}$ and $v_{catalyzed}$ are both equal to 0.5 M s^{-1}.

5. Iron-oxo ions and complexes

The ferryl ion species FeO^{2+} was first proposed in 1932 by Bray and Gorin [33] as one step in the ferric ion catalyzed decomposition of hydrogen peroxide. They connected the ferrous and ferric ion reactions through the postulated equilibrium

$$2Fe^{3+} + H_2O = Fe^{2+} + FeO^{2+} + 2H^+ \quad (20)$$

With hydrogen peroxide in excess, oxygen evolution was proposed as follows:

$$Fe^{2+} + H_2O_2 \rightarrow FeO^{2+} + H_2O \quad (21)$$

$$FeO^{2+} + H_2O_2 \rightarrow Fe^{2+} + H_2O + O_2 \quad (22)$$

Thus their mechanism shows both ferrous and ferryl ions attacked by hydrogen peroxide, but not ferric ions.

Bray and Gorin also stated: "The proposed mechanism explains also the decomposition of H_2O_2 during the very rapid oxidation of Fe^{2+} to Fe^{3+}." This would occur via reactions (21) and (22) when hydrogen peroxide is in excess. When ferrous ion is in excess the stoichiometry is

$$2Fe^{2+} + H_2O_2 + 2H^+ = 2Fe^{3+} + 2H_2O \quad (23)$$

which can be accounted for by reaction (21) followed by reaction (24)

$$FeO^{2+} + Fe^{2+} \xrightarrow{2H^+} 2Fe^{3+} + H_2O \quad (24)$$

Reaction (24) was not included in the Bray and Gorin paper, but the disproportionation of iron(IV) and iron(II) was published by Cahill and Taube in a study of oxygen isotope effects in hydrogen peroxide reactions [34]. The latter authors say that formation of iron(IV) is favored in the reaction of iron(II) with hydrogen peroxide "under the conditions commonly studied."

If reaction (21) is slow and reaction (24) fast, then there is a steady state in FeO^{2+}, and the rate law for the conversion of Fe^{2+} to Fe^{3+} is:

$$v = 2k[Fe^{2+}][H_2O_2] \quad (25)$$

where k, 55.4 ± 0.4 M^{-1} s^{-1} [4], is the rate constant for reaction (21).

According to Barb et al. [2], the mechanism of the reaction of excess ferrous iron with hydrogen peroxide is:

$$Fe^{2+} + H_2O_2 \xrightarrow[H^+]{k_1} Fe^{3+} + {}^•OH + H_2O \quad (26)$$

$$Fe^{2+} + {}^•OH \xrightarrow[H^+]{k_2} Fe^{3+} + H_2O \quad (27)$$

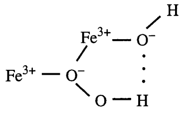
With a steady state in ${}^•OH$ one also obtains the rate law of Eq. (25). Thus there is no obvious kinetic way to distinguish the mechanisms and a conflict of opinion of 70 years duration was born. Koppenol has compared the thermodynamic properties of ${}^•OH$ and FeO^{2+} [35]. The group of Bossmann provides evidence for ferryl ion being the active oxidant in Fenton chemistry [36,37].

6. Reactions of iron(III) with hydrogen peroxide

A free radical chain mechanism for the iron(III)-catalyzed decomposition of hydrogen peroxide was published by Barb et al. [38]. Essentially they added reaction (*i*) (for *initiation*) to their mechanism for the iron(II) reaction.

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^• + H^+ \quad (i)$$

In order to explain the results reaction (*i*) had to be several orders of magnitude slower than reaction (1) for the iron(II) reaction with H_2O_2. For a high ratio of $[Fe^{3+}]/[H_2O_2]$ a second initiation process was postulated by Kozlov et al. [39], second-order in $[Fe^{3+}]$. These authors used tetranitromethane as a radical scavenger in order to study the initiation process directly. Addition of a small amount of tetranitromethane caused an induction period, an indication of a chain mechanism. Their proposal for the transition state containing two Fe^{3+} ions is shown in Scheme 2.



Scheme 2.

Kozlov et al. write the initiation step, first-order in Fe^{3+}, as

$$Fe^{3+} + HO_2^- \rightarrow Fe^{2+} + HO_2^{\bullet} \quad (i')$$

Their value for k_i' is 9×10^5 M^{-1} s^{-1}.

Kremer has also been a staunch supporter of iron-oxo reactive species in the ferric ion reaction with hydrogen peroxide [40–43], in contrast to the views of Walling [44,45]. Kremer found evidence for two iron-oxo intermediates, C_I and C_{II}:

$$Fe^{3+} + HO_2^- \rightleftarrows Fe^{3+}HO_2^- \ (C_I) \tag{28}$$

$$Fe^{3+}HO_2^- \rightarrow HO^- + FeO^{3+} \ (C_{II}) \tag{29}$$

$$FeO^{3+} + H_2O_2 \rightarrow Fe^{3+} + O_2 + H_2O \tag{30}$$

$$2H_2O_2 = O_2 + 2H_2O \tag{31}$$

a non-free radical mechanism. He notes that the reaction can be divided into several phases: (i) the initiation phase; (ii) the steady state phase, that requires sufficient excess hydrogen peroxide; (iii) the transient state; and (iv) the terminal state in which $d[X]/dt = 0$ for all species. A finite amount of FeO^{3+} is present in the terminal state. Spectroscopic evidence exists for iron-oxo ions and complexes [3,25,33,46,47]. Kremer's mechanism, as well as that of Bray and Gorin, indicates that in the simplest inorganic system there exists the equivalent of the compound I species found in peroxidases and catalases. FeO^{2+} contains iron(IV), the same as found in compound I of peroxidases, whereas FeO^{3+} contains iron(V).

6.1. Gif chemistry

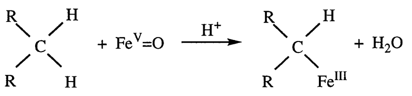
The imaginative experiments started by the group of Barton at the CNRS lab in Gif-sur-Yvette are summarized here [48,49]. Barton wondered about the chemistry that could occur on earth under its early anaerobic atmosphere. Hydrocarbons, especially methane, were abundant, and their oxidation kept oxygen levels low. Hydrogen sulfide, metallic iron and iron(II) were also present. "A form of life took advantage of the new aerobic conditions to oxidize the iron to Fe(III) and deposit it as pure ferric oxide in vast mountain ranges in Australia and Brazil. This new form of life would have obtained far more energy from coupling the oxidation of iron and hydrocarbons together than from just making ferric oxide" [48]. In Barton's model a hydrocarbon in pyridine was added to a mixture of iron, hydrogen sulfide and acetic acid. Dissolution of iron by acetic acid was aided by a surface effect of the hydrogen sulfide. Oxidation of the hydrocarbon was observed even though the excellent reductant hydrogen sulfide was present. In later experiments hydrogen sulfide was replaced by zinc as a source of electrons.

Barton moved to Texas A&M University. "In Aggieland a new chapter of Gif chemistry began: the GoAgg system" [49]. He switched to a hydrogen peroxide (or superoxide) system and postulated that an iron(V) ferryl species could be formed as follows:

$$\left.\begin{matrix} Fe^{II} + H_2O_2 \\ Fe^{II} + KO_2 \end{matrix}\right\} \rightarrow Fe^{III}\text{–O–OH} \overset{H^+}{\rightarrow} Fe^{V} = O + H_2O \tag{32}$$

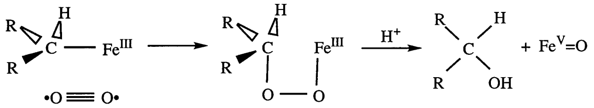
As in Barton's papers the pyridyl rings coordinated to iron are not shown for simplicity.

A hydrocarbon could be activated in what was called the 'Sleeping Beauty effect.' The Fe^V=O group reacted preferentially with secondary positions.



(33)

Addition of dioxygen followed by reduction or hydrolysis leads to alcohol or ketone production. Below is shown the formation of an alcohol, in which the Fe^V=O species is regenerated from dioxygen.



(34)

Barton proposed that this catalytic system could be an improvement over use of enzymes for drug design and production [49].

7. The continuing controversy

A review by Sawyer et al. [50], based on years of meticulous research [51,52], supported the concept that hydroxyl radicals are not formed in Fenton-like chemistry, referring to non-aqueous media, chelated metal ions, and cases where ROOH oxidants replace H_2O_2. Walling [53] and MacFaul et al. [54] claimed the opposite, whereas Goldstein and Meyerstein [55] support the idea that hydroxyl radicals are not generated. Kremer has provided further arguments that hydroxyl radicals are not important in Fenton chemistry [56].

What is the nature of an Fe^{IV}=O species? The mechanism most cited for the action of cytochromes P450, by Groves and McCluskey, may be relevant [57]. In this 'hydrogen abstraction/oxygen-rebound' mechanism, using a saturated hydrocarbon as an example, the following reaction occurs.

$$Fe{=}O + H\text{–}C \rightarrow [Fe \cdots O \cdots H \cdots C] \rightarrow [Fe \cdots HO^{\bullet} \cdots {}^{\bullet}C] \rightarrow Fe + HO\text{–}C \tag{35}$$

They used a modified Fenton model system, and state that the results "are completely consistent with our proposed mechanism of the intermediacy of a metal oxo species, formally a ferryl ion, which is subsequently responsible for hydroxylation."

8. Peroxidases and catalases

There appears to be a better agreement about the mechanism of hydrogen peroxide reactions with catalases and peroxidases than those with ferrous and ferric salts. The first step is the following:

$$\text{Peroxidase/catalase} + H_2O_2 \rightarrow \text{Compound I} + H_2O \quad (36)$$

The rate constant is always of the order of 10^7 M^{-1} s^{-1}. Details for horseradish peroxidase include the following: (i) transfer of a proton from hydrogen peroxide to a distal imidazole side chain as the ^{-}OOH group binds to the heme iron(III); (ii) transfer of the proton to the oxygen atom of the FeOOH group farthest removed from the iron to form a water leaving group; (iii) formation of a formal $Fe^{V}{=}O$ group that is actually $Fe^{IV}{=}O$, with an unpaired electron on the porphyrin ring. Thus compound I contains a ferryl group and a porphyrin π-cation radical [58]. Recent contributions to the mechanism of compound I formation include a density functional analysis [59], and a proposal that electron circuits through the protein play an important role [60]. Subsequent reactions of peroxidases usually involve one-electron reactions with organic substrates, so that compound I is reduced via an intermediate called compound II back to native enzyme [58].

In the case of catalases, compound I formation is followed by further reaction with hydrogen peroxide:

$$\text{Compound I} + H_2O_2 \rightarrow \text{Catalase} + O_2 + H_2O \quad (37)$$

a reaction so exothermic that it is used by the bombadier beetle to generate steam. A very recent explanation of the different reactions of catalases and peroxidases is the following [61]. Water is released in compound I formation and the fate of this water determines the subsequent reaction path. In peroxidases water is retained in the active site so it blocks further access of hydrogen peroxide. (Compound I reduction of peroxidases by organic substrates occurs at the porphyrin edge, not at the iron site, so retained water in the active site does not interfere with compound I reduction [62].) In catalases the water is rapidly released from the active site into bulk water, so that hydrogen peroxide can diffuse into the heme pocket as readily in compound I as it can in native catalase [61].

9. Conclusions

No one has been able to get a Fenton-like reagent nor a heme ferryl species to react with methane, yet hydroxyl radicals readily attack it. The weight of evidence favors ferryl ion formation in the reactions of iron(II)/(III) with hydrogen peroxide, a $2e^-$ reaction. Despite its long popularity the $1e^-$ reaction to form hydroxyl radicals appears not to occur.

Not only does the Haber–Weiss reaction not occur, but the most commonly assumed source of hydroxyl radicals appears not to exist.

Acknowledgements

I am grateful to Peter Jones, Willem H. Koppenol and Mordechai L. Kremer for their helpful comments and discussion.

References

[1] H.J.H. Fenton, J. Chem. Soc. (1894) 899.
[2] W.G. Barb, J.H. Baxendale, P. George, K.R. Hargrave, Trans. Faraday Soc. 47 (1957) 462.
[3] G.G. Jayson, B.J. Parsons, A.J. Swallow, J. Chem. Soc. Faraday Trans. 69 (1973) 236.
[4] M.L. Kremer, Phys. Chem. Chem. Phys. 1 (1999) 3595–3605.
[5] F. Haber, R. Willstätter, Chem. Ber. 64 (1931) 2844.
[6] W.H. Koppenol, Lecture at Oxygen 2000 symposium, San Diego, November 17–20, 2000.
[7] F. Haber, J. Weiss, Proc. R. Soc. Lond. A147 (1934) 332.
[8] A.V. Harcourt, J. Chem. Soc. 14 (1861) 267.
[9] W.C. Bray, J. Am. Chem. Soc. 60 (1938) 82.
[10] P. George, Disc. Faraday Soc. 2 (1947) 196.
[11] B. Halliwell, FEBS Lett. 72 (1976) 8.
[12] J. Weinstein, B.H.J. Bielski, J. Am. Chem. Soc. 101 (1979) 58.
[13] M.J. Gibian, T. Ungermann, J. Am. Chem. Soc. 101 (1979) 1291.
[14] C. Beauchamp, I. Fridovich, J. Biol. Chem. 245 (1970) 4641.
[15] E. Halfpenny, P.L. Robinson, J. Chem. Soc. (1952) 928.
[16] N.V. Blough, O.C. Zafiriou, Inorg. Chem. 24 (1985) 3502.
[17] M. Saran, C. Michel, W. Bors, Free Rad. Res. Comms. 10 (1990) 221.
[18] S. Goldstein, G. Czapski, Free Radic. Biol. Med. 19 (1995) 505.
[19] G. Yang, T.E.G. Candy, M. Boaro, H.E. Wilkin, P. Jones, N.B. Nazhat, R.A. Saadalla-Nazhat, D.R. Blake, Free Rad. Biol. Med. 12 (1992) 327.
[20] G. Merényi, J. Lind, S. Goldstein, G. Czapski, Chem. Res. Toxicol. 11 (1998) 712.
[21] J.W. Coddington, J.K. Hurst, S.V. Lymar, J. Am. Chem. Soc. 121 (1999) 2438.
[22] J.S. Beckman, T.W. Beckman, T.W. Chen, P.A. Marshall, B.A. Freeman, Proc. Natl. Acad. Sci. USA 87 (1990) 1620.
[23] W.H. Koppenol, J.J. Moreno, W.A. Pryor, H. Ischiropoulos, J.S. Beckman, Chem. Res. Toxicol. 5 (1992) 834.
[24] Eight values for reaction 5 quoted by B.H.J. Bielski, D.E. Cabelli, R.L. Arudi, A.B. Ross, J. Phys. Chem. Ref. Data 14 (1985) 1041.
[25] S. Marklund, J. Biol. Chem. 251 (1976) 7504.
[26] Z. Bradic, R.G. Wilkins, J. Am. Chem. Soc. 106 (1984) 2236.
[27] B.H.J. Bielski, A.O. Allen, J. Phys. Chem. 81 (1977) 1048.
[28] B.H.J. Bielski, D.E. Cabelli, Int. J. Radiat. Biol. 59 (1991) 291.
[29] J.M. McCord, I. Fridovich, J. Biol. Chem. 244 (1969) 6049.
[30] F.J. Yost, Jr., I. Fridovich, J. Biol. Chem. 248 (1973) 4905.
[31] R.A. Weisiger, I. Fridovich, J. Biol. Chem. 248 (1973) 3582.

[32] S. Goldstein, C. Michel, W. Bors, M. Saran, G. Czapski, A critical reevaluation of some assay methods for superoxide dismutase activity, Free Rad. Biol. Med. 4 (1988) 295–303.

[33] W.C. Bray, M.H. Gorin, Ferryl ion, a compound of tetravalent iron, J. Am. Chem. Soc. 54 (1932) 2124.

[34] A.E. Cahill, H. Taube, J. Am. Chem. Soc. 74 (1952) 2312.

[35] W.H. Koppenol, J.F. Liebman, J. Phys. Chem. 88 (1984) 99.

[36] S.H. Bossmann, E. Oliveros, S. Göb, S. Siegwart, E.P. Dahlen, L. Payawan, Jr., M. Straub, M. Wörner, A.M. Braun, J. Phys. Chem. A 102 (1998) 5542.

[37] S.H. Bossmann, E. Oliveros, S. Göb, M. Kantor, A. Göppert, A.M. Braun, L. Lei, P.L. Yue, Prog. React. Kinet. 26 (2001) 113.

[38] W.G. Barb, J.H. Baxendale, P. George, K.R. Hargrave, Trans. Faraday Soc. 47 (1957) 591.

[39] Yu.N. Kozlov, A.D. Nadezhdin, A.P. Pourmal, Int. J. Chem. Kinet. 6 (1974) 383.

[40] M.L. Kremer, G. Stein, Trans. Faraday Soc. 55 (1959) 959.

[41] M.L. Kremer, Trans. Faraday Soc. 58 (1962) 702.

[42] M.L. Kremer, Trans. Faraday Soc. 59 (1963) 2535.

[43] M.L. Kremer, Int. J. Chem. Kinet. 9 (1977) 179.

[44] C. Walling, Acc. Chem. Res. 8 (1975) 125.

[45] C. Walling, M. Cleary, Int. J. Chem. Kinet. 9 (1977) 595.

[46] M.G. Evans, P. George, N. Uri, Trans. Faraday Soc. 45 (1949) 230.

[47] P. Jones, R. Kitching, M.L. Tobe, W.F.K. Wynne-Jones, Trans. Faraday Soc. 55 (1959) 79.

[48] D.H.R. Barton, D. Doller, Acc. Chem. Res. 25 (1992) 504.

[49] D.H.R. Barton, Tetrahedron 54 (1998) 5805.

[50] D.T. Sawyer, A. Sobkowiak, T. Matsushita, Acc. Chem. Res. 29 (1996) 409.

[51] C. Sheu, S.A. Richert, P. Cofré, B. Ross, Jr., A. Sobkowiak, D.T. Sawyer, J.R. Kanofsky, J. Am. Chem. Soc. 112 (1990) 1936.

[52] D.T. Sawyer, C. Kang, A. Llobet, C. Redman, J. Am. Chem. Soc. 115 (1993) 5817.

[53] C. Walling, Acc. Chem. Res. 31 (1998) 155.

[54] P.A. MacFaul, D.D.M. Wayner, K.U. Ingold, Acc. Chem. Res. 31 (1998) 159.

[55] S. Goldstein, D. Meyerstein, Acc. Chem. Res. 32 (1999) 547.

[56] M.L. Kremer, J. Inorg. Biochem. 78 (2000) 255.

[57] J.T. Groves, G.A. McCluskey, J. Am. Chem. Soc. 98 (1976) 859.

[58] H.B. Dunford, Heme Peroxidases, Wiley, New York, 1999.

[59] M. Wirstam, M.R.A. Blomberg, P.E.M. Siegbahn, Reaction mechanism of compound I formation in heme peroxidases: a density functional theory study, J. Am. Chem. Soc. 121 (1999) 10178.

[60] H.B. Dunford, J. Biol. Inorg. Chem. 6 (2001) 819.

[61] P. Jones, J. Biol. Chem. 276 (2001) 13791.

[62] P.R. Ortiz de Montellano, Acc. Chem. Res. 20 (1987) 289.

[63] Average of four values obtained from pH 1 to 7, quoted by G.V. Buxton, C.L. Greenstock, W.P. Helman, A.B. Ross in Critical Review of Rate Constants for Reactions of Hydrated Electrons, Hydrogen Atoms and Hydroxyl Radicals in Aqueous Solution, Radiation Chemistry Data Center, Radiation Laboratory, University of Notre Dame, Notre Dame, IN, USA, 1988, p. 7, Table 8.

[64] H. Christensen, K. Sehested, H. Corfitzen, J. Phys. Chem. 86 (1982) 1588.

[65] Ref. [63], p. 13, Table 8.