

Fenton chemistry in biology and medicine*

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Abstract: Various aspects of the participation of Fenton chemistry in biology and medicine are reviewed. Accumulated evidence shows that both hydroxyl radical and ferryl $[\text{Fe(IV)=O}]^{2+}$ can be formed under a variety of Fenton and Fenton-like reactions. Some examples of metal-independent hydroxyl radical production are included. Extracellular Fenton reaction is illustrated by the white rot and brown rot wood-decaying fungi. The natural and practical utilization of catechol-driven Fenton reaction is also presented.

Keywords: Fenton reaction; reactive oxygen species; redox cycling; oxidative stress; free radicals; brown rot fungi; carcinogenesis; extracellular Fenton reaction.

INTRODUCTION

Free radicals and other reactive oxygen and nitrogen species (ROS and RNS) are generated by all aerobic cells and are known to participate in a wide variety of biological and biochemical processes. The ROS designation comprehends not only free radicals, such as superoxide radical anion ($\text{O}_2^{\bullet-}$), carbonate radical anion ($\text{CO}_3^{\bullet-}$), hydroperoxyl radical (HOO^\bullet), hydroxyl radical (HO^\bullet), peroxy radical (ROO^\bullet), and alkoxy radical (RO^\bullet), but also non-radicals, namely, hydrogen peroxide (H_2O_2), singlet oxygen ($^1\text{O}_2$), hypochlorous acid (HOCl), and ozone (O_3). H_2O_2 is a major ROS in living organisms, and its homeostasis can have diverse physiological and pathological consequences. In addition, H_2O_2 can produce reactive HO^\bullet radicals or ferryl intermediate $[\text{Fe(IV)=O}]^{2+}$ by the Fenton or Fenton-like reaction. H_2O_2 and other ROS oxidants are connected to aging and severe human diseases such as cancer, cardiovascular disorders, and Alzheimer's, and related neurodegenerative diseases. On the other hand, emerging evidence supports a physiological role for H_2O_2 as a second messenger in cellular signal transduction. It is well known that the exposition to certain noxious risk factors, such as some xenobiotics, infection agents, pollutants, UV light, cigarette smoke, and radiation, may lead to the production of ROS. On the other hand, ROS, as well as reactive nitrogen species (RNS) like nitrogen monoxide ($^\bullet\text{NO}$), nitrogen dioxide ($^\bullet\text{NO}_2$), and also non-radicals such as peroxyxynitrite anion (ONOO^-), peroxyxynitrous acid (ONOOH), nitrosoperoxycarbonate anion (ONOOCOO^-), nitronium cation ($^\bullet\text{NO}_2$), and dinitrogen trioxide (N_2O_3), are continuously generated in small quantities on normal cellular processes. Endogenously produced ROS and RNS are essential to life, being involved in many different biological functions. However, when overproduced, or when the levels of antioxidants become severely depleted, these reactive species become highly harmful, causing oxidative stress through the oxidation of biomolecules. It is of extreme importance that oxidative stress has been implicated in the etiology of several diseases and in aging. Consequently, in a normal cellular environment, ROS are essential to life,

*Invited contribution to a collection of papers for the IUPAC project 2005-042-1-300 "Chemistry for Biology". Other contributions to the project are published in this issue, pp. 2179–2366.

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while in the case of overproduction or depletion of antioxidants they might become deleterious [1–5]. The very important fact is that Fenton chemistry plays a crucial role in both physiological and pathological processes in living organisms. The Fenton and Fenton-like reactions are probably the earliest chemical means of ROS generation by Nature. The most reactive species, such as hydroxyl radicals, are produced by this way.

FENTON CHEMISTRY

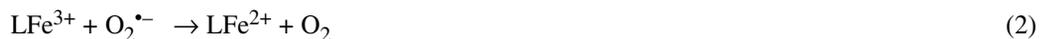
More than 110 years ago, H. J. H. Fenton published his fundamental work entitled “Oxidation of tartaric acid in presence of iron” where he showed that the system Fe(II)–H₂O₂ exhibits strong oxidation effects to some organic acids [6]. It appears later that this mixture, called Fenton reagent, is an efficient oxidation agent for various organic substrates [7]. Forty years later, the occurrence of hydroxyl radical (HO•) in Fenton reaction has been suggested [8]. Later studies have shown [9,10] that the decomposition of H₂O₂ catalyzed with ferrous and ferric ions is a chain mechanism in which Fe(II) is regenerated. An important observation of the work [9] was that the oxidation effects of Fenton reaction strongly depend on the concentration ratio of H₂O₂ and Fe²⁺ in Fenton reagent.

Nowadays, an inner-sphere electron-transfer mechanism is favored in which coordinated ferrous ion (LFe²⁺) is oxidized by H₂O₂ to form LFe³⁺, HO• radical, and HO[−] ion (Fenton reaction, reaction 1a) or an oxoiron (2+) compound (reaction 1b).



Either reactive species is a powerful oxidant, and under some specific biological conditions it can damage biomolecules [11–13]. The formation of higher oxidation iron state intermediate, such as ferryl [Fe(IV)=O]²⁺, was firstly proposed by Bray and Gorin in 1932 [14].

It is assumed that iron was firmly established as a bio-essential element during the anaerobic phase of life on our planet. It is mainly contained in enzymes involved in electron-transfer processes. Iron is also associated with toxic effects. Therefore, the living organism takes great care to sequester iron in safe complex forms. The toxicity of iron, similarly as for other transition metals, may stem from Fenton reaction. Because the iron concentration in biological systems is often very low, the very important factor for Fenton chemistry activity in Nature is the presence of functional metal redox-cycling mechanism [11]. Superoxide radical anion (O₂^{•−}) plays the role of such a reducing agent in biological systems. The reduction of Fe(III) to Fe(II) is a very important biochemical step and proceeds in numerous biological reactions [15]. Thus, the superoxide-driven Fenton reaction [16] belongs to the important reaction in biology.



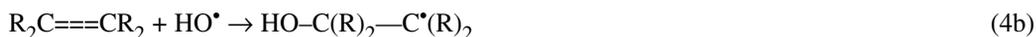
Here, LFe³⁺ or LFe²⁺ presents a coordinated iron with the available biological ligand [17]. In the first step, coordinated Fe(III) is reduced by superoxide to Fe(II), which is necessary for the latter (Fenton) reaction. Iron coordination governs iron reactivity in Fenton reaction and, as a consequence, the final oxidative damage of biological systems [18].

It was mentioned above that the reactive product of Fenton reaction is hydroxyl radical or ferryl. Therefore, Fenton chemistry is above all the chemistry of these reactive intermediates. The considerable reactivity of hydroxyl radicals [19] or ferryl [11] is convincingly documented in their atmospheric reactions with organic substrates. The reactions of hydroxyl radical may be classified with respect to their character to [20]:

- i) reactions proceeding with the abstraction of hydrogen



ii) addition reactions



iii) oxidation reactions



The ferryl ion reactivity has been studied with inorganic and organic compounds [21,22]. The reaction mechanisms in the case of organic compounds are very similar to the reactions of the hydroxyl radical, i.e., H-abstraction.

What is the nature and role of ferryl in the biological system? Although the ferryl ion intermediate has been proposed in Fenton reaction for a long time, direct HO^\bullet radical formation from $\text{H}_2\text{O}_2 + \text{Fe}^{2+}$ seems to be the most generally accepted mechanism, especially at low pH. Despite this, the ferryl ion intermediate is commonly proposed in reactions of Fe^{2+} complexes with H_2O_2 [23–25], in the reaction of H_2O_2 with Fe^{2+} in the presence of organic substrates, and in porphyrin complexes [26,27]. Peroxidases and catalases readily react with H_2O_2 ($\sim 10^7 \text{ M}^{-1} \text{ s}^{-1}$) to give a two-electron oxidized heme (compound I), which is normally a ferryl π -porphyrin radical cation [$\text{Por}^{\bullet+} \text{Fe(IV)=O}$], which upon reduction by substrate is converted into peroxidase compound II, Por Fe(IV)=O . The porphyrin iron-hydroperoxo, Por Fe(III)-OOH or compound O, was detected for horseradish peroxidase (HRP) [26,27]. Thus, for example, incubation of myoglobin (Mb) with H_2O_2 causes slow conversion ($\sim 10^2 \text{ M}^{-1} \text{ s}^{-1}$) of the ferric heme to a ferryl heme [Por Fe(IV)=O], similar to compound II in HRP [27]. On the contrary, peroxidases and catalases readily react with H_2O_2 ($\sim 10^7 \text{ M}^{-1} \text{ s}^{-1}$) to give compound I. Compound I species are key oxidizing intermediates in the enzymatic reactions. The second oxidizing equivalent of H_2O_2 is associated with porphyrin cation radical [$\text{Por}^{\bullet+} \text{Fe(IV)=O}$]. For substrate oxidation by cytochrome P450, the hydrogen abstraction/oxygen rebound mechanism has been proposed [28].



The modified Fenton system ($\text{Fe}^{2+}\text{-H}_2\text{O}_2\text{-CH}_3\text{CN}$) has been used [28]. There appears to be a better agreement about the mechanism of H_2O_2 reactions with catalases and peroxidases than those with ferrous and ferric salts. Compound I is formed in both enzymes in the first step (reaction 7).

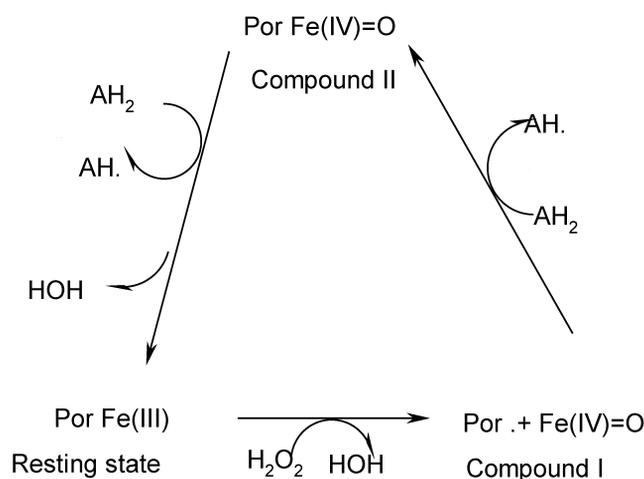


In the case of catalases, compound I formation is followed by further reaction with H_2O_2 .



This reactivity difference was explained by the water association in the active site of peroxidase. Water blocks further access of H_2O_2 . Compound I reduction of peroxidases by organic substrates occurs at the porphyrin radical cation and compound II. Water is retained in the active site opposite to catalases in which the water is rapidly released from the active site, so that H_2O_2 can diffuse into the active site in the heme pocket [29].

HRP, for example, is a member of the plant peroxidases containing heme as a prosthetic group and catalyzes the oxidation of a variety of substrates utilizing H_2O_2 . It has been established that the enzymatic reactions in aqueous buffer normally proceed through the mechanism depicted in Scheme 1, where compounds I and II represent the ferryl intermediates and AH_2 , the HRP substrate [30].



Scheme 1

Compound I is reduced back to the ferric resting state either by the two sequential one-electron-transfer processes from peroxidase substrate or by the two-electron oxidation processes associated with the ferryl oxygen transfer to substrate (e.g., $\text{R-S-R} \rightarrow \text{R-SO-R}$). Thus, the catalytic turnover of HRP with H_2O_2 involves compound I as a two-oxidation equivalent above the resting ferric state [31]. Participation of ferryl intermediates in the enzymatic Fenton-like reactions is of a great importance in biology [1,2], and the mechanism of reaction of the heme-containing peroxidase and catalase enzymes with H_2O_2 is now well established [32].

FENTON CHEMISTRY IN BIOLOGY AND MEDICINE

Nature uses metal ions extensively in functional as well as structural roles. As biological catalysts, metal ions are of crucial importance in electron-transfer reactions and in the activation and transport of small molecules such as dioxygen. Two major factors control the properties of metal ions in biological systems: (i) the structure of the metal, including the geometry of the complex and the nature of the ligands attached to the metal and (ii) the environment of the metal complex.

Iron is an essential constituent of a number of proteins involved in oxygen transport or metabolism. It must also be transported around the body, stored, and made available for synthesis of iron proteins. The ability of iron to undergo redox-cycling is an important aspect of its function. An average adult human male contains some 4.5 g of iron. Iron is stored in cells within two major proteins: ferritin and hemosiderin. In bacteria, it is mainly contained in enzymes involved in electron transfer. The iron content of *Escherichia coli* is, for example, around 10^6 ions per cell. A second salient feature of metal ions with respect to their ability to mediate biological oxidations is the availability of multiple redox states. In the case of iron, the biologically relevant oxidation states are most often +2 and +3.

On the other hand, oxygen is essential for living organisms, and the mechanism by which oxygen expresses its toxicity is linked with the availability to form different ROS. The step-wise one-electron reduction of molecular oxygen can be summarized as follows:



The final compound of oxygen reduction is water. Some reactive ROS, such as hydroxyl radical, are possible to damage different biological target molecules such as DNA, proteins, or lipids. Fenton chemistry plays an important role in these reactions. Due to its strong reactivity with biomolecules, hy-

droxyl radical is probably capable of doing more damage to biological systems than any other ROS [2,4,13,32–34].

Sources and reactions of reactive oxygen and nitrogen species

ROS and RNS are formed and degraded by all aerobic organisms, leading to either normal physiological cell function or to pathological situation called oxidative stress. Vital beneficial physiological cellular use of ROS is now being demonstrated in different areas including intracellular signaling and redox regulation. Nitric oxide (*NO) was identified as a signaling molecule and now is well known as a regulator of transcription factor activities and other determinants of gene expression. H₂O₂, hypochlorous acid (HOCl), and superoxide radical anion have similar intracellular effects [33]. On the other hand, ROS have been shown to be associated with a wide variety of pathological phenomena such as carcinogenesis, inflammation, radiation, and reperfusion injury. Iron, the most abundant transition-metal ion in our body, may work as a catalyst for the generation of ROS in pathological conditions. Iron overload is associated with carcinogenesis. In addition, the Fenton reaction may cause oxidative damage at a specific part of the genome *in vivo*, and this fact led to the novel concept called “genomic sites vulnerable to the Fenton reaction” [35]. Superoxide radical anion is considered as the primary produced ROS which further interact with other molecules to generate secondary ROS, such as hydroxyl radical, either directly or prevalently through enzyme- or metal-catalyzed processes (Fenton reaction). Superoxide is depleted undergoing a dismutation reaction with superoxide dismutase (SOD).

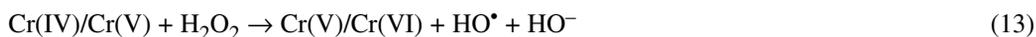


SOD enzymes accelerate this reaction in biological systems by four orders of magnitude. In biological systems, SOD enzymes work in conjunction with the H₂O₂—removing enzymes, such as catalases and glutathione peroxidases [32,34].

Most transition metals have rich coordination and redox chemistry which is closely linked with the generation of various free radicals. The major mechanisms of oxygen activation by transition-metal ions involve Fenton chemistry. Therefore, the redox state of the cell is maintained within strict physiological limits. Under stress conditions, superoxide releases Fe²⁺ ions from iron-sequestering biological molecules. The released Fe²⁺ ions can participate in Fenton reaction to produce reactive hydroxyl radicals.



In the absence of chelators, the Fenton reaction is driven by Cu(I), Fe(II), Co(II), Ti(III), and Cr(V) ions. Chelation may either inhibit or enhance these reactions [36]. Thus, for example, the reduction of Cr(VI) to Cr(III), and the resulting formation of ROS reactive intermediate [e.g., Cr(V) and Cr(IV)] is likely a key component in the toxicity and carcinogenicity of Cr(VI). Reactive Cr intermediates, Cr(V) and Cr(IV), generated by some reductants can participate in Fenton-like reactions with H₂O₂ to generate hydroxyl radical [37]. It has been pointed out that reduction of Cr(VI) by human cytochrome b₅ led to the production both Cr(IV)/Cr(V) and H₂O₂ and thus to the production of HO[•] radical by a Fenton-like reaction.



The Cr(VI) cellular toxicity could be explained in this way [37].

It has been recently also observed that exposure to vanadium in the +2, +3, and +4, but not +5, valence state was accompanied by significant augmentation of hydroxyl radical formation by activated human neutrophils *in vitro*. Similar effects were observed using cell-free systems containing either H₂O₂ or xanthine/xanthine oxidase together with V²⁺, V³⁺, and V⁴⁺ (reaction 14) [38].



Observed results clearly demonstrate that vanadium (+2, +3, +4) interacts prooxidatively with human neutrophils and that the underlying mechanism for the production of hydroxyl radical is a Fenton-like reaction (reaction 14) [38].

On the other hand, some another oxidative ROS molecules like a H_2O_2 can participate in Fenton-like reaction. For Fe(II) and Cu(I), this situation can be generally depicted as follows [20,39],



where X = Cl, ONO, and SCN.

The reduction of HOX molecules by superoxide is very similar to Fenton generation of hydroxyl radicals [40,41],



where X^- is Cl^- , NO_2^- , and SCN^- . The enzyme myeloperoxidase (MPO) can produce back HOX molecules, and thus it can function as a feedback mechanism in the production of ROS [41–46].

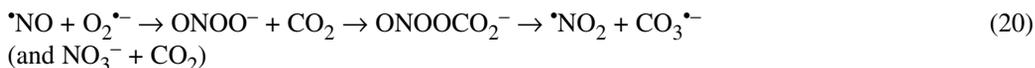


Thus, the MPO– H_2O_2 – X^- systems are very important in biological systems.

In comparison with the Fenton-like reaction (reaction 15), the spontaneous and CO_2 -catalyzed decomposition of peroxyxynitrite ($ONOO^-$) yields HO^\bullet and $CO_3^{\bullet-}$ radicals, respectively, together with *NO_2 radical [47]. Carbonate radical anion, $CO_3^{\bullet-}$, has recently been observed also in biological systems [48]. The reduction potential of $CO_3^{\bullet-}$ is 1.78 V at pH 7.0. Besides, from the reaction of CO_3^{2-}/HCO_3^- with the hydroxyl radical (reactions 18 and 19),



$CO_3^{\bullet-}$ is also generated from homolytic O–O bond cleavage of $ONO-OCO_2^-$ adduct (reaction 20).



Similarly, as hydroxyl radical, $CO_3^{\bullet-}$, can oxidize many biological targets, but its formation requires strong oxidizing species that will be limited to a few biological routes. In addition, the reaction of hydroxyl radical with bicarbonate at neutral pHs has a rate constant that is orders of magnitude smaller than the rate constants of HO^\bullet radical reactions with most organic compounds ($\sim 10^9 \text{ M}^{-1} \text{ s}^{-1}$). A more likely Fenton-type mechanism for the production of the carbonate radical anion from CO_2 can be proposed for enzymatic reactions with nucleophilic addition of deprotonated peroxides (ROO^-) to carboxylic compounds.



In biology, the most significant and important ROS/RNS-generating system in living organisms is constituted by the pool of neutrophils. In the course of their defense activities, they produce a vast amount of oxidants. The whole spectrum of oxidants generated by neutrophils is more or less due to the action of four different enzymes, catalyzing different reactions. NADPH-oxidase by which the ROS generation is initiated by respiratory burst to produce superoxide. This $O_2^{\bullet-}$ is a substrate for the SOD, which catalyzes the formation of H_2O_2 from $O_2^{\bullet-}$. H_2O_2 is relatively stable and is known for its capacity to diffuse and to cross cellular membranes. In neutrophils, however, most of the H_2O_2 (up to 70 %) is consumed by the enzyme MPO. MPO is the most abundant protein in neutrophils and catalyzes the conversion of H_2O_2 into HOCl and other ROS precursors, which are connected under transition-metal

catalysis with Fenton chemistry to produce HO• or other RO_x• radicals. Nitric oxide synthase (iNOS) catalyzes the production of •NO from L-arginine, oxygen, and reduced nicotinamide adenine dinucleotide phosphate (NADPH). Because of their reactivity, these products will constantly interact with each other, causing the formation of a myriad of oxidants among which the hydroxyl radical is recognized as being the most harmful for biological target molecules [4,5,34,49].

Metal-independent production of hydroxyl radicals

Different radical anions, X^{•-}, a one-electron reduction intermediate of neutral molecules, are very common species in cell metabolism. The main forms of such compounds in relation to ROS production are superoxide radical anions (O₂^{•-}), semiquinone radical anions (Q^{•-}), and different one-electron reduced xenobiotics, for example, RNO₂^{•-} radical anions [1–4]. Generally, when neutral molecule A accepts one electron, the responsible radical anion intermediate A^{•-} will be formed. Electron-transfer reactions in living system are very important biochemical processes. If we have a system containing different solutes A, B, C, and D, each of which react with electrons at near diffusion-controlled rates and which are present in concentrations such that [A] >> [B] >> [C] >> [D], the electron adduct A^{•-} will be formed initially. However, it is possible that subsequent electron-transfer processes may occur as follows [50]:



This picture is very similar to the real situation in living biosystems. Generally, in the cases of stable radical anions, they can be source of electrons for other biological electron-transfer reactions. On the other hand, some of these radical anions decompose rapidly to the free radical and anion (e.g., halogenated xenobiotics). Thus, quinone (Q) or oxygen (O₂) after one-electron reduction form the relatively stable semiquinone radical anion (Q^{•-}) or superoxide radical anion (O₂^{•-}), respectively. In opposite, one-electron reduction of CCl₄ gives CCl₄^{•-} radical anion which immediately splits to the •CCl₃ radical and Cl⁻ ion. Depending on their electron affinity, some radical anions such as O₂^{•-}, semiquinone (Q^{•-}), and RNO₂^{•-} may contribute to Fenton chemistry by the participation in redox-cycling of transition metals or by the redox-decomposition of HOX molecules (HOOH, HOCl, HOONO, HOSCN, ROOH, etc.) by one-electron transfer (reactions 23–25),



where X⁻ is HO⁻, Cl⁻, NO₂⁻, or SCN⁻ ions. It is clear from given examples that dissociative electron transfer in biology is very often the situation when the radical anions are produced. From a Fenton chemistry point of view, the production of hydroxyl radical is mainly of great importance. In phagocytosis, besides the Fenton-like reaction (reaction 26), very important metal-independent source of the hydroxyl radical is a reaction of superoxide with HOCl (reaction 27) [5,41].



Reaction 27 will produce HO• radicals at an appreciable rate ($k_{27} = 10^7 \text{ M}^{-1} \text{ s}^{-1}$) which is very important for the ROS production in phagocytosis.

Adriamycin has been shown to behave in this way, and its reduction by xanthine oxidase under nitrogen therefore provides a convenient method for continuous production of the semiquinone (Q^{•-}). In addition, the reaction of adriamycin with xanthine oxidase and xanthine under N₂ in the presence of H₂O₂ resulted in the production of hydroxyl radicals [51].



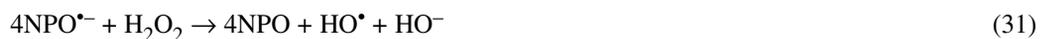
Neither a metal catalyst nor $O_2^{\bullet-}$ are required for this reaction. The characteristics of this reaction are such that it could be of major significance in the mechanism of antitumor activity of adriamycin [51]. The reaction of the adriamycin semiquinone with H_2O_2 appears to be fast, with hydroxyl radical as a major product. Similar behavior has been observed in the case of ubiquinol (reduced coenzyme Q) [52]. The ubisemiquinone intermediate was observed as a first reaction product of ubiquinol in the study of lipid peroxidation. The simultaneous formation of semiquinone and H_2O_2 led to the production of hydroxyl radical (reaction 28). In addition to inorganic peroxide such as H_2O_2 , both cumol (CumOOH) and lipid (LOOH) hydroperoxides were also found to interact with ubisemiquinone. The reaction products of the reductive homolytic cleavage were alkoxy radicals, which are strong promoters of lipid peroxidation [52].



The observed interaction of semiquinones ($Q^{\bullet-}$) with various compounds present during oxidative stress (O_2 , H_2O_2 , LOOH, HOX) demonstrates that $Q^{\bullet-}$ may initiate a variety of prooxidative reactions. Further example presents the metal-independent production of hydroxyl radicals from H_2O_2 and tetrachloro-1,4-benzoquinone (TCBQ), a carcinogenic metabolite of the widely used wood-preservative pentachlorophenol [53]. The electron spin resonance (ESR) trapping was used in this study. It is interesting that 2,5-dichloro- and 2-chloro-1,4-benzoquinone were more efficient than TCBQ in producing hydroxyl radicals. In contrast, no hydroxyl radical production was detected from H_2O_2 and the nonhalogenated quinone, 1,4-benzoquinone, and the methyl-substituted quinones 2,6-dimethyl- and tetramethyl-1,4-benzoquinone [53]. A comparative study with ferrous ion and H_2O_2 , the classic Fenton system, strongly supports the conclusion that hydroxyl radical is produced by TCBQ and H_2O_2 through a metal-independent mechanism. Another important evidence of such a mechanism is that the TCSQ $^{\bullet-}$ ESR signal was markedly decreased by the addition of H_2O_2 , accompanied by hydroxyl radical formation. These observations suggest that TCSQ $^{\bullet-}$ semiquinone radical anion directly reacts with H_2O_2 and reduces it to hydroxyl radical. It has been previously proposed [54] that if a quinone/semiquinone couple has a reduction potential of between -330 and $+460$ mV, it can theoretically bring about a metal-independent Fenton reaction. Such reactions are thermodynamically feasible and do not require metal ions for catalysis. This may well be the case of the quinone/semiquinone couples for 2-chloro-, 2,5-dichloro-, and TCBQ where the reduction potentials are -100 , $+60$, and $+250$ mV, respectively. In contrast, the reduction potentials for 2,6-dimethyl- and tetramethyl-1,4-benzoquinone of -430 and -600 mV, respectively, are outside this range, and indeed hydroxyl radical formation has not been detected [53]. The observation that chlorinated quinones can react with H_2O_2 to produce hydroxyl radical in a metal-independent manner has interesting biological consequences. For example, many widely used chlorinated aromatic compounds, such as hexachlorobenzene, chlorophenols, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2,4-dichlorophenoxyacetic acid (2,4-D), the well-known priority environmental pollutants, can be metabolized in vivo to chlorinated quinones [55] which may exert toxic effects through hydroxyl radical production.

Metabolism of xenobiotics occurs via various pathways and is catalyzed by a wide variety of enzymes. One-electron transfer by enzymes can produce a xenobiotic radical anion that can lead to the cascade which various radicals capable of causing cellular damage can arise. This is a case of enzyme glucose oxidase, which can catalyze one-electron reduction of several different classes of xenobiotics such as 1,4-naphthoquinone (1,4NQ) and 4-nitropyridine-*N*-oxide (4NPO), resulting in generation of radical anion products [56]. The coupling of oxygen reduction to glucose oxidation by the enzyme glucose oxidase results in the production of H_2O_2 with no detectable one-electron reduced intermediates (e.g., superoxide). If 4NPO and 1,4NQ were incubated together at an equimolar concentration in the presence of glucose oxidase and glucose, both 1,4NQ $^{\bullet-}$ and 4NPO $^{\bullet-}$ radical anions have been detected by ESR. The signal of 1,4NQ $^{\bullet-}$ greatly predominates the spectrum of the 4NPO $^{\bullet-}$. The addition of the

ferric ion led to its reduction to the ferrous ion and to its participation on a Fenton reaction to generate hydroxyl radical from H_2O_2 . On the other hand, the possibility of metal-independent production of hydroxyl radical by the reaction of $\text{Q}^{\bullet-}$ or $\text{RNO}_2^{\bullet-}$ radical anions with H_2O_2 cannot be rigorously excluded [51,56].

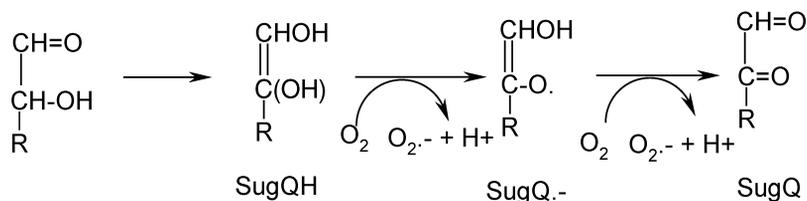


The participation of any suitable radical anion $\text{X}^{\bullet-}$ in this type of metal-independent Fenton-like reaction can be described as follows (reaction 32).



Further study to characterize the metal-independent hydroxyl radical production is needed for the deeper understanding of the mutagenic and carcinogenic properties of some environmentally important pollutants.

Sometimes, a surprising problem of the short-chain sugars mutagenic activity is another example of Fenton chemistry proceeding in biology. The short-chain reducing sugars are indeed able to induce mutagenesis. The production of hydroxyl radicals by metal-independent or -dependent Fenton-like reactions are the underlying mechanisms of their activity. Short-chain sugars such as glycolaldehyde, glyceraldehyde, and dihydroxyacetone, for example, are produced at the initial stages of nonenzymatic glycosylation. Because their carbonyl groups cannot be blocked by cyclization, such compounds tautomerize to enediols (SugQH). The superoxide is a product of their air oxidation with the final formation of H_2O_2 , on the one hand [57,58]. On the other hand, enediols after one-electron transfer to oxygen form semiquinone-like intermediate (SugQ $^{\bullet-}$), and after a transfer of a second electron, the α,β -dicarbonyl is formed (SugQ) (Scheme 2).



Scheme 2



The one-electron oxidation can be caused slowly by oxygen yielding superoxide or more rapidly by superoxide yielding H_2O_2 (reaction 33). The short-chain sugars can damage DNA. Their mutagenic effect is oxygen-dependent and is inhibited by SOD. This suggests that $\text{O}_2^{\bullet-}$ plays a central role in the short-chain sugar-induced mutagenicity [57,58]. The production of hydroxyl radicals is now possible by two ways, (i) by metal-independent hydroxyl radical production (reaction 34)



or (ii) by Fe(II) released from the [4Fe-4S] clusters by superoxide, through the Fenton reaction. This is a part of our knowledge, how diabetes mellitus-induced oxidative stress may lead to the development of accelerated atherosclerosis [59].

Basidiomycetes utilize extracellular Fenton reaction

The hypothesis that wood decay fungi use extracellular reactive oxygen species (ROS) to degrade lignocellulose dates from the middle of the last century. There are three major types of wood decay by fungi, and ROS have been implicated in all of them. White rot basidiomycetes degrade both wood polysaccharides and lignin. There are reports that white rot fungi produce extracellular HO[•] radicals, but they also secrete peroxidases, laccases, and cellulases that undoubtedly participate in lignocellulose degradation. Brown rot basidiomycetes degrade wood polysaccharides. Soft rot ascomycetes and deuteromycetes resemble brown rotters in that they degrade wood polysaccharides more readily than lignin. Little is known about their decay mechanism. The basidiomycetes that cause brown or white rot of wood are major recyclers of lignocellulose in terrestrial ecosystems, where they make essential contributions to humus formation and soil fertility. A prerequisite to gaining access to the cellulose and hemicellulose components of woody biomass is the circumvention of the lignin barrier. Filamentous fungi, the predominant degraders of wood, have evolved at least two mechanisms to circumvent this barrier. White rot fungi circumvent the lignin barrier by degrading it with extracellular peroxidases such as lignin peroxidase (LiP) and manganese peroxidase (MnP), laccases, and oxidases producing H₂O₂ [60]. LiP and MnP are classical heme-protein peroxidases. Both share mechanistic properties and form the oxidized intermediates, compounds I and II. Like LiP, MnP can also react with H₂O₂ in the absence of reducing substrate to yield compound III. The mechanism of MnP is similar to LiP except for its reducing substrate. LiP mechanism is as follows:



Manganese peroxidase (MnP) mechanism can be described as follows:



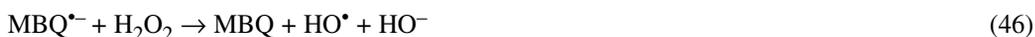
The initial reaction in both systems involves a two-electron cleavage of the peroxide dioxygen bond to produce water and compound I, which oxidizes a substrate by one-electron transfer to produce a radical cation intermediate and compound II. Transfer of a second electron from substrate to compound II is the final step of the catalytic cycle, reducing the enzyme back to its native state. In the case of wood decay, radical cation induces lignin polymer degradation to the simple molecules such as veratryl alcohols. On the basis of the above-mentioned LiP and MnP mechanisms and on the basis of our knowledge of some details revealed during the white rot fungi degradation studies, the brief wood decay mechanism, including the participation of the Fenton reaction, can be summarized as follows. It is well known that veratryl alcohol (3,4-dimethoxybenzyl alcohol) and oxalate are the main secondary metabolites of white rot fungi. It is also observed that the oxidation of lignin-derived hydroquinones by laccase leads to the accumulation in the reaction mixture of H₂O₂ and the hydroxyl radical production by Fenton reaction in the presence of iron has been occurred. Although iron in most biological systems is generally sequestered in redox-inactive complexes to prevent oxidative damage via Fenton chemistry, this is not the case in wood, which contains enough iron to make HO[•] radical generation feasible, provided chelators or reductants are available to solubilize the metal [60,61]. Oxygen activation during oxidation of the lignin-derived hydroquinones 2-methoxy-1,4-benzohydroquinone (MBQH₂) and 2,6-dimethoxy-1,4-benzohydroquinone (DBQH₂) by laccase leads to the production of superoxide, H₂O₂ and finally hydroxyl radical, respectively [61].



Redox-cycling of Fe(III) to Fe(II) in this system can occur by superoxide or by MBQ^{•-} semiquinone. It is observed that between O₂^{•-} and MBQ^{•-}, the latter is the main agent reducing Fe(III) during the oxidation of MBQH₂ [61].



On the other hand, it can be also proposed that metal-independent production of HO[•] radicals by the reaction of MBQ^{•-} semiquinone with H₂O₂ is possible.



The production of hydroxyl radical by laccase suggests novel uses for this enzyme and also for white rot fungi for the degradation of organopollutants [62].

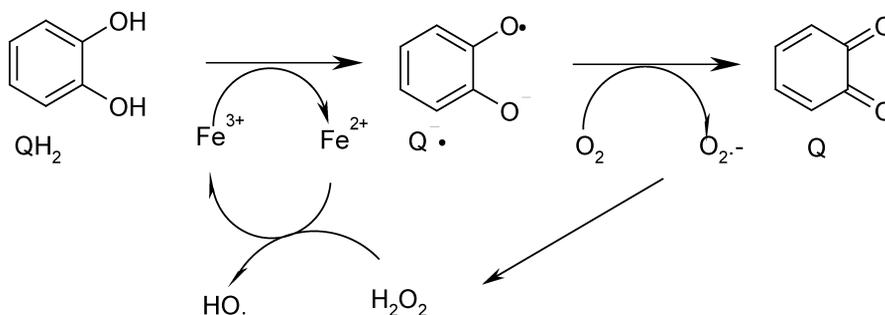
Brown rot basidiomycetes, the most interesting group of rotters, cause a highly destructive type of wood decay and are important lignocellulose recyclers in forest ecosystems. They produce ROS which might be the agents that initiate wood decay. Most of the work to date has focused on hydroxyl radical, but other ROS, such as peroxy radicals (ROO[•]) or hydroperoxy radicals (HOO[•]), might also be produced by fungi to attack wood polymers. Numerous studies indicate that hydroxyl radicals are produced via Fenton chemistry [60,63,64]. It follows that brown rot fungi require extracellular mechanism to reduce Fe³⁺ and O₂, the forms of iron and oxygen they generally encounter, if they are to degrade wood by this mechanism. Three different reducing agents for the iron have been suggested for brown rot fungi. One is an enzyme, cellobiose dehydrogenase, and the other two are oxalate and 2,5-dimethoxyhydroquinone (2,5-DMHQ). Most of the studies have been done with the brown rot basidiomycete *Gloeophyllum trabeum*. It is now well known that *G. trabeum* uses a quinone redox cycle to generate extracellular Fenton reagent, a key component of the biodegradative wood decay fungus. The production of extracellular hydroxyl radicals enables brown rot fungi to oxidize a large number of different xenobiotic chemicals. The substrates of the hydroxyl radical in the wood decay are cellulose and hemicellulose. Cleavage of these polymers into small, diffusible fragments allows the fungus to circumvent the lignin barrier. The formation of the hydroxyl radicals requires a metal (typically ferric ions), molecular oxygen, and a reducing agent. In biological systems such as wood, free iron and molecular oxygen are readily available. For example, the iron concentration in the wood samples of the *Pinus taeda* was 105 mg kg⁻¹ for the undecayed control and in the aqueous extracts, the soluble iron species concentration varied from 1.0 to 2.6 mg l⁻¹, which would be enough for reduction reactions during in vivo wood degradation. Another remarkable characteristic of the wood extracts was a strong Fe³⁺-reducing ability. High Fe³⁺-reducing activity and high catechol concentrations were detected in the wood extracts from the undecayed control [65]. Thus, for brown rot fungi, secretion of a reducing agent can result in extracellular hydroxyl radical formation. The delivery of extracellular electrons by 2,5-DMHQ and the subsequent reactions with the ferric ion and molecular oxygen may be summarized as follows:





At a low pH, secreted 2,5-DMHQ is stable for autoxidation but is a good reductant for ferric ion. One-electron reduction yields the ferrous ion and the semiquinone radical anion (2,5-DMBQ $^{\bullet-}$, reaction 47). The semiquinone radical anion is further oxidized to the quinone (2,5-DMBQ) by molecular oxygen to yield O $_2^{\bullet-}$ (reaction 48). H $_2$ O $_2$ formed by dismutation reaction (reaction 43a) reacts with Fe $^{2+}$ in the Fenton reaction (reaction 44) to produce hydroxyl radicals.

It is also observed that further dihydroxybenzenes (DHBs) such as catechol and its derivatives reduce Fe(III) to Fe(II) and enhance the formation of hydroxyl radicals in Fenton reaction. In Nature, the DHB-driven Fenton reaction is efficiently used by wood-degrading fungi, especially by brown rot fungi. These organisms use a quinone reductase enzyme to reduce quinones (e.g., 2,5-DMBQ) to hydroquinones (e.g., 2,5-DMHQ), closing the cycle [66]. Nowadays, the catechol-driven Fenton reaction (Scheme 3) is of a great value in the practical degradation of different recalcitrant compounds [67–69]. Fe(III) is reduced by QH $_2$ to Fe(II) which reacts with H $_2$ O $_2$ by the Fenton reaction to produce hydroxyl radicals. The degradation efficiencies of recalcitrant pollutants have been increased in this way.



Scheme 3

CONCLUSION

Differentiation between the hydroxyl radical or ferryl participation in Fenton chemistry is not so important in biology because of the similar chemistry of both. But more important is our knowledge of Fenton chemistry occurrence in biological systems, mainly its participation in pathological processes such as carcinogenesis, neurodegenerative diseases, atherosclerosis, etc. It is also of great importance to know the type of Fenton reaction which proceeds in the given biological system. Finally, it could be said that the Fenton reaction has played an important role in biology for all of the time that life has existed on our planet, but its role in modern civilization diseases is new.

ACKNOWLEDGMENT

This work was supported by VEGA Grant No. 1/2462/05 and by APVT Grant No. 20-029804.

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