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Hydroxyl Radical

by

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Abbreviations

$HO^{\cdot}$: hydroxyl radical

2,3-DHBA: 2,3-dihydroxybenzoate

2,4-DHBA: 2,4-dihydroxybenzoate

2,5-DHBA: 2,5-dihydroxybenzoate

HPLC: high performance liquid chromatography

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Abstract

Hydroxyl radical ($HO^{\cdot}$) is highly reactive species and is electrophilic in nature. Kinetics selectivity studies presented in this report support the hypothesis that $HO^{\cdot}$ is the transient oxidant in the photo-Fenton reaction. Kinetics experiments for photo-Fenton reactions further indicate that Fe(II) and its oxalate, citrate, and phosphate complexes react with H_2O_2 efficiently to produce $HO^{\cdot}$ in aqueous solutions with pH ranging from 3 to 8. The present study has also investigated metal-binding to various $HO^{\cdot}$ detectors, its effects on $HO^{\cdot}$ measurement and scavenging, and the occurrence of site-specificity in the reaction of $HO^{\cdot}$ with molecules.

Introduction

Previous studies indicate that direct photolysis of nitrate and nitrite can be important sources of hydroxyl radical ($HO^{\cdot}$) in natural waters. In atmospheric water drops, photolysis of hydroxide complexes of Fe(III) is an important sources of $HO^{\cdot}$. In addition, abundant evidence exists that the Fe(II)-H_2O_2 reaction is a major way for oxidation of Fe(II) and production of $HO^{\cdot}$ in sunlit regions of surface waters and the sea [1].

Chemical properties

The property of $HO^{\cdot}$ has been developed by aqueous radiation chemistry. Evidence from table 1 shows that $HO^{\cdot}$ is an oxidant. The $HO^{\cdot}$ has a standard reduction potential of ~ 2.7 V in acidic solution and ~ 1.8 V in neutral solution [2].

Table 1. Properties of $HO^{\cdot}$ in aqueous solution

Absorption maximum (nm)	~ 225
Extinction coefficient, ε (L mol^{-1} cm^{-1})	540 (188 nm)
Charge	0
Radius (cm x 10^8)	2.2
Primary yield (molecules per 100 eV), pH 7	2.7
Diffusion coefficient (cm^2s^{-1} x 10^5)	2 ~ 2.3
Reduction potential (V)	1.77 ~1.91 ($HO^{\cdot} + e^- \rightarrow OH^-$)
	2.59 ~ 2.74 ($HO^{\cdot} + e^- + H^+ \rightarrow H_2O$)
pK_a	11.9
ΔH (ionization) (kJ mol^{-1})	42
Electron affinity (eV)	1.83
ΔG_f^o (kJ mol^{-1})	13 ~ 35.7
ΔH_f^o (kJ mol^{-1})	-7 ~ -4
S^o (J mol^{-1} K^{-1})	96
ΔG (hydration)(kJ mol^{-1})	-21 ~ -10
ΔH (hydration)(kJ mol^{-1})	-42

The simplest way to represents the reaction of $HO^{\cdot}$ with ions is shown in equation 1 which contains a simple electron transfer. S means ion and n means the charge on the ion [2].

$$HO^{\cdot} + S^n \rightarrow S^{n+1} + OH^- \quad (1)$$

Also, intermediate adducts may be formed during the oxidation process of $HO^{\cdot}$ and halide ions (X^-), as shown in equation 2 [2].

$$HO^{\cdot} + X^{-} \rightarrow HOX^{-} \quad (2)$$

In aqueous solution, $HO^{\cdot}$ may react with metal ion (M) such as Tl^{+}, Ag^{+}. Cu^{2+}, Sn^{2+}, Fe^{2+} and Mn^{2+}. Hence, its coordination number (n) will be increased, as shown in the following equation [2].

$$[M(H_2O)_k]^{n+} + HO^{\cdot} \leftrightarrow [(H_2O)_k\ M\text{-}OH]^{n+} \rightarrow [M(H_2O)_k]^{(n+1)+} + OH^{-} \quad (3)$$

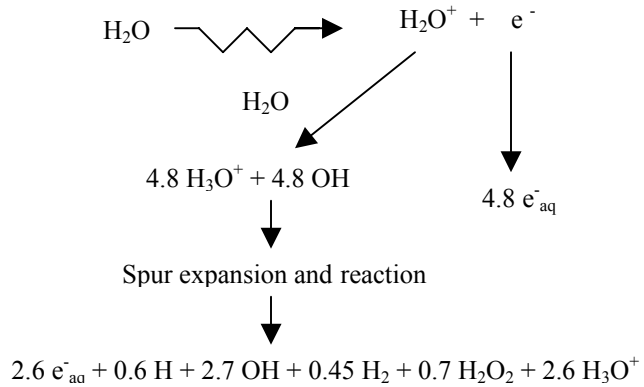
In strongly alkaline solution, $HO^{\cdot}$ rapidly converted to its conjugate base O^{-} with k = 1.2 x 10^{10} L mol^{-1} s^{-1} , and pK_a ($HO^{\cdot}$) = 11.9 [2].

$$HO^{\cdot} + HO^{-} \leftrightarrow {}^{\cdot}O^{-} + H_2O \quad (4)$$

When $HO^{\cdot}$ reacts with organic molecules, it behaves as an electrophile. It adds to unsaturated bonds and abstracts H from C-H bonds readily. Finally, different products can be formed [2].

Radiolysis of water

There are two basic methods of generating $HO^{\cdot}$ .One is the radiolysis of water, and the other is Photo-Fenton reaction that relates to the iron-salt-dependent decomposition of H_2O_2. Scheme 1 represents the radiolysis of neural water at 10^{-16} to 10^{-7} s after irradiation. During above process, the initial radiolysis product is called spur. It expands through diffusion and reacts continuously. Also, one can regard 10^{-7} s as the lifetime of $HO^{\cdot}$ which reacts with solutes at the diffusion-controlled rate in neural water [2].



Scheme 1. Radiolysis of water

Photo-Fenton reactions

On the other hand, Fenton reaction occurs under acidic condition such as in atmospheric water droplets, freshwater and seawater. This reaction involves the generation of $HO^{\cdot}$ from Fe(II) and H_2O_2. Previous studies indicate that Fe(II) complexes react with H_2O_2 at neural pH might not result in $HO^{\cdot}$. To determine Fenton reaction result in $HO^{\cdot}$ occurs in acidic condition, kinetic approach was performed. Results showed Fe(II) complexes including Fe(II)-oxalates and Fe(II)-citrates can react with H_2O_2 and produce $HO^{\cdot}$ in aqueous solutions with pH ranging from 3 to 8 [1].

To further demonstrate $HO^{\cdot}$ is the transient oxidant in the Fenton reaction, steady-state approach could be performed. One can irradiate aqueous system containing different probe compound until the reactive transients reach a steady state in which the transient's generation rates equal their decay rates. The model for Fenton reactions can be described by equations 5-8. In equation 5 and 6, $Fe(III)L_n$ represents the photoreactive complexes of Fe(III) in the system. In equation 7, $\sum k_{HO,\,i}[S_i]$ is the pseudo-first-order rate constant (s^{-1}) for $HO^{\cdot}$ scavenging by all constituents of the solution except the probe compound. In equation 8, $\sum k_{HO,p}$ is the second-order rate constant ($M^{-1}s^{-1}$) for reaction of $HO^{\cdot}$ with the probe (P). As a result, the lifetime of the hydroxyl radical is determined by its reactions with the probe compounds and with scavengers (S*i*) [1].

$$Fe(III)Ln + light \rightarrow Fe(II) + e^-_{aq} \quad (5)$$

$$Fe(II) + H_2O_2 \rightarrow HO^{\cdot} + Fe(III) \quad (6)$$

$$HO^{\cdot} \xrightarrow{\sum k_{HO,\,i}[S_i]} \text{scavenging products} \quad (7)$$

$$HO^{\cdot} \xrightarrow{\sum k_{HO,\,p}[P]} \text{scavenging products} \quad (8)$$

Fenton reactions can also be accelerated by the addition of reducing agents such as superoxide radical, ascorbate, and paraquat radical. Iron complex will become reduced forms and $HO^{\cdot}$ will be decomposed. Both effects will generate the damaging species and cause more damage to the biological molecule [3].

Rate constants

To demonstrate the rate constant of $HO^{\cdot}$, direct method and competition kinetics are used. In the direct method, the data are obtained by using pulse radiolysis and by measuring the decay of $HO^{\cdot}$ directly or observing the formation of products, as shown in the following equation [2].

$$HO^{\cdot} + S \rightarrow P \quad (9)$$

Actually, it's hard to measure the decay of $HO^{\cdot}$ because of its weak ultraviolet absorption spectrum. Also, the formation of the product can only be observed when it has proper absorption spectrum. That's why competition kinetic method is generally used. There are three advantages of this method. 1) higher concentration of solutes can be performed. 2) eliminate the possibility of $HO^{\cdot}$ reacting with itself. 3) shorten the timescale of observation and minimize the extent of continuous reactions of the products. Table 2 shows some established rate constants for $HO^{\cdot}$. Generally, for oxidation of many aquated metal cations, the rate constant of $HO^{\cdot}$ is usually less than 3×10^8 L $mol^{-1}s^{-1}$ [2].

Table 2. Selected rate constants for $HO^{\cdot}$

Reactant	k (L $mol^{-1}s^{-1}$)
Bicarbonate ion	8.5×10^6
Thiocyanate ion	1.1×10^{10}
Carbonate ion	3.9×10^8
Ferrocyanide ion	1.05×10^{10}
Iodide ion	1.1×10^{10}
Benzoate ion (BzO^-)	5.9×10^9
N, N-Dimethyl-4-nitrosoaniline (RNO)	1.25×10^{10}
Ethanol	1.9×10^9
Formate ion	3.2×10^9
Methanol	9.7×10^8
2-Methyl-2-propanol (*tert*-BuOH)	6.0×10^8
Nitrobenzene (NB)	3.9×10^9
4-Nitrobenzoate ion ($PNBA^-$)	2.6×10^9
2-Propanol	1.9×10^9
Thymine (5-MeU)	6.4×10^9

Although there are several examples of $HO^{\cdot}$ reacting with inorganic ions at the diffusion controlled rate, rate constants for oxidation many aquated metal cations usually less than 3×10^8 L mol^{-1} s^{-1} [2] .

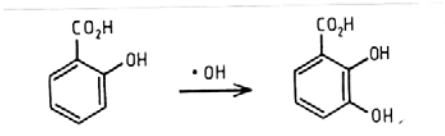
It is to be noted that the rate constant of $HO^{\cdot}$ can still be measured by direct observation of the formation of the product $Fe(CN)_6^{3-}$ which is more stable compared to other reactants [4].

$$Fe(CN)_6^{-4} + HO^{\cdot} \rightarrow Fe(CN)_6^{-3} + HO^{-\cdot} \quad (10)$$

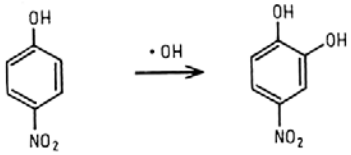
Moreover, ferrocyanide and thiocyanate ions have been used extensively as reference solutes in measuring rate constant of $HO^{\cdot}$ by competition methods. For example, the values of rate constant for the reaction of hydroxyl radicals with ferrocyanide ions can be calculated from the psuedo-first order growth of the ferricyanide [5].

Detection

Aromatic compounds are good detectors because they are hydroxylated to stable derivatives by $HO^{\cdot}$ readily. In addition, the position of attack on the ring depends on the electron-donating or the withdrawing properties of the substitutents. Shown in equation 11 and 12 are examples of the formation of hydroxylated aromatic products, salicylate and 4-nitrophenol [6].



(11)



(12)

As a result, one can use hydroxylation assay to detect $HO^{\cdot}$ produced by various iron complexes in the Fenton reaction. This assay depends on the ability of these iron complexes to hydroxylate the aromatic substrate. As shown in Figure 1, the substrate (I) doesn't react with $HO^{\cdot}$ quantitatively, it still can be used to compare $HO^{\cdot}$ production in various solutions. On hydroxylation, the predominant products formed are the *ortho* and *para* hydroxy compounds (II) and (III). Both can be quantified by standard high performance liquid chromatography (HPLC) techniques [6].

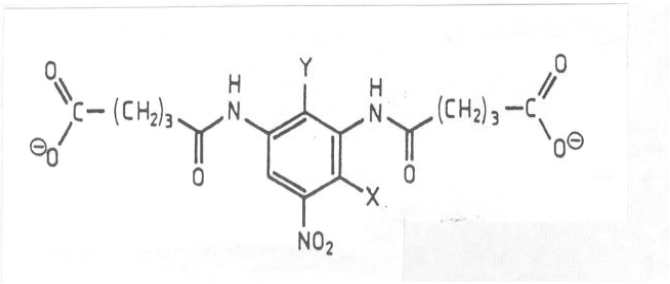


Figure1. Structure of N, N'-(5-nitro-1,3-phenylene) bisglutaramide (I), X=H, Y=H
N, N'-(4-hydroxy-5-nitro-1,3-phenylene)- bisglutaramide (II), X=OH, Y=H
N,N'-(2-hydroxy-5-nitro-1,3-phenylene) bisglutaramide (III), X=H, Y=OH

Spin trap labels is another method for $HO^{\cdot}$ detection. The use of salicylate as a reporter is also based on the ability of $HO^{\cdot}$ to attack aromatic compounds. Figure 2 shows the major products of $HO^{\cdot}$ attack on salicylate are 2,3-dihydroxybenzoate (2,3-DHBA), 2,4-dihydroxybenzoate (2,4-DHBA) and 2,5-dihydroxybenzoate (2,5-DHBA). Different DHBAs can be separated and detected by HPLC.

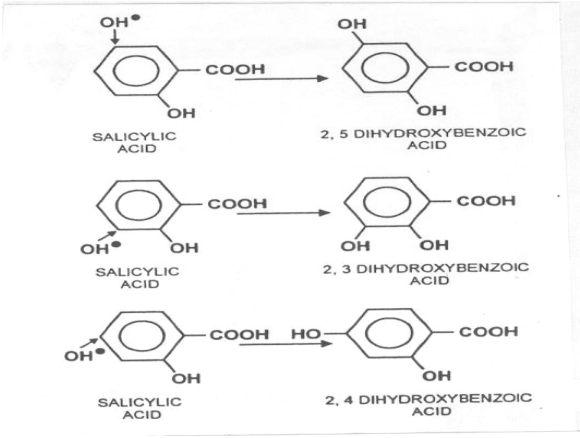


Figure2. Chemical trapping of $HO^{\cdot}$ by salicylate

Other biochemical assays for $HO^{\cdot}$ detection include the production of formaldehyde from dimethylsufoxide, the production of ethylene from methional, oxidation of formate to carbon dioxide, bleaching of p-nitrodimethylaniline [6].

One should keep in mind is that when two or more $HO^{\cdot}$ detectors or scavengers are present in an assay system they will compete for available $HO^{\cdot}$. For example, when phenylalanine competed with salicylate, DHBA production was consistently lower than calculated for random competition. This suggested that other factors may be involved in the competition for $HO^{\cdot}$ [5].

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