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Hydroxyl Radical

by

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Abbreviation:

DMPO:5, 5-dimethylpyrroline-N-oxide; UV: ultraviolet;
EPR: electron paramagnetic resonance;
DEPMPO: 5,5-dimethyl-pyrroline-N-oxide and 5-diethoxyphosphoryl-5- methyl-pyrroline-N-oxide

Contents

I. Abstract:

Hydroxyl radical is a highly oxidizing free radical. It is widely produced in many biological processes, and causes various oxidative damage in organisms. Hydroxyl radical is generated from one-electron reduction of hydrogen peroxide by metal ions and /or organic molecules *in vivo*, therefore it is highly electrophilic in nature. Understanding the properties of hydroxyl radical can help develop methods to reduce oxidative damage. The generation, detection, and reactions of this radical are reviewed in this paper.

Introduction

Hydroxyl radical is a highly reactive and toxic radical with one unpaired electron. It is formed through one-electron reduction of hydrogen peroxide [1]. It is highly electrophilic in nature [2]. Thus it is a powerful oxidizing agent. It is formed in living cells during physiological processes or after environment stimulation. Since it is a highly reactive radical, once it is formed, it will react with various biomolecules. This then causes different biological damage, which may eventually cause many disease states. Aging, heart disease, Parkinson's disease, and rheumatoid arthritis are thought to be partial outcomes of hydroxyl radical damage. In order to make progress in our understanding of these areas, it is necessary to study the chemical properties of this radical, such as the generation, detection, and reactivity.

Generation

There are a number of ways to generate hydroxyl radical, some of them are used *in vitro* as research tools. Water radiolysis, Fenton-type reaction, photolysis, sonolysis, and ozonation are briefly introduced below.

Radiolysis of water

When water is exposed to ionizing radiation, such as x-ray and γ-ray, hydroxyl radical and other radicals are produced [4]. Cell killing caused by ionizing radiation is mostly due to the hydroxyl radicals produced by water radiolysis [3]. The reactions are listed below:

$2H_2O$? $H_2O^+ + H_2O^* + e_{aq}^-$ 10^{-16} s (1)

H_2O^* ? $H + HO^?$ 10^{-14} s (2)

$H_2O^+ + H_2O$? $H_3O^+ + HO^?$ 10^{-16} s (3)

H_2O^* is an excited water molecule, and e_{aq}^- is a hydrated electron. When N_2O is present, the reaction is [3]:

$$e_{aq}^- + N_2O + H^+ ? \quad HO^? + N_2 \qquad (4)$$

Fenton reaction

Classical Fenton reaction is referred as superoxide interaction with hydrogen peroxide to produce hydroxyl radical. This reaction is thermodynamically favored, (?G = -18.2 kcal/mol) [22] but the activation energy prevents it from happening spontaneously. The rate constant can be increased dramatically in the presence of catalysts, such as iron or copper. The over all reaction was first proposed by Haber and Weiss in 1934 [5], therefore it is also named as Haber-Weiss reaction.

$$O_2^{?-} + Fe\ (III) ? \quad O_2 + Fe\ (II) \qquad (5)$$

$$O_2^{?-} + H^+ ? \quad H_2O_2 + O_2 \qquad (6)$$

$$Fe\ (II) + H_2O_2 ? \quad HO^? + OH^- + Fe\ (III) \qquad k_2 = 761\ M^{-1}S^-1 \qquad (7)$$

$$O_2^{?-} + H_2O_2 ? \quad HO^? + OH^- + O_2 \qquad (8)$$

In 1998, hydroxyl radical was detected when H_2O_2 reacted with nitric oxide in the absence of transition metals in Vass' lab [6]. Recently, M. Chevion found tetrachlorohydroquinon (TCHO) and hydrogen peroxide complex also had the ability to generate hydroxyl radical in a metal-independent way. The mechanism is unclear [7].

Photolysis

Photolysis is another important way to produce hydroxyl radical. UVB (280-320 nm) is responsible for this reaction. Under UV light exposure, intracellular H_2O_2 production is increased, which in turn forms in the presence of transition metals in a Fenton-type reaction [8].

$$H_2O_2 + Fe\ (II) ? \quad HO^? + OH^- + Fe\ (III) \qquad (9)$$

UV can directly split H_2O_2 into two HO? [23]:

$$H_2O_2 + h? ? \quad 2HO^{?} \qquad (10)$$

Ozonation

Ozone is a slowly reacting oxidant. It can transform into other more reactive oxygen species in aqueous solution, such as hydroxyl radical, and carbon centered radicals [9]. Hoigen and Bader first reported that hydroxyl radicals were oxidizing intermediates in ozonation of water. [10]. The reaction equation is:

$$O_3 + e_{aq}^- + H^+ ? \quad HO^{?} + O_2 \qquad (11)$$

Sonolysis

Water vapor at very high temperatures and pressures can be decomposed to HO?. These temperatures and pressures are achieved in the gas bubbles by ultrasound. The decomposition reaction is [4]:

$$H_2O_2 \ ? \quad 2HO^{?} + 2H^+ + O_2 \qquad (12)$$

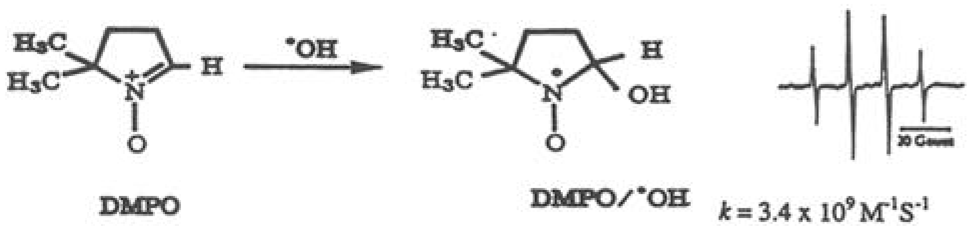
In summary, hydroxyl radical is produced in various physiological and pathological processes, and it can also be produced after certain chemical and physical stimulations, such as sunlight, smoke. The basic reactions are radiolysis and Fenton-type reaction.

Detection

Hydroxyl radical is extremely reactive. Its half-life is approximately10^{-9} second [11]. It cannot be detected directly. Electron paramagnetic resonance (EPR) spin-trapping and high performance liquid chromatography electrochemical detection (HPLC-EC) are two widely used detection methods.

EPR

Free radicals have one or more unpaired electrons. This is the reason for its paramagenetic property. In order to detect the hydroxyl radical, spin trap agents should react with it to make more stable nitroxide radicals, these relatively stable radicals can be observed by EPR. Some commonly used spin trapping agents are DMPO [12], DEPMPO [13], salicylate [15]. Here is the reaction between DMPO and $HO^{?}$:



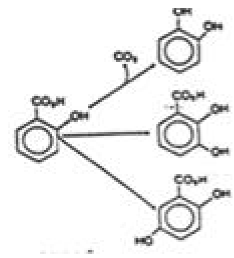
(13)

"The low stability of Hydroxyl radical-derived nitroxides if a limiting factor for directly spin trapping of $HO^{?}$ in biological systems." Addition of DMSO can partly solve this problem because $HO^{?}$ oxidizes DMSO to methyl radical. The nitroxide derivatives of methyl radical are more stable than other spin-trapped nitroxides [14]. Equation (14) shows the chemistry.

$$HO^{?} + CH_3SOCH_3 \; ? \; {}^{?}CH_3 + CH_3SO_2H \qquad (14)$$

HPLC-EC

Hydroxyl radical reacts with a number of molecules by hydroxylation. These hydroxylation products can be separated in HPLC, then detected by electrochemical detection, or fluorescence. Salicylate, tyrosine, and Phenylalanine [16] are some commonly used agents. The figure showed below is hydroxyl radical reacting with salicylate. The rate constant is 1.2×10^{10} $M^{-1}s^{-1}$ [17]:



(11%) Catechol, (49%) 2,3-Dihydroxybenzote, (40%) 2,5-Dihydroxybenzote

Figure 1: Chemical trapping of $HO^{?}$ by salicylate. [Adapted from 17].

Reactions

Hydroxyl radical rapidly reacts with other molecules in three main types reactions. All of these reactions are very fast, the rate constants are10^9 $M^{-1}s^{-1}$ magnitude [3]:

1). Hydrogen atom abstraction:

For example, when hydroxyl radical reacts with ethanol, it abstracts a hydrogen atom from ethanol to form water [3]:

$$CH_3CH_2OH + HO^{?} \ ? \ CH_3C^{?}OH + H_2O \qquad k = 2.2 \times 10^9 \ M^{-1}s^{-1} \qquad (16)$$

2). Electron- transfer:

Sometimes, hydroxyl radical transfers its electron to organic/inorganic compounds to make OH^-:

$$ClO_2^- + HO^{?} ? \ ClO_2^{?} + OH^- \qquad k = 6.6\times 10^9 \ M^{-1}s^{-1} \qquad (17)$$

3) Addition reaction:

Hydroxyl radical can add to ring structures with unsaturated bonds. .

(18)

II. Radical-radical reactions

Hydroxyl radical reacts with other radicals at nearly diffusing limiting rates because these reactions require no activation energy [4]. Some of these reactions are listed below:

$HO^{?} + HO^{?} ?\ \ H_2O_2$ $\qquad 2k = 1.1\times 10^{10}\ M^{-1}s^{-1}$ (19)

$HO^{?} + O_2^{?-} ?\ \ HO_2^{-}$ $\qquad k = 2\times 10^{9}\ M^{-1}s^{-1}$ (20)

Hydroxyl radical in cell function

Activated neutrophils release hypochlorous acid (HOCl) and $O_2^{\cdot-}$, which in turn forms hydroxyl radical. This is due to the cytotoxic effects of the neutrophils [18]. The reaction is:

$O_2^{?-} + HOCl\ ?\ \ HO^{?} + Cl^{-} + O_2$ (20)

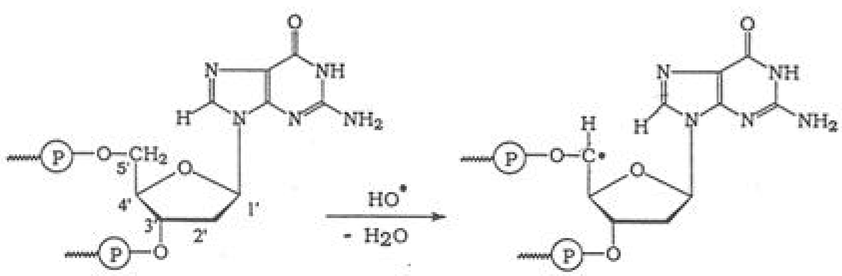
NADH, GSH and H_2O_2 are involved in respiration, it is reported that GSH and NADH can react with H_2O_2 to produce hydroxyl radical, which has considerable biological significance [19].

$NADH + H_2O_2\ ?\ \ 2HO^{?} + 2H_2O + NAD^{+}$ (21)

Hydroxyl radical can quickly reacts with all kinds of cell components, such as protein, lipid, and DNA/RNA. It reacts with amino acid by decarboxylation [20]. Equation 22 shows the chemistry. It reacts with DNA by abstracting a hydrogen atom from the deoxyribose sugars along the DNA backbone, and cut down the DNA backbone. Equation 23 shows the reaction [24]. COO

$CH + HO^{?}\ ?\ \ HO^{-} + CO_2 + R^{?}CHNH_2$ (22)

NH_2



(23)

Summary

In short, hydroxyl radical is generated in various conditions including physiological and pathological processes. It is powerful oxidant, it reacts with most molecules in a nearly diffusion limiting rate. Though direct detection of this radical is impossible, a number of indirect methods have been developed. Further research in its chemical and biological properties can lead to better understand of many pathological processes.

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