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Hydrogen Peroxide (H_2O_2)

by

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Abbreviations

A°, angstrom
CO_2, carbon dioxide
DCFH-DA, 2′, 7′-dichlorofuoresceine diacetate
ε, extinction coefficient
esu, electrostatic unit
Fe^{3+}, ferric iron
Fe^{2+}, ferrous iron
GSH, glutathione
GSSG, glutathione disulfide
H^+, proton
H_2O, water
H_2O_2, hydrogen peroxide
$HO^{\bullet}$, hydroxyl radical
OH^-, hydroxyl anion
O_2^{2-}, peroxide dianion
$O_2^{\bullet-}$, superoxide
O_2, ground state dioxygen
SOD, superoxide dismutase

Table of contents *Page number*

Abstract

Hydrogen peroxide (H_2O_2) is produced during normal aerobic cell metabolism, and its formation involves a number of enzymatic reaction especially superoxide dismutase enzyme (SOD). H_2O_2 is decomposed to oxygen (O_2) and water (H_2O) by enzymes such as catalase and glutathione peroxidase. Other non-enzymatic reactions are involved in H_2O_2 metabolism. In the presence of transition metals H_2O_2 can be reduced by the Haber-Weiss reaction to the hydroxyl radical ($HO^{\bullet}$), which is highly reactive and can result in lipid peroxidation and many biological effects.

Introduction

Hydrogen peroxide is a protonated form of peroxide ion (O_2^{2-}), which is not a radical since it has no unpaired electrons in its outer shell. Free radical researchers are interested in H_2O_2 because it can be converted into $HO^{\bullet}$ radical by a chain reaction with other molecules that then can induce toxic effects. Inactivation of critical enzymes by H_2O_2 can also induce toxicity. Understanding the chemical and physical properties of H_2O_2 and its metabolism is so important for understanding its toxic effects. This paper will focus on the chemistry, metabolism and the detection of H_2O_2.

Description of H_2O_2

Hydrogen peroxide is a two-electron reduction of dioxygen molecule [1]:

$$O_2 + 2e^- + 2H^+ \longrightarrow H_2O_2 \quad \text{(protonated form of } O_2^{2-}\text{)}$$

The diatomic O_2 molecule in a ground state is a free radical that has two unpaired electrons in a π^*2p antibonding orbital. The addition of of two electrons to O_2 will give peroxide ion (O_2^{2-}), which is not a radical as shown in Figure 1 [1]. In ground state O_2 atoms are bonded by two covalent bonds, but in O_2^{2-} by one bond only. Therefore, the oxygen-oxygen bond in O_2^{2-} ion is much weaker as compared to O_2 in ground state [2]. At physiologic pH, H_2O_2 with pKa's of 11.65 and about 16 [3] remains uncharged and protonated.

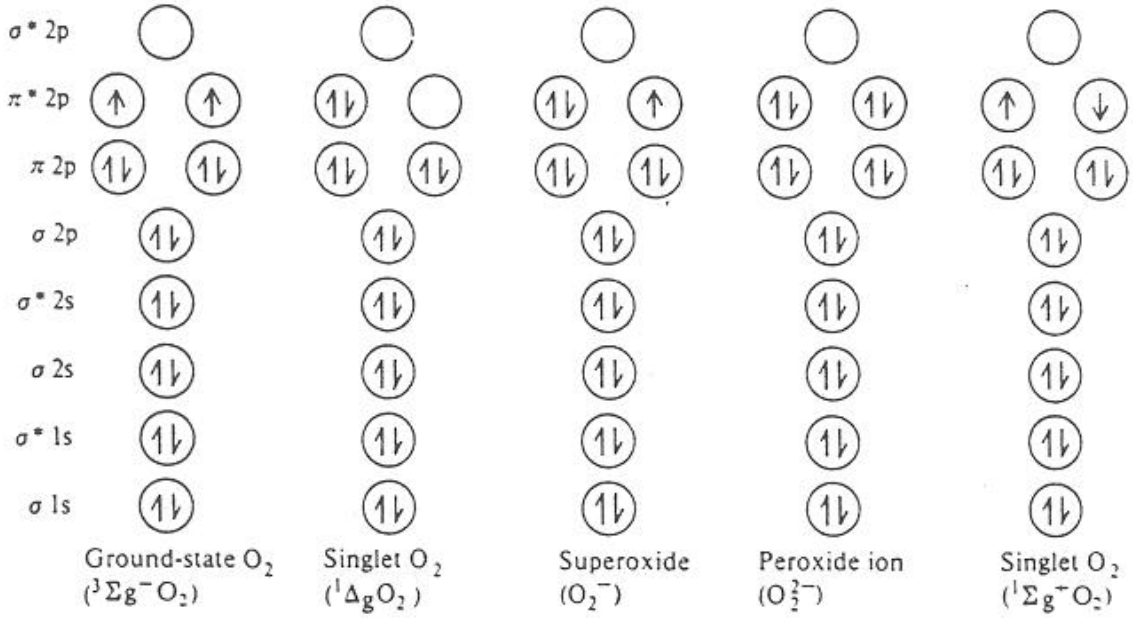


Figure 1. Electronic configuration of the diatomic oxygen molecule [1].

H_2O_2 structure

Hydrogen peroxide is a polar molecule. The dipole moment is 2.26×10^{-18} esu, which was determined by the Stark effect in microwave spectrum [4]. This polarity dictates a linear or trans-planar configuration (Figure 2a, b) [4].

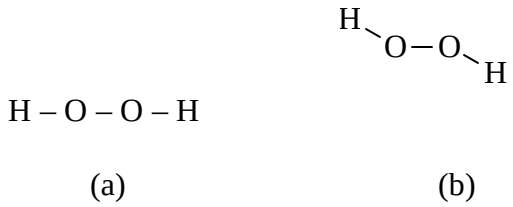


Figure 2. Linear (a), and trans.-planar (b) configuration of H_2O_2

However, three-dimensional skew chain structure is preferred, which also possess a dipole moment (Figure 3) [4]. This preferred structure was confirmed experimentally by infrared and X-ray diffraction measurements [4]. The 2p electrons of the oxygen atom are the main contributors to the chemical bonds in H_2O_2. One of the 2p electrons of each oxygen atom forms the oxygen-oxygen σ bond and another will combine with the 1s of the hydrogen to form another σ bond. This leaves the third p orbital occupied by a lone electron pair in each oxygen atom. As a result, these bonds are expected to favor an O-O-H angle θ of about 90° and two O-H bonds angle ϕ of about 90°, thereby minimizing repulsion between the lone electron pairs [4].

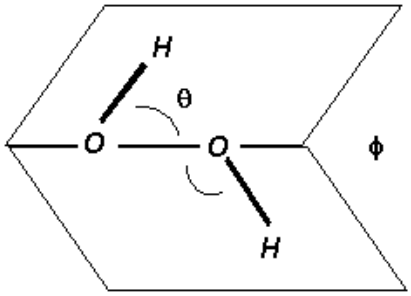


Figure 3. Skew chain structure of H_2O_2 molecule with $q = 90°$, and $f = 90°$ [4].

Physical and chemical properties of pure H_2O_2

Physical and chemical properties of pure H_2O_2 are summarized in Table 1 [5].

Table 1. Physical and chemical properties of H_2O_2.

Property	**Description**
Color, and taste	Colorless liquid with a bitter taste.
Boiling point	150.2°C
Melting point	-0.43°C
Solubility	Very soluble in water, soluble in diethyl ether, insoluble in petroleum ether.
Vapor pressure	665 Pa at 30°C
Reactivity	May decompose violently if traces of impurities are present-decompose by many organic solvents.

Chemical reactivity of H_2O_2

Hydrogen peroxide has an oxidizing and reducing power. It can oxidizes molecules such as nitrite, cyanides and hydrogen sulfide by two electrons acceptance which changes its oxidation number from –1 to –2 [4,6]:

$$H_2O_2 + 2H^+ + 2e^- \longrightarrow 2H_2O$$

It can also serve as a reducing agent for compounds such as hypochlorites, permanganates and cerium salts with increase in oxidation number of O_2 from –1 to 0 and liberation of molecular oxygen [4,6]:

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$$

Biologically, H_2O_2 is a weak oxidizing and reducing agent and is poorly reactive. H_2O_2 is toxic to more cells at concentrations at or above the 10-100 μM range [1,3]. H_2O_2 is capable of inactivating critical enzymes directly by oxidation of thiol groups at the active site of the enzyme [1,13].

H_2O_2 formation in biological systems

Hydrogen peroxide is normally found in each aerobic cell. It is generated during normal cell respiration by different metabolic process and by oxidative stress [7]. H_2O_2 is formed intracellularly by mitochondria, endoplasmic reticulum and peroxisomes, which contain a number of H_2O_2 generating enzymes. These enzymes include superoxide dismutase (SOD), several oxidases such as glycolate oxidase, urate oxidase and fatty acyl CoA-oxidase, several peroxidases, and flavin dehyrogenases [1,8]. During mitochondrial respiration O_2 acts as a terminal acceptor of electrons with 4-electron reduction yielding H_2O. However, there is a finite probability that 1-electron reduction of O_2 to form $O_2^{\bullet -}$ will occur, probably at site I or site III of electron transport chain [9]. $O_2^{\bullet -}$ then rapidly dismute to form H_2O_2. This reaction can occur spontaneously or is catalyzed by SOD [1,10]:

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \longrightarrow H_2O_2 + O_2$$

Spontaneous dismutation is a second-order reaction, which depends on the pH, and the overall rate constant is about 5×10^5 $M^{-1}s^{-1}$ at physiologic pH [1,3,10]. Whereas the SOD catalyzed reaction is

first order and is almost independent of pH in the range of 5.3-9.5, and the rate constant is about $1.9\times10^9\ M^{-1}s^{-1}$ [1,10].

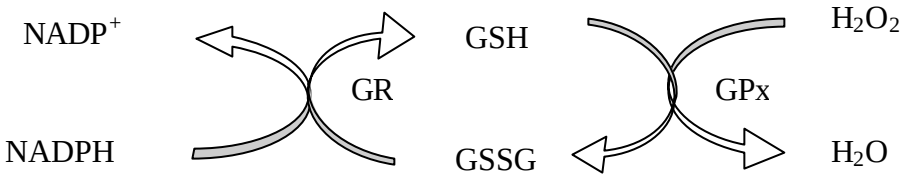
H_2O_2 metabolism

Hydrogen peroxide is decomposed enzymatically by catalase and glutathione peroxidase, and non-enzymatically by pyruvate and metal-catalyzed Fenton reaction [5]. Furthermore, H_2O_2 can be decomposed easily by UV light. In the following paragraphs a breif description of these reactions:

The decomposition of H_2O_2 by catalase is shown as follows [11]:

$$H_2O_2 + H_2O \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Catalase is present in nearly all mammalian cells and is particularly efficient in dealing with large amounts of H_2O_2 [11]. On the other hand, glutathione peroxidase is specific for GSH but not for H_2O_2. Glutathione peroxidase is more efficient in dealing with low concentration of H_2O_2 as compared to catalase [12]. GSH reduces H_2O_2 to water with formation of GSSG. Then, GSH can be generated by GSSG-reductase by consuming NADPH [1,12]:



Pyruvate and other α-ketoacids have the capacity to undergo non-enzymatic decarboxylation in the presence of H_2O_2 [13]. H_2O_2 is detoxified to water, while α-keto acid is converted to carboxylic acid and CO_2 is liberated:

$$\underset{(\alpha\text{-keto acid})}{R\text{-}C(O)C(O)OH} + H_2O_2 \longrightarrow R\text{-}C(O)OH + H_2O + CO_2 \quad [13]$$

In the presence of transition metals particularly ferrous ions (Fe^{2+}), H_2O_2 can be reduced to $HO^{\bullet}$ radical by metal catalyzed Fenton reaction [1,15]:

$$H_2O_2 + Fe^{+2} \longrightarrow HO^{\bullet} + OH^{-} + Fe^{+3}$$

Then Fe^{+3} is reduced by the $O_2^{\bullet-}$ anion:

$$O_2^{\bullet-} + Fe^{+3} \longrightarrow Fe^{+2} + O_2$$

The overall net reaction is called Haber-Weiss reaction:

$$O_2^{\bullet-} + H_2O_2 \longrightarrow HO^{\bullet} + OH^{-} + O_2$$

The generation of $HO^{\bullet}$ radicals depends on the availability of H_2O_2 and in the presence of Fe^{+2} ions. Since the concentration of H_2O_2 in cells and tissues is low, the rate of the Haber-Weiss reaction is nearly zero [1].

Heat an UV light can induce photochemical decomposition of H_2O_2 with a chain mechanism that leads to the formation of free radicals as shown below [1,4]:

Initiation $H_2O_2 + h\nu \longrightarrow 2HO^{\bullet}$

Propagation $HO^{\bullet} + H_2O_2 \longrightarrow H_2O + HO_2^{\bullet}$

$2HO_2^{\bullet} \longrightarrow H_2O_2 + O_2$

$H_2O_2 + HO_2^{\bullet} \longrightarrow HO^{\bullet} + H_2O + O_2$

The absorption of UV radiation by H_2O_2 occurs in the region extending from 4000 A° to 1950 A° as shown in Figure 4:

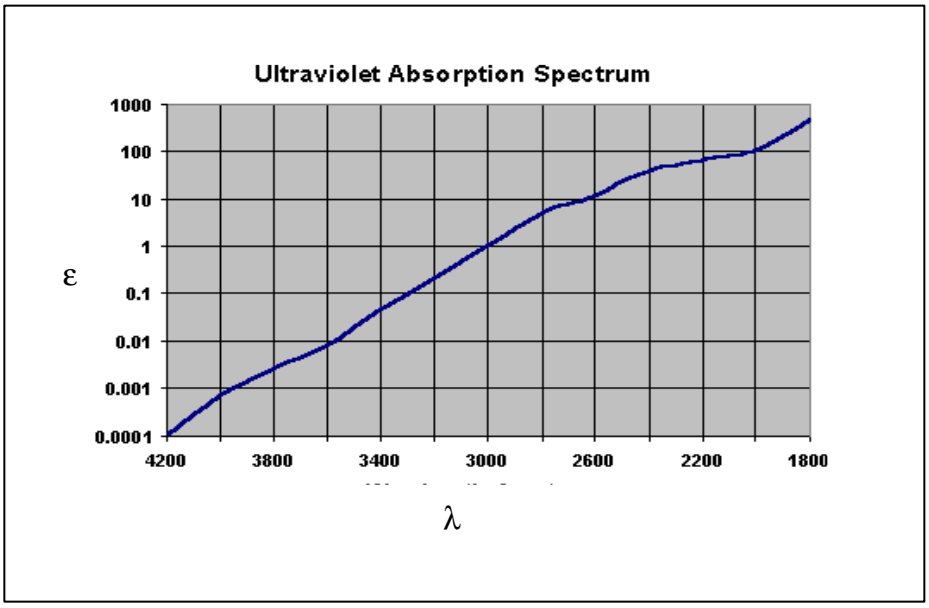


Figure 4. The UV absorption curve of H_2O_2 in distilled water. ε = Extinction coefficient, l = Wavelength, A°. (http://www.H_2O_2.com /intro/properties/radiation.html.)

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H_2O_2-mediated thiol oxidation

H_2O_2 acts on proteins and other thiol-containing molecules through the oxidation of sulphydryl (-SH) groups contained within a cysteine of the protein structure. For example, Radi's group found that the reaction of H_2O_2 with protein BSA-SH or cysteine obeyed a second order reaction with rate constants of $1.14 \pm 0.03\ M^{-1}s^{-1}$ for BSA-SH and $4.64 \pm 0.06\ M^{-1}s^{-1}$ for cysteine at 37°C and pH 7.4 [16]. Also, they found that sulphydryl oxidation with H_2O_2 is pH dependent due to the dissociation of the thiol group to thiolate anion:

$$RSH \longleftrightarrow RS^- + H^+$$

Thiolate anion

$$RS^- + H_2O_2 \longrightarrow RSOH + H_2O$$

Sulfenic acid

As a result, it was found that the rate-determining reaction for cysteine oxidation by H_2O_2 to cystine is the formation of the cysteine sulfenic acid intermediate [16].

H_2O_2 detection

Several methods for H_2O_2 detection in biological systems are developed and some of these methods are going to be discussed.

1) Horseradish peroxidase method (HRP):

A fluorescent chemical such as phenol red is used where it is oxidized by HRP and H_2O_2 to form a chromophore that absorbs light at 610 nm. It is a straightforward analytic measure of H_2O_2 concentration [1,3]. Scopoletin can be substituted for phenol red. Sensitivity is increased with strong emission at 450 nm with excitation 360 nm. However, the correlation between fluorescence and H_2O_2 concentration is not consistent over a broad range. Since $O_2^{\bullet-}$ can decrease peroxidase activity and may compromise measurement of H_2O_2 in systems generating $O_2^{\bullet-}$, SOD addition is needed to be included in the reaction [1,3]. There are other substrates, which have been substituted for scopoletin such as, homovanillic acid and baggiotoni [1,3].

2) Dichlorofluorescein diacetate (DCFH-DA):

Conversion of the non-fluorescent compound 2′, 7′-dichlorofuoresceine to the fluorescent 2′, 7′-dichlorofuoresceine was first described in 1965 as a fluorimetric assay for H_2O_2. Phagocytes and endothelial cells in combination with flow cytometry and other techniques use this method to detect the intracellular generation of H_2O_2 [1,3]. However, the exact mechanism and species responsible for oxidizing 2′, 7′-dichlorofuoresceine in biological systems is not understood [3].

3) Inhibition by catalase:

If a reaction requires H_2O_2, then it should be inhibited by catalase, since catalase converts H_2O_2 into H_2O and O_2. As a result, the determination of catalase-enhanced O_2 generation provides a direct measure of H_2O_2 production [1,14].

4) Aminotriazole inhibition:

A minotriazole is used to inhibit catalase activity. Therefore, the extent of inactivation of catalase by aminotriazole is used to calculate H_2O_2 production in an isolated cells and organs. Since this agent is ineffective in antagonizing glutathione peroxidase, this method leads to an underestimation of the cellular flux of H_2O_2 [1,3].

Summary

Although H_2O_2 is difficult to visualize and measure, researchers found that H_2O_2 can damage many cell components such as DNA, lipid and protein either directly or indirectly. Therefore, it is important to understand the characteristics, chemical reactivity and function of H_2O_2 as a reactive oxygen species.

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