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Hydrogen Peroxide: An Ubiquitous Reactive Oxygen Species

by

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Abbreviations

CAT, catalase
GPx, glutathione peroxidase
GSH, reduced glutathione
GSSG, oxidized glutathione
H_2O_2, hydrogen peroxide
HOCl, hypochlorous acid
HRP, horseradish peroxidase
LM-PCR, ligation-mediated polymerase chain reaction
MPO, Myeloperoxidase
$O_2^{\bullet-}$, superoxide anion
OH^-, hydroxide anion
$OH^{\bullet}$, hydroxyl radical
$^1O_2$, singlet oxygen
PHPA, *p*-hydroxyphenylacetic acid
SOD, superoxide dismutase

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Abstract

Hydrogen peroxide is a reactive oxygen species commonly found in biological sources. It can be formed through the dismutation of superoxide, destroyed in reactions catalyzed by catalase and glutathione peroxidase. It can also be manipulated by macrophages to form hypochlorous acid and hydroxyl radical. Although it is a normal byproduct of cellular metabolism, it is also a potentially damaging species, capable of disrupting polysaccharides, proteins, lipids, and DNA.

Introduction

Hydrogen peroxide is a small, ubiquitous, strongly oxidizing agent. Cells may be exposed to it as a result of exogenous stresses, or as a byproduct of normal metabolism. The purposes of this paper are to describe: (1) the chemical properties of the hydrogen peroxide molecule, (2) the catabolic and anabolic reactions of hydrogen peroxide, (3) methods for detection and quantification, and (4) the effects of hydrogen peroxide exposure on cells.

Physical and Chemical Properties of Hydrogen Peroxide

Hydrogen peroxide is a small molecule (MW = 34.01) commonly produced by aerobic biological systems [1]. It has a melting point of -0.43°C and a boiling point of 152°C [2], making it a liquid at room temperature. This colorless, bitter-tasting, syrupy liquid is completely miscible with water, and soluble in ether [2]. 30% hydrogen peroxide has a specific gravity of 1.11, a vapor density of 1 g/L [1], and a density of 1.463 g/cm^3[2]. Hydrogen peroxide is available as a 3% or 30% solution, and the densities of these are 1.00 g/cm^3 and 1.11 g/cm^3, respectively [2].

Hydrogen peroxide is a fairly unstable molecule when in a water solution and decomposes easily, particularly when metal contaminants are present. This is because the O-O bond is very weak, its bond dissociation energy being only 51 kcal/mol [4]. If one examines a molecular orbital diagram of the peroxide anion, the reason for this weakness becomes easily apparent (See Figure 1.) Since each oxygen contributes a total of five electrons to the 12 molecular orbitals, two of the antibonding orbitals are filled, leaving only 1 net bonding orbital.

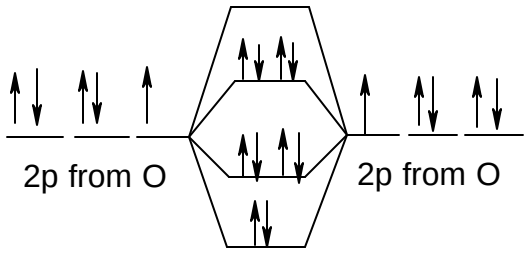

Figure 1: Molecular orbital diagram for the O-O bond in hydrogen peroxide.

Many compounds are known to hasten the decomposition of H_2O_2, including albumin, alkalies, alkali citrates, ammonia, carbonates, chlorides, ferrous salts, gold, hypophosphites, iodides, mercurous salts, permanganates, phenol, sulfites, and a variety

of organic compounds [2]. H_2O_2 solutions must also be stored at 4°C in a darkened container to prevent decomposition, as the energy from photons can break the weak O-O bond [1]. Even simple agitation of the solution or contact with rough surfaces can cause decomposition [2]. Stabilizers such as acetanilide (or similar organic compounds) and mineral acids are often added to hydrogen peroxide solutions to increase shelf life [2].

Hydrogen peroxide is a fairly strong oxidizer. For the reaction:

$$H_2O_2 + 2H^+ + 2e^- \text{-------}\rightarrow 2\ H_2O \qquad (1)$$

$E° = +1.77$ V for an aqueous H_2O_2 at 25°C [3]. Hydrogen peroxide is also weakly acidic, with a $pK_a = 11.7$ (K_a of 2.4×10^{-12}) [3].

Hydrogen Peroxide-Forming Reactions

Two important reactions involving the dismutation of superoxide cause the formation of H_2O_2 in a cell. At acidic (non-physiologic) pH, superoxide anion reacts with an acid to give hydroperoxyl radical, which spontaneously forms hydrogen peroxide and oxygen (dioxygen) [4].

$$2HO_2^\bullet \rightarrow H_2O_2 + O_2 \qquad (2)$$

This reaction occurs quickly (k for hydroperoxyl radical to hydrogen peroxide is $8.3 \times 10^5\ M^{-1}s^{-1}$) [5]. At physiologic pH, however, most of the superoxide anion is in its deprotonated form, so the dominant biological reaction is actually [4]:

$$O_2^{\bullet-} + HO_2^\bullet + H^+ \rightarrow H_2O_2 + O_2 \qquad (3)$$

Therefore, most of the chemical dismutation of superoxide to hydrogen peroxide actually occurs via reaction (3). The k for this reaction is $9.7 \times 10^7\ M^{-1}s^{-1}$ [5].

Finally, there is a third possible superoxide dismutation reaction [4].

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (4)$$

However, since the rate constant for this reaction is very slow ($k < 0.3\ M^{-1}s^{-1}$), the enzyme superoxide dismutase (SOD) is used to drastically increase the rate of superoxide catabolism ($k = 2$ to $4 \times 10^9\ M^{-1}s^{-1}$) [5].

In addition to these reactions, hydrogen peroxide may be produced due to the action of oxidase enzymes. For example, during the oxidation of glucose, an enzyme called glucose oxidase makes hydrogen peroxide from dioxygen [6].

Hydrogen Peroxide-Catabolizing Reactions

Hydrogen peroxide is catabolized in several important reactions in a biological system. The first of these reactions is catalyzed by catalase (CAT). The k for this reaction (below) is $1.7 \times 10^7\ M^{-1}\ s^{-1}$ [4]:

$$2\ H_2O_2 \xrightarrow{\text{CAT}} 2\ H_2O + O_2 \quad (5)$$

Hydrogen peroxide may also be altered in a peroxidase-driven reaction as follows [4]:

$$H_2O_2 + 2\ GSH \xrightarrow[\text{peroxidase}]{\text{Glutathione}} 2\ H_2O + GSSG \quad (6)$$

In this reaction, hydrogen peroxide is removed by oxidation of a tripeptide thiol called glutathione (GSH) in a reaction catalyzed by glutathione peroxidase (GPx). The oxidized glutathione (GSSG) is recycled by means of glutathione reductase, which uses NADPH as a source of electrons for the reduction [7]. The glutathione peroxidase system is responsible for the catabolism of most of the hydrogen peroxide in a cell [6].

Two additional hydrogen peroxide-consuming reactions occur in macrophages. The first involves the enzyme myeloperoxidase (MPO), which combines hydrogen peroxide and chloride ion to form HOCl(hypochlorous acid or bleach). In a second reaction, hydrogen peroxide oxidizes Fe(II) to Fe(III) and produces $OH^{\bullet}$ [7]. Both of these substances are highly toxic to invading microorganisms.

Methods for Detecting and Measuring Hydrogen Peroxide

There are numerous ways of detecting and quantifying hydrogen peroxide in a solution. HPLC-ECD has been successfully used [4]. It can also be quantified by measuring its absorbance at 240 nm in a standard spectrophotometer [I].

Others have used an oxygen electrode to measure the release of O_2 following the addition of catalase to a system. For every two hydrogen peroxide molecules in a sample, one dioxygen molecule will be produced by the catalase reaction. This method is sensitive enough to measure concentrations as low as 5 μM hydrogen peroxide [6].

Another method of quantification involves the use of an aromatic compound called *p*-hydroxyphenylacetic acid (PHPA). In this method, horseradish peroxidase is used to catalyze a hydrogen peroxide-dependent dimerization reaction between PHPA molecules as seen in Figure 2 [9].

Interactions of Hydrogen Peroxide with Biological Systems

Hydrogen peroxide freely diffuses across biological membranes [8]. As a result it is a very important and potentially damaging molecule to biological molecules, including

DNA, polysaccharides, proteins, and lipids [10, 11, 12, 13]. It can also activate or inhibit a variety of cellular pathways (see table 1).

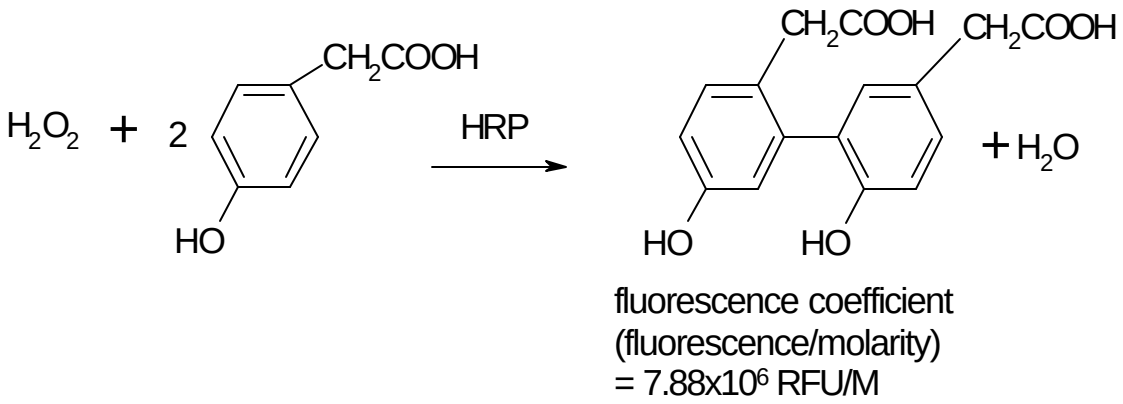

Figure 2: Oxidative dimerization of PHPA by HRP [9].

Cellular Consequences of Hydrogen Peroxide Exposure
1. Hexose monophosphate shunt activation
2. Glutathione redox cycle activation
3. Oxidation of intracellular sulfhydrals
4. Decreased intracellular ATP
5. DNA damage
6. Loss of NAD^+
7. Poly (ADP-ribose) polymerase activation
8. Increased free Ca^{2+}
9. Cytoskeletal alterations
10. Plasma membrane alterations
11. Inhibition of glycolysis

Table 1: Cellular Effects of H_2O_2 [13]

As mentioned, H_2O_2 is capable of damaging cellular polysaccharides. One group found that exposure to H_2O_2 caused breakdown of pectin, sodium-carboxycellulose, and xylan in a reaction which was optimal at physiologic pH (6.5 to 7.5) to yield reducing compounds which were ethanol-soluble [12]. They proposed that this mechanism could be important in cell wall breakdown in plant cells.

DNA can also be damaged as a result of exposure to H_2O_2. In 1947, investigators noted that the ability of *Arbacia punctulata* sperm were less able to fertilize eggs when the sperm had been treated with H_2O_2. Additionally, when the eggs were H_2O_2-treated, they were less fertilizable by untreated sperm. This effect could be attenuated by addition of catalase to the H_2O_2-containing solution [10]. At that point, however, the mechanism for DNA damage was not understood. Later, it was suggested that H_2O_2 does not directly cause DNA damage, but rather works by reacting with a ferrous catalyst which is complexed to DNA. The iron catalyzes H_2O_2 breakdown to $HO^{\bullet}$, which is the species actually responsible for the oxidation of a base, ultimately resulting in strand breakage [14]. Later yet, sites of DNA damage were mapped using the *E. coli* restriction enzymes endonuclease III (Nth protein), and formamidopyrimidine glycosylase (Fpg protein) combined with LM-PCR. Nth and Fpg are special restriction enzymes which only cut at oxidized bases. This group found that Cu(I)-H_2O_2-mediated DNA damage is sequence dependent, primarily occurring at the 5' bases of d(pGn) and d(pCn) and at the internal guanines of d(pCCCGGG) and d(pGGGCCC) [11]. See Figure 3 for a summary of copper(I)-H_2O_2-mediated DNA damage.

Hydrogen peroxide is capable of damaging proteins. The primary way this occurs is through the oxidation of the sulfhydryl groups in cysteines. This oxidation creates a disulfide bond, which can interfere with the protein's conformation and function, especially when the cysteines are found in the active site of an enzyme [15].

Finally, hydrogen peroxide can damage unsaturated fatty acids in a process known as lipid peroxidation [16], which in itself can alter membrane structure and fluidity. Xanthine oxidase can cause the production of singlet oxygen from hydrogen

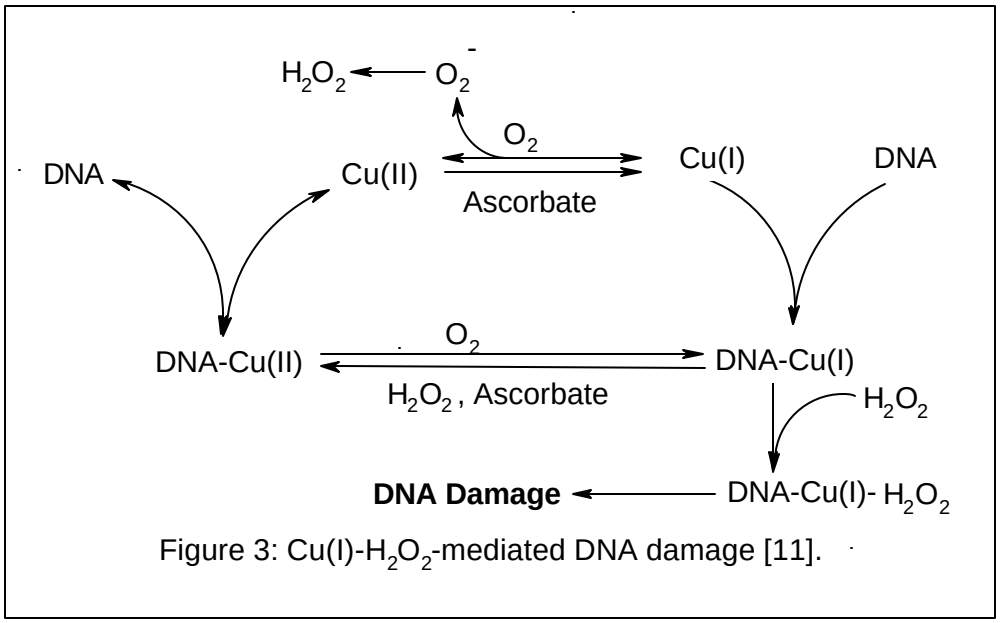

Figure 3: Cu(I)-H_2O_2-mediated DNA damage [11].

peroxide and singlet oxygen in the reaction [16]:

$$O_2^{\bullet-} + H_2O_2 \text{ --------} \rightarrow OH^- + OH^{\bullet} + {}^1O_2 \qquad (7)$$

In addition to this, lipid peroxidation itself results in the production of $HO^{\bullet}$, H_2O_2, $^1O_2$, peroxyl radicals and alkoxyl radicals, which can then be damaging to DNA [17].

Summary

While H_2O_2 is a reactive oxygen species commonly found in cells, it has many potentially toxic effects, including damage to DNA, lipids, carbohydrates, and proteins. However, it is also a critical part of cellular metabolism, particularly in the detoxification of superoxide and the production of more damaging reactive oxygen species in macrophages. Thus, it is apparent that we must understand the reactions hydrogen peroxide undergoes in the cell.

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