ROS

REVIEW ARTICLES

Hydrogen Peroxide in Biology and Medicine: An Overview

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ABSTRACT | Hydrogen peroxide (H_2O_2) is one of the most extensively studied reactive oxygen species (ROS) in biology and medicine. It is generated constitutively from various cellular processes either directly via two-electron reduction of molecular oxygen indirectly via dismutation of superoxide. The notable direct cellular sources for H_2O_2 include xanthine oxidoreductase, monoamine oxidase, endoplasmic reticulum oxicireductin 1, oxidases in peroxisomes, and possibly certain members of the NOX/DUOX family. Because of the high activation energy, H_2O_2 reacts poorly with most cellular constituents. However, it may oxidize the thiol groups in certain proteins and enzymes, including these involved in cell signaling transduction. The potential of H_2O_2 to cause oxidative stress and tissue injury primarily results from its reactions with other molecules to form secondary reactive species, including hydroxyl radical and hypochlorous acid. While the tightly controlled production of H_2O_2 plays important roles in various physiological responses, overproduction of this ROS contributes to the pathophysiology of a variety of disease processes and related conditions, including cardiovascular diseases, diabetes, neurodegeneration, cancer, and aging, among many others.

KEYWORDS | Disease process; Hydrogen peroxide; Immunity; Redox signaling

ABBREVIATIONS | CYP, cytochrome P450; ERO1, endoplasmic reticulum oxidoreductin 1; MAO, monoamine oxidase; MPO, myeloperoxidase; NOX, NADPH oxidase; ROS, reactive oxygen species; SOD, superoxide dismutase

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1. OVERVIEW

Hydrogen peroxide (H_2O_2) was discovered in 1818 by Louis Jacques Thénard (1777–1857), a French chemist [1], and the biological catalyst of H_2O_2 was identified and named as catalase in 1900 by Oscar Loew (1844–1941), a German chemist [2]. In the early 1970s, H_2O_2 was shown to be produced by animal cells and tissues [3–5]. It is now known that formation of H_2O_2 occurs ubiquitously in both animal and plant cells, as well as microorganisms. The past two decades have witnessed the explosion of knowledge on H_2O_2 in biology and medicine, ranging from its well established ability to cause oxidative stress and tissue injury to its emerging roles in cell signaling and normal physiology. Indeed, H_2O_2 is also among the most extensively investigated reactive oxygen species (ROS) in biology and medicine.

In biological systems, H_2O_2 is the primary product of superoxide dismutation, which occurs either spontaneously or catalyzed by superoxide dismutase. Hence, the sources of production and the biological activities of these two ROS overlap significantly. Different from superoxide, H_2O_2 is a non-radical species with a relatively long half-life in biological milieu and is able to readily cross cell membranes and diffuse into different cellular compartments. As such, H_2O_2 may act as a novel second messenger in cell signal transduction. This review article considers the source, chemistry and biochemistry, as well as biology and medicine of this simple, but biologically unique molecule.

2. SOURCES

H_2O_2 is a major ROS formed in animal cells from various intracellular sources, which are discussed next. It is noteworthy that H_2O_2 is also formed in plant cells, with mitochondria and chloroplasts being the major sources [6]. In addition to the animal and plant kingdoms, H_2O_2 is found in Earth's atmosphere as well as interstellar space [7]. Regarding the cellular production of H_2O_2 in animals, including humans both direct and indirect mechanisms have been identified (**Figure 1**).

2.1. Indirect Formation via Superoxide Dismutation

H_2O_2 is formed through either the spontaneous or superoxide dismutase (SOD)-catalyzed dismutation of superoxide. Therefore, the chief sources for superoxide formation are also the main ones for H_2O_2. In this regard, NADPH oxidases (also known as NOXs) and mitochondrial electron transport chain are widely considered the primary sources of superoxide-derived H_2O_2 in mammalian cells. Other sources of superoxide-derived H_2O_2 include xanthine oxidoreductase, cytochrome P450 enzyme system, and uncoupled endothelial nitric oxide synthase (eNOS), as well as the redox cycling of environmental chemicals and drugs by cellular one-electron reduction systems (e.g., cytochrome P450 reductase).

2.2. Direct Formation via Two-Electron Reduction

Some enzymes in mammals including humans may directly catalyze the two-electron reduction of molecular oxygen to form H_2O_2 or predominately produce H_2O_2 via an unclear mechanism. These include xanthine oxidoreductase, monoamine oxidase, some members of the NOX/DUOX family (e.g., NOX4, DUOX1, DUOX2), and multiple oxidases in peroxisomes, among others.

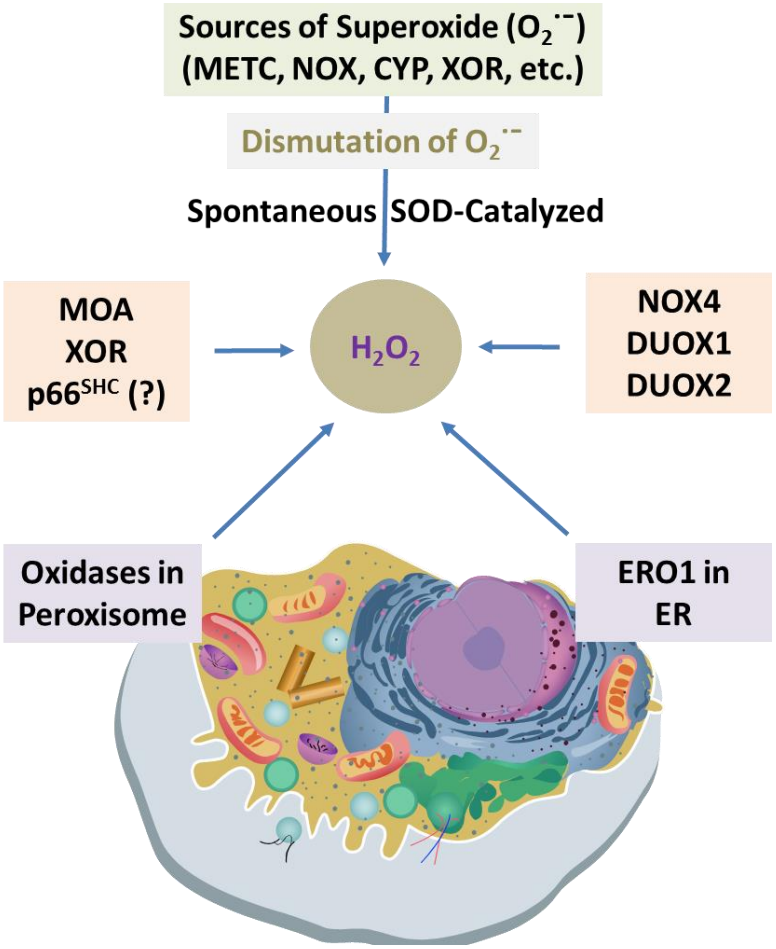

FIGURE 1. Cellular sources of hydrogen peroxide (H_2O_2). Cellular H_2O_2 production may result from either dismutation of superoxide or directly via two-electron reduction of molecular oxygen. Enzymes that directly catalyze two-electron reduction of molecular oxygen to form H_2O_2 include xanthine oxidoreductase (XOR), monoamine oxidase (MAO), endoplasmic reticulum (ER) oxidoreductin 1 (ERO1), and oxidases in the peroxisome, as well as possibly $p66^{SHC}$ and some members of the NADPH oxidase (NOX)/DUOX family (e.g., NOX4, DUOX1, DUOX2). XOR is also capable of catalyzing one-electron reduction of molecular oxygen to superoxide. METC, mitochondrial electron transport chain; CYP, cytochrome P450 system.

2.2.1. Xanthine Oxidoreductase

As noted above, xanthine oxidoreductase catalyzes the one-electron reduction of molecular oxygen to form superoxide. This enzyme also directly catalyzes the two electron-reduction of molecular oxygen to form H_2O_2. Hence, xanthine oxidoreductase is capable of causing both one- and two-electron reduction of molecular oxygen to form superoxide and H_2O_2, respectively [8].

2.2.2. Monoamine Oxidase

Another enzyme capable of directly producing H_2O_2 is the flavin-dependent monoamine oxidase (MAO). This enzyme catalyzes deamination of dopamine through a two-electron reduction of molecular oxygen to H_2O_2 [9]. There are two types of MAO, namely, MAOA and MAOB, and both are located in (or bound to) the outer membrane of mitochondria in most cell types in the body.

2.2.3. NOX/DUOX Family

While superoxide is the primary product of most NOX enzymes, NOX4 may predominantly produce H_2O_2 rather than superoxide [10, 11]. Dual oxidases 1 and 2 (DUOX1 and DUOX 2), members of the NOX/DUOX family, may also primarily generate H_2O_2 [12]. However, it remains unclear whether these enzymes can directly catalyze the two-electron reduction of oxygen to H_2O_2 or they produce H_2O_2 via a possible superoxide intermediate that may not be detected by current techniques due to rapid intramolecular dismutation or inaccessibility to the superoxide-detecting probes [12, 13].

2.2.4. Endoplasmic Reticulum

Endoplasmic reticulum (ER) is a significant source of cellular H_2O_2 due to the presence of various oxidoreductases in this organelle. While the cytochrome P450 enzyme (CYP) system associated with ER is a major indirect source of H_2O_2 (from dismutation of CYP-derived superoxide), oxidoreductases present in the ER lumen can directly reduce oxygen to form H_2O_2. For instance, the endoplasmic reticulum oxidoreductin 1 (ERO1), also known as endoplasmic reticulum oxidase 1, is a major source of H_2O_2 formed in the ER lumen [14]. The H_2O_2 produced by ERO1 plays an important role in oxidative protein folding in the ER. However, in cells lacking ERO1, H_2O_2 is also formed in the ER lumen and fuels peroxiredoxin 4-mediated oxidative protein folding, suggesting the existence of an unrecognized luminal source of H_2O_2 [15].

2.2.5. Oxidases in Peroxisomes

Peroxisomes contain various enzymes that produce H_2O_2 as part of their normal catalytic cycle. These enzymes include acyl-CoA oxidases, urate oxidase, D-amino acid oxidase, D-aspartate oxidase, L-pipecolic acid oxidase, L-α-hydroxyacid oxidase, polyamine oxidase, and xanthine oxidase [16].

2.2.6. Others

The mitochondria-associated redox protein $p66^{SHC}$ is a genetic determinant of lifespan in mammals [17]. This redox protein may reduce oxygen to H_2O_2 by utilizing the reducing equivalents of the mitochondrial electron transport chain via oxidation of cytochrome c [18].

Although not naturally occurring in mammalian tissues, glucose oxidase, an enzyme expressed in certain fungal species, is perhaps among the best know enzymes for producing H_2O_2. Glucose oxidase catalyzes the oxidation of beta-D-glucose to gluconic acid, by utilizing molecular oxygen as an electron acceptor with simultaneous production of H_2O_2 [19, 20]. This enzyme has a number of industrial and biotechnological applications, with its use in measurement of blood glucose being most notable [19, 20].

3. CHEMISTRY AND BIOCHEMISTRY

3.1. General Chemical Properties

H_2O_2 is a strong two-electron oxidant, with a standard reduction potential of 1.32 V at pH 7.0 (H_2O_2/H_2O). It is therefore more oxidizing than hypochlorous acid (OCl^-/Cl^-) and peroxynitrite ($ONOO^-/NO_2^-$), for which the standard reduction potentials are 1.28 and 1.20 V, respectively. However, in contrast to the above two oxidants, H_2O_2 reacts poorly or not at all with most biological molecules, including proteins, nucleic acids, and lipids, as well as low-molecular-weight antioxidants. This is because a high activation energy barrier must be overcome to release its oxidizing power, or in other words, the reactions of H_2O_2 are kinetically rather than thermodynamically driven [21].

Nevertheless, as discussed below, via two-electron oxidation, H_2O_2 reacts readily with certain biological molecules especially protein thiols to account for much of its signaling function. On the other hand, H_2O_2 is a weak one-electron oxidant with the standard reduction potential of 0.32 V ($H_2O_2/ OH^{\bullet}$). But, its reaction with transition metals (e.g., iron and copper) generates the highly reactive hydroxyl radical which may account for much of the detrimental effects of H_2O_2 in biological systems.

3.2. Oxidation of Protein Sulfhydryl Groups

Although in general the reaction between H_2O_2 and proteins is much limited, the cysteine thiol groups (also known as sulfhydryl groups) in certain proteins are readily oxidized by H_2O_2. These proteins include antioxidant enzymes (e.g., peroxiredoxins) and cell

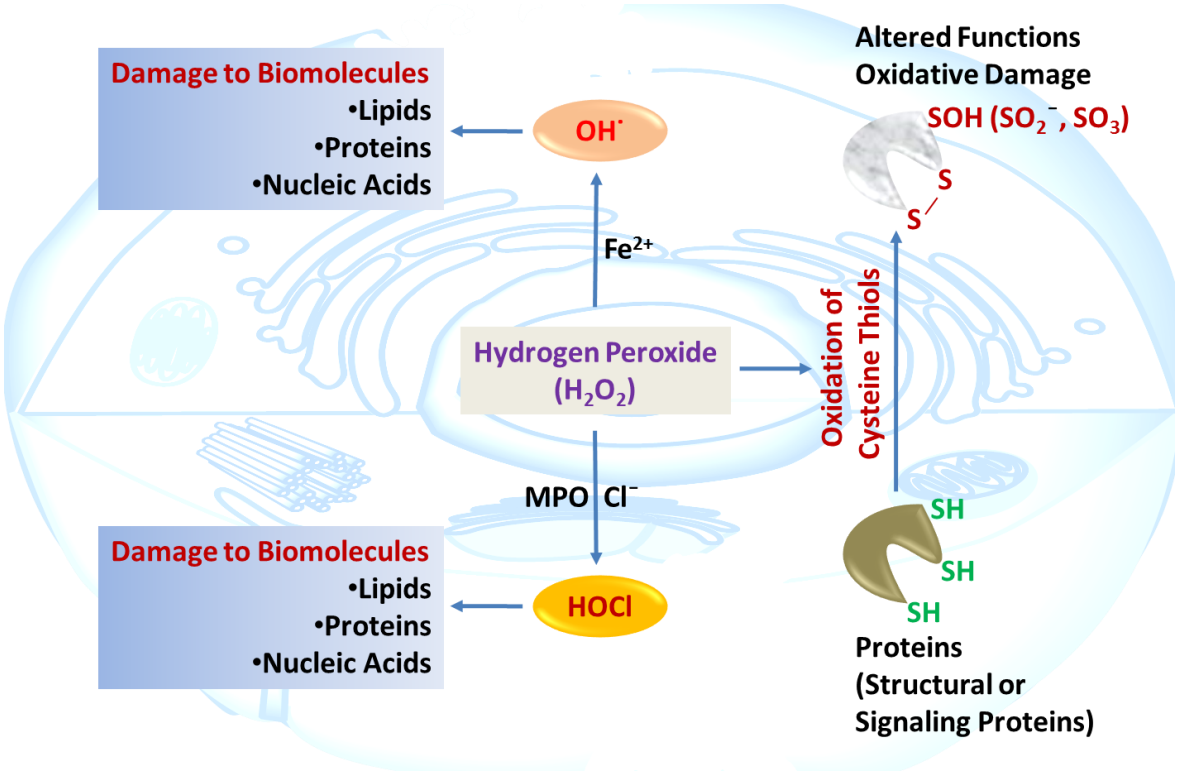

FIGURE 2. Chemical and biochemical reactivity of hydrogen peroxide (H_2O_2). As illustrated, H_2O_2 may directly react with the thiol groups of the cysteine residues in certain proteins, resulting in the formation of protein sulfenic acid (protein-SOH), sulfinic acid (protein-SO_2H), and sulfonic acid (protein-SO_3H). These modifications may cause altered protein function and oxidative protein damage depending on the levels and duration of H_2O_2 exposure (also see the legend of Figure 9.3). H_2O_2 reacts with transition metal ions, such as ferrous iron ion (Fe^{2+}), producing the highly reactive hydroxyl radical ($OH^{\cdot}$). Likewise, reaction between H_2O_2 and chloride ion (Cl^-) in the presence of myeloperoxidase (MPO) results in the formation of hypochlorous acid (HOCl), another potent oxidant. Production of these secondary reactive species is largely responsible for H_2O_2-induced oxidative damage in biological systems. Although hydrogen peroxide at high levels causes damage to cells and tissues, under certain circumstances, at lower levels it can act as a signaling molecule to participate in cell signal transduction.

signaling molecules (e.g., certain transcription factors) [22–24]. Protein thiol oxidation is now recognized as a major chemical basis behind H_2O_2 sensing and signaling [25, 26] (see section below). However, extensive oxidation of protein thiols by large amounts of H_2O_2 causes irreversible oxidative protein damage, resulting in cell injury. **Figure 2** depicts the redox modifications of protein thiols by H_2O_2.

As illustrated in the above figure as well as **Figure 3**, mild oxidation of protein thiols by H_2O_2 results in the formation of protein sulfenic acid, which is unstable and readily reacts with an adjacent protein thiol group (either on the same protein or another protein) to form protein disulfides or with reduced form of glutathione (GSH) to become glutathionylated. The above redox modifications of proteins are reversible via the actions of antioxidant enzymes, including thioredoxin and glutaredoxin systems. Such a reversible nature is instrumental in H_2O_2-mediated redox signaling.

On the other hand, prolonged exposure to large amounts of H_2O_2 can cause further oxidation of the

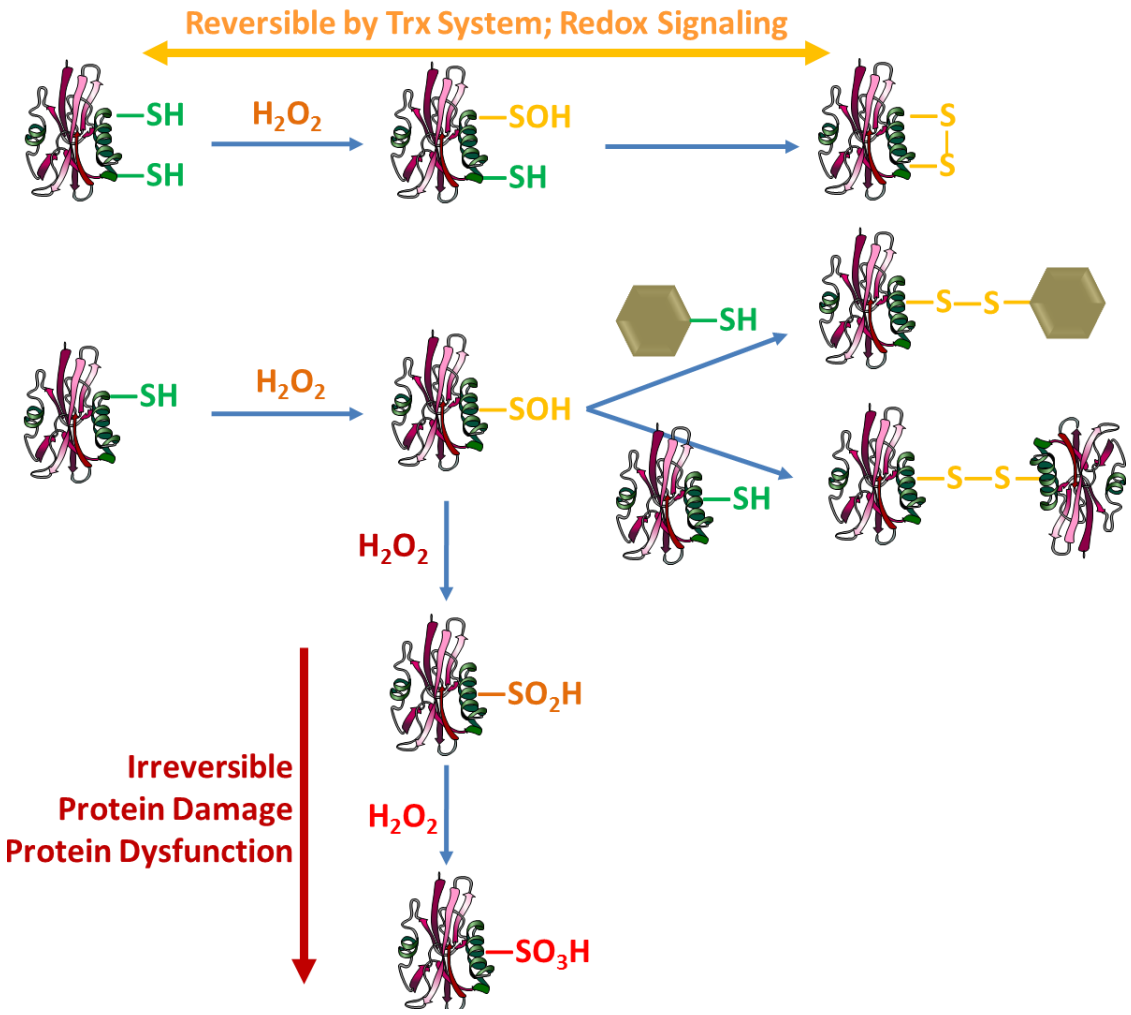

FIGURE 3. Thiol-dependent redox mechanisms of cell signaling mediated by hydrogen peroxide (H_2O_2). Oxidation of protein cysteine thiol groups by H_2O_2 is a major chemical basis for this ROS-mediated redox signaling. In this regard, a moderate and transient increase in the levels of H_2O_2 may cause oxidation of the cysteine thiols in certain signaling proteins, resulting in the formation of protein sulfenic acid (protein-SOH). Due to its high reactivity, the protein-SOH reacts with another cysteine thiol either on the same or another protein, forming protein disulfides (protein-S-S-protein). These reactions are reversible via the action of thioredoxin (Trx) system. The reversibility of the above reactions makes it possible for H_2O_2 to transiently alter the functionality of the protein (e.g., a protein kinase or a transcription factor), ensuring redox signaling. On the other hand, high levels and prolonged duration of H_2O_2 exposure may cause further oxidation of protein sulfenic acid to form protein sulfinic acid (protein-SO_2H) and sulfonic acid (protein-SO_3H). These hyperoxidative reactions are generally irreversible and thereby cause protein dysfunction and oxidative damage.

protein sulfenic acid to form sulfinic acid and oxidation of sulfinic acid to form sulfonic acid. Such hyperoxidative modifications of protein thiols typically result in irreversible damage to the protein (**Figure 3**). Hence, H_2O_2 serves as a signaling molecule only when its formation is tightly regulated. In this context, multiple families of enzymes are involved in the decomposition of H_2O_2 (see Section 4).

3.3. Fenton Reaction to Form Hydroxyl Radical

Reaction of H_2O_2 with transition metal ions gives rise to the formation of hydroxyl radical ($OH^{\bullet}$), an extremely potent oxidant. The Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^-$) is an important mechanism for H_2O_2-mediated oxidative damage. Other metal ions such as cuprous ion (Cu^{1+}) can also catalyze the formation of hydroxyl radical from H_2O_2 via a similar reaction called Fenton-type reaction: $Cu^{1+} + H_2O_2 \rightarrow Cu^{2+} + OH^{\bullet} + OH^-$).

3.4. Reaction with Chloride Ion Forming Hypochlorous Acid

Reaction of H_2O_2 with chloride (Cl^-) generates hypochlorous acid (HOCl), a potent oxidant ($H_2O_2 + Cl^- \rightarrow HOCl + OH^-$). This reaction is catalyzed by myeloperoxidase (MPO) found in phagocytic cells. The HOCl formed is involved in the killing of invading microorganisms by phagocytic cells. On the other hand, abnormal formation of HOCl also contributes to tissue injury, such as atherosclerosis [27].

3.5. Reaction with Other Molecules

H_2O_2 oxidizes pyruvate to form acetate and CO_2 with a reaction rate constant of 2.2 $M^{-1}s^{-1}$ and as such, pyruvate may act as an efficient biological scavenger of H_2O_2 [28]. Indeed, pyruvate present in cell culture media or inside the cells has been shown to inhibit the biological activity of H_2O_2 [29–31].

H_2O_2 reacts with CO_2 to form peroxymonocarbonate ($H_2O_2 + CO_2 \rightarrow HCO_4^- + H^+$), which is much more reactive to thiols and methionine [21]. The biological significance of this reaction remains to be elucidated though peroxymonocarbonate may give rise to carbonate radical ($CO_3^{\bullet}$), a potent oxidizing species. Reaction of H_2O_2 with Cu,ZnSOD has also been shown to produce secondary oxidants and inactivation of the enzyme [32, 33].

3.6. Half-Life, Diffusion, and Membrane Permeability

In biological systems, H_2O_2 has a relatively long half-life in the range of minutes depending on the levels of surrounding H_2O_2-decomposing enzymes (e.g., catalase, glutathione peroxidase, peroxiredoxin). It has been long known that H_2O_2 readily crosses mammalian cell membranes. Recently, several specific aquaporin (water channel) isoforms (e.g., AQP3, AQP8, AQP9) are found to facilitate the passive diffusion of H_2O_2 across cell membranes and influence the cellular effects (e.g., cytotoxicity) of this ROS [34–36]. This is not surprising as water and H_2O_2 share similar physicochemical properties.

Notably, aquaporin-facilitated H_2O_2 transport may also regulate H_2O_2 signaling [35, 37]. For example, a recent study shows that aquaporin-3-mediated H_2O_2 transport is required for nuclear factor kappa B (NF-κB) signaling in keratinocytes and development of psoriasis in an animal model [37]. Additionally, aquaporin-3 also controls breast cancer cell migration and metastasis by regulating hydrogen peroxide transport and its downstream cell signaling (e.g., the Akt pathway) [38].

4. CELL AND TISSUE DEFENSES

H_2O_2 is decomposed to water by several enzymes in mammals including humans. These include catalase, glutathione peroxidase, and peroxiredoxin. As noted earlier in Section 3.5, pyruvate (or pyruvic acid) present in biological systems can spontaneously detoxify H_2O_2 via a nonenzymatic decarboxylation reaction. In addition to pyruvate, other α-keto acids, such as α-ketoglutarate, oxaloacetate, glyoxylate may also scavenge H_2O_2 via a similar mechanism [39, 40].

5. BIOLOGY AND MEDICINE

As mentioned above, H_2O_2 is among the most extensively investigated ROS in biology and medicine. Substantial evidence points to the important roles played by this ROS, ranging from both innate and adaptive immunity to cell signaling involved in stem cell proliferation and wound healing. On the other hand, abnormal production of H_2O_2 causes oxidative stress and tissue injury, thereby contributing to disease pathophysiology.

5.1. Innate Immunity

As a major product of phagocytic respiratory burst, H_2O_2 is involved in the killing of the invading pathogens via the formation of hypochlorous acid, a much

more potent oxidant (see Section 3.4). H_2O_2 may also kill the microorganisms via the formation of hydroxyl radical through the Fenton reaction (see Section 3.3). In addition to its antiseptic role, a recent study using zebrafish shows that H_2O_2 formed by dual oxidase (DUOX) at the wound margin and the resulting H_2O_2 concentration gradient are required for the rapid recruitment of leukocytes to the wound [41]. This finding reveals a novel role for H_2O_2 to potentially act as a leukocyte chemoattractant in innate immunity.

5.2. Adaptive Immunity

Mitochondria-derived ROS have recently been demonstrated to play important roles in adaptive immunity, including regulation of T cell activation and $CD8^+$ memory T cell formation, as well as B cell fate determination upon activation [42–44]. Although the exact ROS involved in the above processes remain unclear, H_2O_2 appears to be the most likely ROS that acts as a signaling molecule to regulate adaptive immunity [45]. In this regard, H_2O_2 is among the best characterized ROS involved in cell signal transduction.

5.3. Redox Signaling

It is well recognized that the regulated formation of H_2O_2 from various sources (including NOX and mitochondria) serves as an important mechanism of cell signaling. Oxidation of the cysteine thiol by H_2O_2 in signaling proteins (e.g., proteins kinases /phosphatases, receptors, and transcription factors) appears to be a major molecular basis underlying H_2O_2-mediated redox signaling [25, 26] (**Figure 3**).

Notably, a recent study shows that peroxiredoxin-2 (Prx2, a H_2O_2-decomposation enzyme) and STAT3 form a redox relay for H_2O_2 signaling. Specifically, H_2O_2 oxidizes Prx2, and the oxidized Prx2 forms a redox relay with the transcription factor STAT3 in which oxidative equivalents flow from Prx2 to STAT3. The redox relay generates disulfide-linked STAT3 oligomers with attenuated transcriptional activity. Cytokine-induced STAT3 signaling is accompanied by Prx2 and STAT3 oxidation and is modulated by Prx2 expression levels [46]. The redox signaling role of H_2O_2 explains its involvement in diverse conditions, such as stem cell proliferation and wound healing.

5.4. Stem Cell Biology

H_2O_2 is involved in stem cell biology. While high levels of H_2O_2 cause injury and shorten the lifespan of stem cells [47], regulated production of H_2O_2 may be essential for stem cell proliferation. In this regard, Dickinson et al. show that adult hippocampal stem/progenitor cells generate H_2O_2 through NOX2 to regulate intracellular growth signaling pathways, which in turn maintains their normal proliferation in vitro and in vivo [48].

5.5. Wound Healing

As noted above, wounded epithelial cells release H_2O_2 and generate a tissue-scale gradient of H_2O_2, which guides leukocyte recruitment to the wound site to kill pathogens, minimize infection, and promoting healing [41]. In addition, low levels of H_2O_2 may also cause proliferation of keratinocytes as well as promote angiogenesis via augmenting epithelial growth factor and endothelial growth factor signaling, respectively [49, 50].

5.6. Circadian Rhythm

Light is the key entraining stimulus for the circadian clock, but several features of the signaling pathways that convert the photic signal to clock entrainment remain to be deciphered. Hirayama et al. show that light induces the production of H_2O_2 that acts as the second messenger coupling photoreception to the circadian clock in zebrafish [51]. Recent studies suggest that mitochondrial release of H_2O_2 is also likely a circadian event that conveys temporal information on steroidogenesis in the adrenal gland and on energy metabolism in the heart and brown adipose tissue to cytosolic signaling pathways [52, 53].

5.7. Disease Process

Due to its readily commercial availability, H_2O_2 is perhaps the most widely used chemical for studying oxidative stress in experimental models. Indeed, much of our current knowledge in oxidative stress results from studies using exogenous H_2O_2. Studies on the involvement of endogenously generated H_2O_2 in disease process have been frequently done with animal models of catalase gene knockout or overexpression. In this regard, like SOD for selective me-

tabolizing superoxide, catalase is a highly selective enzyme for the detoxification of H_2O_2. As such, the impact of manipulating cellular or tissue catalase on disease pathogenesis can be reasonably interpreted as a causal involvement of H_2O_2 in the disease process.

Using primarily catalase gene knockout or overexpression animal models, extensive studies over the past decades suggest an important role for H_2O_2-induced oxidative stress in a wide variety of disease processes and related conditions. These include various forms of cardiovascular disorders [54–58], diabetes and metabolic syndrome[59–61], multistage tumorigenesis [62–64], neurodegeneration[65, 66], pulmonary injury [67, 68], hepatic injury [69], and osteoporosis [70], as well as aging [71–74], among many others.

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