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Biomolecules Oxidation by Hydrogen Peroxide and Singlet Oxygen

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Abstract

Hydrogen peroxide (H$_2$O$_2$) and singlet oxygen (¹O$_2$) are important reactive oxygen species (ROS) for biological and medicinal fields. Oxidation processes of chemical materials by molecular oxygen are important H$_2$O$_2$ source, whereas photochemical reaction is important for ¹O$_2$ production. Reactivity and biomolecule damage by these ROS depend on the surrounding conditions and targeting molecules. In this chapter, production mechanisms of H$_2$O$_2$ and ¹O$_2$, biomolecule oxidation by these ROS, their detection methods, and production control of ¹O$_2$ are briefly reviewed.

Keywords: hydrogen peroxide, singlet oxygen, DNA damage, protein damage, photooxidation

1. Introduction

Biomolecule damage, for example, oxidation of DNA and/or protein, by reactive oxygen species (ROS) is closely related to carcinogenicity [1–3] and/or toxicity [4–6]. Furthermore, oxidative damage to unwanted tissue can be applied to the treatment of disease including cancer treatment [7–9], and similar reaction is applied to sterilization [10–14]. Hydrogen peroxide (H$_2$O$_2$) is a relatively long-lived ROS compared with a short-lived ROS such as superoxide anion radicals (O$_2^{-}$) [15]. One of the most important producing mechanisms of H$_2$O$_2$ is a dismutation of O$_2^{-}$, which is easily formed though oxidation of various materials by dioxygen molecule (O$_2$). Various carcinogenic chemical compounds produce H$_2$O$_2$ through their oxidation processes. Relationship among molecular oxygen and ROS is shown in Figure 1. Oxygen molecules are easily reduced by surrounding materials, and various ROS and the intermediates are formed (Figure 1A). In the case of photosensitized reaction, excited states of oxygen molecules are produced (Figure 1B). Singlet oxygen (¹O$_2$), which is also an important ROS, can
be easily generated via photosensitized reaction [16–18]. The \( ^1\Sigma_g^+ \) state \( (^1\text{O}_2(1\Sigma_g^+)) \) is mainly produced through the excitation energy transfer from the excited state, in general triplet excited (T1) state, of photosensitizer [16–18]. The \( ^1\text{O}_2(1\Sigma_g^+) \) has higher energy, 1.6 eV, corresponding to the ground state of oxygen molecule \( (^3\text{O}_2) \). The lifetime of \( ^1\text{O}_2(1\Sigma_g^+) \) is several picoseconds, and \( ^1\text{O}_2(1\Delta_g) \) is rapidly converted to the \( ^1\Delta_g \) state \( (^1\text{O}_2(1\Delta_g)) \) [16–18]. Because the lifetime of \( ^1\text{O}_2(1\Delta_g) \) (several microseconds) is markedly longer than that of \( ^1\text{O}_2(1\Sigma_g^+) \), \( ^1\text{O}_2(1\Delta_g) \) is a more important ROS. After that, \( ^1\text{O}_2 \) indicates \( ^1\text{O}_2(1\Delta_g) \) without explanation in this chapter. Visible light, other than ultraviolet radiation, has sufficient energy to produce \( ^1\text{O}_2 \) from the ground state of oxygen molecule. Therefore, \( ^1\text{O}_2 \) production is an important mechanism of phototoxicity and/or photo-carcinogenicity under strong light illumination with phototoxic materials. The purpose of this chapter is a review of the ROS-mediated biomolecule damage and the related topics.

2. Hydrogen peroxide

Hydrogen peroxide itself is not strongly ROS. However, other ROS including hydroxyl radicals \( (^\cdot\text{OH}) \) are produced from \( \text{H}_2\text{O}_2 \). In general, \( \text{H}_2\text{O}_2 \) is produced from the dismutation of \( \text{O}_2^- \), and, in vivo, production of \( \text{H}_2\text{O}_2 \) and \( \text{O}_2^- \) occurs in mitochondria [19]. In this section, \( \text{H}_2\text{O}_2 \) formation from compounds, specifically artificial materials, is introduced.

2.1. Hydrogen peroxide formation through oxidation of chemical compounds

One of the most important processes of \( \text{H}_2\text{O}_2 \) production is a dismutation of \( \text{O}_2^- \). Various chemical compounds or metals can be oxidized by oxygen molecules. In the case of a simple electron transfer-mediated oxidation, \( \text{O}_2^- \) is produced by the electron extraction from chemical compounds or metals. The lifetime of \( \text{O}_2^- \) in aqueous solution is about several milliseconds [15]. The produced \( \text{O}_2^- \) in aqueous media is converted to \( \text{H}_2\text{O}_2 \) through the dismutation by proton (H+) as follows:

---

**Figure 1.** Relationship among ground-state oxygen molecule \( (^3\text{O}_2) \) and ROS (A) and the energy levels of oxygen molecule (B). HOMO and SOMO are the abbreviations of highest occupied molecular orbital and semi-occupied molecular orbital, respectively. The “arrows” in (B) indicate the electron spin.
2O$_2$$^•$ + 2H$^+$ → H$_2$O$_2$ + O$_2$  \hspace{1cm} (1)

For example, hydroquinone, which is one of the metabolites of benzene, can produce H$_2$O$_2$ through the autoxidation process (Figure 2) [20]. This process is markedly enhanced by the presence of metal ions, specifically Cu$^{2+}$ ions [20]. In the presence of sacrificial reductants, for example, nicotinamide adenine dinucleotide (NADH), the oxidized form of hydroquinone, $p$-benzoquinone, is reduced to the parent hydroquinone. Consequently, the redox cycle is formed, leading to the production of H$_2$O$_2$ abundantly. It has been also reported that hydrazine analogues produce H$_2$O$_2$ through their autoxidation processes (Figure 3) [21–23].

2.2. Hydrogen peroxide production through photochemical processes

Photochemical processes also contribute to the formation of H$_2$O$_2$. Because the reorganization energy of the reduction of small molecule, such as O$_2$ molecules, through electron transfer becomes large due to the Marcus theory [24, 25], the O$_2$$^•$ production through photoinduced electron transfer is energetically difficult [26, 27]. However, ultraviolet radiation to reductive photosensitizer, such as NADH (Figure 4), can produce O$_2$$^•$ as follows [28]:

**Figure 2.** Autoxidation process of hydroquinone and ROS production.
where NADH* is the photoexcited state of NADH and NAD* is the radical form. NAD* undergoes further oxidation by oxygen molecules to NAD⁺, the final oxidized product. The formed O₂⁻ is also converted to H₂O₂ through the dismutation process of Eq. (1).

Photocatalytic reaction can also produce H₂O₂ [29–34]. For example, the surface of titanium dioxide (TiO₂) can reduce relatively oxidative molecules under ultraviolet A (UVA; wavelength, 315–400 nm) irradiation [29–32]. Two crystalline forms of TiO₂, anatase and rutile with band gap energies of 3.26 and 3.06 eV, respectively, are well-known semiconducting photocatalyst [29–32]. The adsorbed oxygen molecules on the TiO₂ surface is reduced to O₂⁻ by the electron of conduction band, which is excited from the valence band by UVA energy (Figure 5).
Similarly to the abovementioned reaction, $O_2^{•−}$ is also converted to $H_2O_2$ through the dismutation process of Eq. (1). In addition, oxidation reaction of TiO$_2$ photocatalyst also produces $H_2O_2$. The formed hole ($h^+$) in the valence band by UVA irradiation oxidizes water molecules on the surface of TiO$_2$ to $•OH$. The reaction of two $•OH$ species can produce $H_2O_2$ as follows:

$$2•OH \rightarrow H_2O_2$$

Although TiO$_2$ particles are barely incorporated into cell nucleus [35], cellular DNA damage was reported [36–39]. Because $H_2O_2$ has a transparency for nuclear membrane, the cellular DNA damage can be explained by $H_2O_2$-mediated mechanism [32]. The activation of $H_2O_2$ and DNA damage by $H_2O_2$ are described later.

### 2.3. Secondary formation of hydrogen peroxide through photocatalytic reaction

Photocatalytic reaction can produce oxidized intermediates other than final oxidized products of chemical compounds. For example, photooxidized amino acids [40] and sugars [41] by TiO$_2$ photocatalyst produce $H_2O_2$ through secondary oxidation reaction in the presence of metal ions (Figure 6). Titanium dioxide can photocalyze the production of $•OH$, a strong oxidant, through the decomposition of $H_2O$. The formed $h^+$ in the valence band by UVA irradiation can also oxidize various materials adsorbed on TiO$_2$ surface. Hydroxyl radicals and $h^+$ can oxidize these biomolecules, resulting in the production of oxidized intermediates. The formation of partly oxidized molecules leads to the secondary $H_2O_2$ production in the presence of metal ions. This $H_2O_2$ production process may cause a remote $H_2O_2$ generation in cells.

It has been reported that the photooxidized phenylalanine and tyrosine by TiO$_2$ produce $H_2O_2$ in the presence of copper(II) ion [40]. Since TiO$_2$ photocatalysis induces a hydroxylation of
aromatic compounds [42], the formation of benzenediol derivatives from these aromatic amino acids is possible (Figure 7). As mentioned above, hydroquinone can produce H$_2$O$_2$ through the autoxidation (Figure 2) [20, 43]. The amount of H$_2$O$_2$ production from the photooxidized phenylalanine is significantly larger than that from tyrosine [40]. The difference of their autoxidation rates should affect the H$_2$O$_2$ production. It has been reported that the autoxidation rate of 1,4-form of benzenediol is markedly faster than that of 1,2-form [20]. Phenylalanine can be oxidized into various types of benzenediol, including the 1,4-form; however, tyrosine cannot be converted to the 1,4-form. Consequently, phenylalanine can produce relatively large amount of secondary H$_2$O$_2$ through the photocatalysis of TiO$_2$. Furthermore, other amino acids can be also oxidized by TiO$_2$ photocatalyst and induce the secondary ROS production. Specifically, photocatalyzed cysteine by anatase form produces significantly large amount of secondary H$_2$O$_2$. In the case of sugar oxidation by TiO$_2$ photocatalyst, the activity of secondary H$_2$O$_2$ production by anatase form TiO$_2$ is larger than that of rutile form [41].

Figure 7. Tyrosine oxidation by TiO$_2$ photocatalysis and the copper ion mediated ROS production. Phenylalanine is also oxidized by the similar processes, leading to the secondary ROS production.
3. DNA damage by hydrogen peroxide

Hydrogen peroxide itself barely induces DNA damage; however, it can oxidize nucleobases and cleave sugar-phosphate backbone in the presence of metal ions. In this section, the sequence-specific DNA damage by the H$_2$O$_2$-derived ROS and its biological effect are briefly introduced.

3.1. Sequence-specific DNA damage by hydrogen peroxide

Hydrogen peroxide causes alkali-labile products at guanine, thymine, and cytosine in the presence of copper ion (Cu$^{2+}$) [44]. Since copper ions are associated with chromatin [45] to form stable complexes with DNA [46–49], Cu$^{2+}$ can play an important role in the activation of H$_2$O$_2$ in cell nucleus. Polyacrylamide gel electrophoresis studies demonstrated that H$_2$O$_2$ itself cannot cleave and oxidize DNA [44]. However, the incubation of DNA with H$_2$O$_2$ and Cu$^{2+}$ induce base modifications at guanine, thymine, and cytosine residues. These base modification sites can be cleaved by hot piperidine treatment [20–22, 44]. The derived reactive species from H$_2$O$_2$, for example, copper-peroxyl species (Cu(I)-OOH), are responsible for this DNA damage:

$$\text{H}_2\text{O}_2 + \text{Cu}^+ \rightarrow \text{Cu(I)-OOH} + \text{H}^+$$  (5)

Cu(I)-OOH is not strongly reactive compared with •OH; however, its lifetime is relatively long to induce DNA base modification. Single-stranded DNA is easier oxidized by these ROS. Therefore, DNA damage by H$_2$O$_2$ is enhanced by denaturation of DNA [44]. Abovementioned chemical compounds, benzenediol [20] and hydrazine [21], induce these base modification in the presence of Cu$^{2+}$. In the case of relatively low concentration of TiO$_2$ particles, similar sequence-specific DNA damage was observed after UVA irradiation with Cu$^{2+}$ [32]. DNA damage mediated by H$_2$O$_2$ is effectively inhibited by catalase [50], which is an enzyme to decompose H$_2$O$_2$ to H$_2$O and O$_2$. Chelating molecules for copper ions also effectively suppress this DNA damage. In addition, 3-methylthiopropanal (methional) is an effective inhibitor of Cu(I)-OOH [20, 32, 44]. Cu(I)-OOH cannot be scavenged by free •OH scavengers, such as sugars and alcohols [20, 22, 32, 44]. In the presence of Cu$^{2+}$, UVA-irradiated NADH also induces DNA damage by the similar process through H$_2$O$_2$ production [28]. In general, photosensitized DNA damage could be explained by •O$_2$ formation mechanism or electron transfer-mediated oxidation [51]. The H$_2$O$_2$-mediated DNA is a rare case in the photochemical DNA damage.

Hydrogen peroxide and Cu$^{2+}$ can induce tandem lesion at guanine and thymine residues [32]. Clustered DNA lesions including tandem damage have important mutagenic potential [52–54]. Furthermore, the repair of such DNA damage is more difficult than single-base damage [55–60]. Therefore, oxidative DNA damage through H$_2$O$_2$ production may play an important role in carcinogenesis.
In the presence of iron ions (Fe$^{2+}$), *OH is formed as follows:

$$\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \cdot\text{OH} + \text{OH}^- + \text{Fe}^{3+}$$ \hspace{1cm} (6)

Formed *OH induces base oxidation with non-sequence specificity, because *OH can oxidize all nucleobases [44, 61]. In addition, direct cleavage of sugar-phosphate backbone is caused by *OH. Hydroxyl radical-mediated DNA damage was reported by the case of ascorbate with Cu$^{2+}$ [62]. As mentioned above, in the case of TiO$_2$ photocatalysis, *OH is directly produced from water decomposition [29–32], and DNA damage without sequence specificity can be induced in the absence of metal ions [32]. Relatively high concentration of anatase form of TiO$_2$ induce non-sequence-specific DNA damage under UVA irradiation without metal ions through *OH production [32]. DNA damage by *OH is effectively inhibited by sugars and alcohols [32, 44]. However, in the presence of metal ions, the addition of *OH scavengers rather enhances DNA damage through the secondary generation of H$_2$O$_2$ from the oxidized products of scavengers themselves by *OH [32, 41]. Base modifications can cause carcinogenesis. Because H$_2$O$_2$ can penetrate into nuclear membrane, DNA modification can be induced by H$_2$O$_2$ originally formed in the sphere of outer cell nucleus through the assistance of metal ions.

3.2. Mutagenicity and cytotoxicity caused by hydrogen peroxide production

As oxidized products of nucleobases by the H$_2$O$_2$-mediated mechanism, 8-oxo-7,8-dihydroguanine (8-oxo-G; oxidized guanine, Figure 8) [63–65]; 5,6-dihydroxy-5,6-dihydrothymine (OH-thy; oxidized thymine, Figure 9) [58, 66, 67]; 5-hydroxyuracil (OH-Ura; oxidized cytosine, Figure 9) [67, 68]; 5-hydroxyhydantoin (OH-Hyd; oxidized cytosine, Figure 9) [68], and 5-hydroxycytosine (OH-Cys; oxidized cytosine, Figure 9) [67] are well-known compounds.

![Guanine oxidation by ROS](image)

**Figure 8.** Guanine oxidation by ROS. This scheme is an example of the guanine oxidation by 1O$_2$ to 8-oxo-G. Other H$_2$O$_2$-derived ROS, *OH and Cu(I))-OOH, also produce 8-oxo-G through the oxidation of guanine.
In a certain case, oxidative DNA damage induces cell death [69, 70]. As a minor oxidized product of guanine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy-G, Figure 10) can be formed by H$_2$O$_2$ and metal ions [63, 71]. Mutagenicity of Fapy-G is low [72]; however, a related product, methyl-Fapy-G formation, is a lethal lesion [73]. Furthermore, a theoretical study suggested that the formation of Fapy-G contributes to mutation [74]. Cytotoxicity of TiO$_2$ photocatalyst can be explained by oxidative damage of membrane protein [75–77]. In addition, cellular DNA damage was also reported [78, 79]. Because H$_2$O$_2$ has a transparency for nuclear membrane, the cellular DNA damage by TiO$_2$ photocatalysis can be explained by H$_2$O$_2$ production. The formed H$_2$O$_2$ through TiO$_2$ photocatalysis is incorporated into cell nucleus and activated by endogenous metal ions, leading to oxidative DNA damage [32]. Examples of the mutations caused by the oxidized guanines are described in Section 4.
4. Singlet oxygen

In general, the production mechanism of \(^1\text{O}_2\) involves photochemical processes. Various photooxidation processes can be explained by \(^1\text{O}_2\) production. In this section, the production mechanism of \(^1\text{O}_2\), its application, and biomolecule oxidation by \(^1\text{O}_2\) are briefly introduced.

4.1. General property of singlet oxygen

Singlet oxygen is an excited state of \(^3\text{O}_2\), ground triplet state of molecular oxygen [16–18]. In general, singlet excited (\(S_1\)) states of \(\text{O}_2\) are \(^1\Delta_g\) and \(^1\Sigma_g^+\); they have excitation energy of 0.98 eV and 1.63 eV above \(^3\text{O}_2\), respectively [16–18]. Because of the short lifetime of \(^1\Sigma_g^+\) (a few picoseconds), \(^1\Delta_g\), the lower \(S\) state of \(\text{O}_2\), plays an important role in various oxidation reactions. In this chapter, \(^1\Delta_g\) is denoted throughout as \(^1\text{O}_2\). The highest occupied molecular orbital (HOMO) of \(^3\text{O}_2\) is a semi-occupied molecular orbital (SOMO), whereas this molecular orbital of \(^1\text{O}_2\) becomes the lowest unoccupied molecular orbital (LUMO) (Figure 1B). The oxidative activity of \(^1\text{O}_2\) is stronger than that of \(^3\text{O}_2\) due to the vacant molecular orbital. Commonly, \(^1\text{O}_2\) is produced through photosensitized reaction. Since the excitation energy of \(^1\text{O}_2\) is relatively small, which corresponds to the energy of photon with the wavelength of 1270 nm (smaller than that of visible light photon), photoexcited states of various dyes can sensitize the generation of \(^1\text{O}_2\) under visible light or ultraviolet irradiation. Various molecules become photosensitizer (PST) to generate \(^1\text{O}_2\). In general, the photosensitized reaction of \(^1\text{O}_2\) generation is an electron exchange energy transfer (the Dexter mechanism) [80]. These processes are presented as follows:

\[
PST + \nu \rightarrow PST^*(S_1) \quad (7)
\]

\[
PST^*(S_1) \rightarrow PST + \text{fluorescence} \quad (8)
\]

\[
PST^*(S_1) \rightarrow PST^*(T_1) \quad (9)
\]

\[
PST^*(T_1) + ^3\text{O}_2 \rightarrow PST + ^1\text{O}_2 \quad (10)
\]

where \(PST^*(S_1)\) and \(PST^*(T_1)\) are the \(S\) and \(T\) states of PST, respectively. In general, since the lifetime of \(PST^*(T_1)\) is markedly longer (several microseconds) than that of \(PST^*(S_1)\) (several nanoseconds), \(^1\text{O}_2\) is produced by \(PST^*(T_1)\). However, the formation of \(^1\text{O}_2\) by \(PST^*(S_1)\) is not impossible. The lifetime of \(^1\text{O}_2\) (\(\tau_\Delta\)) is relatively long (Table 1). Generated \(^1\text{O}_2\) can oxidize various materials, including biomolecules, within its long lifetime. The \(\tau_\Delta\) strongly depends on the surroundings, and a solvent deuterium effect on the reactivity of \(^1\text{O}_2\) is significant (Table 1). For example, the \(\tau_\Delta\) in deuterium oxide (D\(_2\)O) is markedly longer than that in H\(_2\)O, and the biomolecule oxidation by \(^1\text{O}_2\) is significantly enhanced in D\(_2\)O compared with that in H\(_2\)O.
4.2. Photodynamic therapy

One of the most important medicinal applications of $^1\text{O}_2$ is photodynamic therapy (PDT) (Figure 11) [7–9]. Photodynamic therapy is a promising and less invasive treatment for cancer [7–9] and photosterilization [10–14]. For cancer PDT, in general, porphyrins are used for photosensitizers, for example, porfimer sodium [87] and talaporfin sodium [88]. Photosterilization, antimicrobial PDT, is also carried out using dyes, for example, methylene blue (MB) [11, 14, 89]. The important mechanism of PDT processes including photosterilization is oxidation of biomolecules of cancer cell or bacteria through $^1\text{O}_2$ production under visible light irradiation. Visible light, especially longer wavelength visible light (wavelength > 650 nm), is less harmful for the human body and can penetrate into the tissue deeply. As mentioned above, $^1\text{O}_2$ can be generated by longer wavelength visible light. Administered photosensitizers, porphyrins, or other dyes produce $^1\text{O}_2$ through energy transfer to oxygen molecules with relatively large quantum yield ($\Phi_{^1\text{O}_2}$).

### Table 1. Solvent dependence of the lifetime of singlet oxygen.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Photosensitizer</th>
<th>$\tau_{\Delta}$/µs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (H$_2$O)</td>
<td>Cationic porphyrin</td>
<td>3.5</td>
<td>[81]</td>
</tr>
<tr>
<td></td>
<td>Rose bengal</td>
<td>3.77</td>
<td>[82]</td>
</tr>
<tr>
<td>Phosphate buffer (pH 7.6)</td>
<td>P(V) porphyrin</td>
<td>3.5</td>
<td>[83]</td>
</tr>
<tr>
<td>Ethanol (C$_2$H$_5$OH)</td>
<td>Rose bengal</td>
<td>15.4</td>
<td>[82]</td>
</tr>
<tr>
<td>Ethanol/H$_2$O (1/1)</td>
<td>Rose bengal</td>
<td>6.37</td>
<td>[82]</td>
</tr>
<tr>
<td>Water (D$_2$O)</td>
<td>Berberine with DNA</td>
<td>72</td>
<td>[84]</td>
</tr>
<tr>
<td></td>
<td>Methylene blue</td>
<td>32</td>
<td>[83]</td>
</tr>
<tr>
<td></td>
<td>Phenalenone</td>
<td>64.4</td>
<td>[86]</td>
</tr>
<tr>
<td></td>
<td>Tris(bipyridine)Ru(II)</td>
<td>59.47</td>
<td>[82]</td>
</tr>
<tr>
<td>Chloroform (CHCl$_3$)</td>
<td>Phenalenone</td>
<td>232</td>
<td>[86]</td>
</tr>
<tr>
<td>Tetrachloromethane (CCL$_4$)</td>
<td>Phenalenone</td>
<td>34,000</td>
<td>[86]</td>
</tr>
</tbody>
</table>

Figure 11. Scheme of the general procedure of PDT.
4.3. Photocatalytic singlet oxygen generation

As mentioned above, TiO\textsubscript{2} photocatalyzes the generation of various ROS. Singlet oxygen can be also produced through the photocatalysis of TiO\textsubscript{2} [31, 90–96]. In general, photogenerated electron in the conduction band reduces the surface-adsorbed oxygen molecules to O\textsubscript{2}\. Through the reoxidation of O\textsubscript{2}\textsuperscript{•−}, ¹O\textsubscript{2} is formed. The possible reactions of photocatalytic ¹O\textsubscript{2} productions are as follows:

\[
\text{O}_2\textsuperscript{•−} + h^+ \rightarrow ¹\text{O}_2
\]  

(11)

and

\[
\text{O}_2\textsuperscript{•−} + \cdot\text{OH} \rightarrow ¹\text{O}_2 + \text{OH}^–
\]  

(12)

The photogenerated h\textsuperscript{+} in the valence band and *OH can act as the oxidants to produce ¹O\textsubscript{2}. In addition, hydroperoxyl radical (*OOH) generated from O\textsubscript{2}\textsuperscript{•−} and H\textsuperscript{+} also produces ¹O\textsubscript{2} as follows:

\[
\text{O}_2\textsuperscript{•−} + \cdot\text{OOH} + \text{H}^+ \rightarrow ¹\text{O}_2 + \text{H}_2\text{O}_2
\]  

(13)

The reported values of Φ\textsubscript{Δ} are depending on the experimental condition, for example, around 0.2 (0.2, Degussa P25 in water [92], and 0.22, rutile particle in chloroform [95]). Other cases reported relatively small values, for example, 0.003 [96] and 0.02 [94]. In the cases of airborne ¹O\textsubscript{2}, quite small value (10\textsuperscript{−8}–10\textsuperscript{−9}) was reported [93]. It has been reported that the τ\textsubscript{Δ} value of ¹O\textsubscript{2} produced by Degussa P25 aqueous suspension is 5 μs [92]. Other photocatalytic materials, for example, zinc oxide (ZnO) can photocatalyze ¹O\textsubscript{2} production through the similar reaction of TiO\textsubscript{2} photocatalysis [97]. Recently, carbon quantum dots, which have been paid attention as interesting nano-materials, also photocatalyze ¹O\textsubscript{2} production [33].

Singlet oxygen is an important ROS for PDT. Other than ¹O\textsubscript{2}, H\textsubscript{2}O\textsubscript{2} production can be also applied for PDT mechanism. Photocatalytic materials can produce these ROS under photoirradiation. Therefore, application of photocatalysts, specifically TiO\textsubscript{2} nanoparticles, for PDT has been also studied [29, 98–101]. To realize the TiO\textsubscript{2}-utilized PDT, direct administration of small TiO\textsubscript{2} powders into tumor assisted with an optical fiber was proposed [29]. In addition, it was reported that oral-administrated TiO\textsubscript{2} nanoparticles are transported into the tumor of nude mouse skin transplanted from a human prostate cancer cell line [98]. As mentioned above, in general, TiO\textsubscript{2} nanoparticles can be excited by UVA irradiation. To utilize visible light for TiO\textsubscript{2} excitation, upconversion technique was also studied [100].

4.4. DNA oxidation by ¹O\textsubscript{2} and mutation

Singlet oxygen can oxidize only guanines without sequence specificity; however, it does not have the ability to induce the oxidation of other nucleobases or to cleave the sugar-phosphate backbone [44]. The main oxidized product of guanine by ¹O\textsubscript{2} is 8-oxo-G (Figure 8) [63–65]. Guanines undergo the Diels-Alder reaction by photoproduced ¹O\textsubscript{2}, leading to the formation
of [4 + 2] cycloaddition product with the imidazole ring to produce an endoperoxide. Through the subsequent proton transfer, this peroxide is converted to 8-hydroperoxyguanine [102, 103], which becomes 8-hydroxyguanine [63]. The keto-enol tautomerism produces 8-oxo-G from 8-hydroxyguanine. Because single-stranded DNA is easily oxidized by ROS, 8-oxo-G formation by \(^{1}O_2\) is increased by DNA denaturation [44]. The 8-oxo-G formation causes DNA misreplication (Figure 12), which can lead to mutations such as G-C:T-A transversion caused by the stable base-pair formation between 8-oxo-G and adenine [104, 105]. Since 8-oxo-G is more easily oxidized than guanine, 8-oxo-G undergoes further reaction, leading to the formation of imidazolone and oxazolone (Figure 13) [63, 106, 107]. Imidazolone forms more stable base pair with guanine than cytosine [106, 107]. Therefore, guanine oxidation by \(^{1}O_2\) may cause G-C:C-G transversion [108, 109] through imidazolone formation, a further oxidized product of 8-oxo-G. Indeed, it has been reported that UVA can induce these mutations [110].

4.5. Protein oxidation by \(^{1}O_2\)

Protein oxidation is also induced by \(^{1}O_2\). The following amino acids, tryptophan, tyrosine, cysteine, histidine, and methionine, can be oxidized by \(^{1}O_2\) [111]. In the case of tryptophan oxidation by \(^{1}O_2\), \(N\)-formylkynurenine (Figure 14) is a major oxidized product [112, 113]. The reported reaction rate coefficient between tryptophan and \(^{1}O_2\) is \(3.0 \times 10^7\) \(s^{-1} M^{-1}\) [114]. Oxidation of tryptophan residue in a certain protein can be examined with a fluorometer [115]. For example, human serum albumin (HSA) has one tryptophan residue, and the intrinsic fluorescence of tryptophan at around 350 nm can be diminished by the oxidative damage. Porphyrin phosphorus(V) complexes (Figure 15), of which the \(\Phi\) is larger than 0.5, can induce oxidative damage to the tryptophan residue of HSA [116]. Photosensitized HSA damage is enhanced in \(D_2O\), in which the lifetime of \(^{1}O_2\) is markedly elongated compared in \(H_2O\) (Table 1). Furthermore, sodium azide (NaN\(_3\)), a strong physical quencher of \(^{1}O_2\) [117], effectively suppresses this HSA damage. From the analysis of the effect of NaN\(_3\) on the HSA damage, the contribution of \(^{1}O_2\)-mediated oxidation to the total quantum yield of protein damage

![Figure 12. Hydrogen bonding between 8-oxo-G and adenine.](http://dx.doi.org/10.5772/intechopen.71465)
Figure 13. Structures of imidazolone and oxazolone and the hydrogen bonding between guanine and imidazolone.

Figure 14. Structures of tryptophan and N-formylkynurenine, an oxidized product of tryptophan by $^{1}\text{O}_2$.

Figure 15. Example of P(V)porphyrin photosensitizer.
can be determined [115]. Photosensitized $^{1}\text{O}_2$ production by porphyrin phosphorus(V) complexes induces the damage of tyrosinase, which is an enzyme to catalyze the hydroxylation of tyrosine, resulting in the deactivation of tyrosinase [118]. Oxidation of the amino acid residue by $^{3}\text{O}_2$ can cause the deactivation of protein function. The protein oxidation photosensitized by porphyrins through ROS production is an important mechanism of PDT.

Photocatalyzed $^{1}\text{O}_2$ production by TiO$_2$ may not play an important role in the oxidation reaction [31, 94]. Formed $^{3}\text{O}_2$ on the TiO$_2$ surface is quenched by TiO$_2$ itself with relatively large quenching rate coefficient (e.g., $2.4 \times 10^9 \text{ M}^{-1} \text{s}^{-1}$ [95]). In the presence of bovine serum albumin, $^{1}\text{O}_2$ produced by TiO$_2$ photocatalysis is effectively quenched, suggesting the protein oxidation [94]. However, in the case of TiO$_2$ photocatalyst, other ROS are more important for protein oxidation than $^{1}\text{O}_2$-mediated reaction [29–32].

5. Detection of ROS

ROS detection is an important theme to investigate a biological effect of ROS or evaluation of the activity of PDT photosensitizers [119–122]. Fluorometry is one of the most important and effective methods of ROS detection. For example, 5-carboxyfluorescein-based probe has been developed (Figure 16) [123]. This probe can detect H$_2$O$_2$ in the living cell. As an inexpensive method, the fluorometry using folic acid (Figure 17) was reported [23, 119, 124]. Folic acid can be decomposed by H$_2$O$_2$ in the presence of Cu$^{2+}$, resulting in the fluorescence enhancement. The limit of detection (LOD, at signal/noise = 3) for this method was 0.5 μM H$_2$O$_2$. This method is based on the oxidative decomposition of folic acid by Cu(I)-OOH. In the presence of Fe$^{2+}$, •OH slightly induces the folic acid decomposition; however, the effect of •OH on this folic acid decomposition is negligibly small because of the very short lifetime [125, 126]. In addition, O$_2^*$ does not

Figure 16. Structure of 5-carboxyfluorescein-based fluorescence probe for H$_2$O$_2$ [123].
have the activity of folic acid decomposition. Using folic acid or its analogue, $^1$O$_2$ can be also detected \[124\]. Specifically, in D$_2$O, folic acid or methotrexate (Figure 17), an analogue of folic acid, is effectively decomposed by $^1$O$_2$ resulting in the fluorescence enhancement \[124\]. Using this method, the values of $\Phi$, of various water-soluble photosensitizers can be determined.

6. Control of singlet oxygen production

Control of photosensitized $^1$O$_2$ is an important theme for biology or medicine, for example, to realize target-selective PDT \[127\] or “theranostics” (therapy and diagnosis) \[128\]. The pH-dependent control \[129\] and target-selective control \[127, 128, 130–132\] methods have been reported. It has been reported that free base porphyrins were synthesized to control their photosensitized $^1$O$_2$ generating activity by pH \[128\] \[129\]. The $S_1$ state of this porphyrin

![Figure 18. Example of the reported pH-responsive porphyrin \[129\].](image-url)
is quenched by the electron-donating moiety in neutral or alkali solution. However, protonation of this electron-donating moiety under acidic condition suppresses the electron transfer, leading to the recovery of the \( \text{^1O}_2 \) production activity of porphyrin ring. Because cancer cell is slightly a more acidic condition compared with normal cells \(^{133-135}\), this pH-based control of photosensitized \( \text{^1O}_2 \) production can be applied to cancer-selective PDT. DNA-targeting control of photosensitized \( \text{^1O}_2 \) generation has been also reported \(^{127, 128}\). For example, electron donor-connecting porphyrins have been studied (Figure 19) \(^{81, 130-132}\). These compounds can be photoexcited by visible light irradiation, and their \( S_1 \) states are effectively quenched through intramolecular electron transfer. The charge-transfer state energy can be raised through the binding interaction with DNA, an anionic polymer, resulting in the inhibition of the intramolecular electron transfer and enhancement of \( \text{^1O}_2 \) generation.

### 7. Conclusions

Hydrogen peroxide is easily produced from the oxidation processes of chemical compounds by oxygen molecules. In addition, UVA-irradiated NADH and semiconductor photocatalytic materials can also produce H\(_2\)O\(_2\). Formed H\(_2\)O\(_2\) in cells can be incorporated into cell nucleus and activated by endogenous metal ions. Copper ion induces Cu(I)-OOH formation from H\(_2\)O\(_2\).
whereas ‘OH is produced from $\text{H}_2\text{O}_2$ and iron ion. These ROS cause base oxidation, and ‘OH can induce strand break of DNA. Base modifications lead to carcinogenesis or lethal effect. Photoirradiation to various sensitizing materials induces $\text{O}_2^*$ production. Visible light has sufficient energy to produce $\text{O}_2$. Therefore, $\text{O}_2$ is easily produced by various dyes under photoirradiation. Photocatalytic $\text{O}_2$ formation through reoxidation of $\text{O}_2^*$ is also possible. Formed $\text{O}_2$ can oxidize guanine residues of DNA without sequence specificity and several amino acid residues of protein within its lifetime, which depends on the surroundings. Various detection methods of these ROS have been developed. In addition, the target-selective or condition-selective productions of ROS become important strategies for PDT and cancer “theranostics.”

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