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An introduction to singlet oxygen chemistry

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Abbreviations:

EPR	Electron Paramagnetic Resonance
IR	Infrared
LASER	Light Amplification by Stimulated Emission of Radiation
LOOH	Lipid hydroperoxide
PDT	Photodynamic therapy
ROS	Reactive Oxygen Species
$^1O_2$	Singlet Oxygen

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1. Abstract

Singlet oxygen ($^1O_2$) is the high-energy reactive state of oxygen. It has all paired electrons and is therefore not a free radical. However $^1O_2$ can be viewed as an "ene" molecule with a double bond between the two oxygen atoms. Most of the chemical reactions of $^1O_2$ can be explained using this model. Although a short-lived species, it has characteristic physical and chemical properties. These properties have been used in designing various detection methods for singlet oxygen. $^1O_2$ is toxic to cells. This forms the underlying basis of photodynamic therapy for cancer in the clinic. This report is a review of the sources, detection methods, chemical reactions and biological effects of singlet oxygen.

2. Introduction

The term singlet oxygen refers to the two, high-energy, excited states of dioxygen. These two states have the same number of electrons as molecular oxygen but paired spins. Quantum mechanically these are denoted as $^1\Delta_g$ and $^1\Sigma_g^+$ (excitation energies 23.4 kcal mol^{-1} and 37.6 kcal mol^{-1} above ground state respectively) [1]. Since both forms of singlet oxygen have paired electrons, they are NOT free radicals. However, being high-energy species, they are extremely reactive and form a part of what is collectively called the Reactive Oxygen Species (ROS). Since $^1\Delta_g$ is lower in energy than $^1\Sigma_g^+$, it is chemically and biologically the more prevalent form. When $^1\Sigma_g^+$ is formed in a reaction, it quickly converts to $^1\Delta_g$. Thus, this report is restricted to the discussion of $^1\Delta_g$ form of oxygen.

3. Generation of Singlet oxygen

Singlet oxygen can be generated by chemical reactions or by physical methods. Some of these are shown below [1].

3.1 Chemical methods

3.1.1 Reaction of bleach with hydrogen peroxide produces singlet oxygen. It is an exothermic chemiluminiscent reaction.

$$H_2O_2 + NaOCl \longrightarrow {}^1O_2 + NaCl + H_2O$$

3.1.2 Certain ozonide molecules such as triphenyl phosphene ozonide and 1-phospha 2,8,9-trioxa-adamantane ozonide, spontaneously decompose to produce phosphate and singlet oxygen in CH_2Cl_2 solvent. The yield in these cases can be as good as 95%.

3.2 Physical Methods

3.2.1 Photosensitization:

Photosensitization is the most common method of singlet oxygen production. A photosensitizer is a molecule that can be activated by light to a high-energy state. It may then collide with oxygen and transfer its extra energy to oxygen forming $^1O_2$. In the process, the sensitizer molecule itself comes back to ground state [2]. This pathway of singlet oxygen production is called Type II reaction. A less significant pathway of photosensitizer deactivation is the Type I reaction, which may or may not produce $^1O_2$ [3]. Most photosensitizers work *via* Type II mechanism. Production of singlet oxygen by a photosensitizer forms the basis of PDT for cancer. The steps of a typical Type II pathway are shown in below:

$$\underset{\text{(Ground state sensitizer)}}{^1S} \xrightarrow{h\nu} \underset{\text{(Excited state sensitizer)}}{^3S^*}$$

$$\underset{\text{(Ground state oxygen)}}{^3O_2} + {}^3S^* \longrightarrow {}^1S + \underset{\text{(Excited state oxygen } ^1\Delta_g)}{^1O_2}$$

3.2.2 Microwave discharge

Singlet oxygen can also be produced by discharge of microwave directly on O_2 [1]:

$$O_2 + h\nu \longrightarrow {}^1O_2$$

3.3 Miscellaneous

Various other sources of singlet oxygen have been reported in the past, *e.g.* generation of $^1O_2$ from non-photosensitive dyestuffs (*e.g.* Benzanthrone), food additives (*e.g.* metanil yellow) [4] and reaction of ozone with the leaves of *Sedum album* (low yield) [5].

4. Detection of Singlet Oxygen

4.1 Near IR Phosphorescence

Singlet oxygen has a characteristic emission at wavelength ~1270 nm. Production of singlet oxygen can thus be detected directly by emission in the near infrared region of the spectrum [6]. In a study to test the effectiveness of fullerenes (C_{60}) as photosensitizers for PDT, Yamakoshi *et.*al. confirmed the generation of singlet oxygen after the photoactivation of C_{60} in benzene as solvent [7] (**Figure 1**).

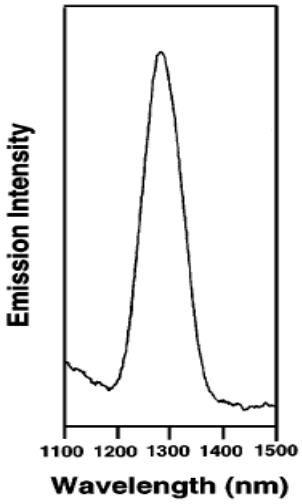

Figure 1: Characteristic emission of $^1O_2$ at 1270 nm. The spectrum was generated in C_{60} activated by Ar laser light at 514.4 nm with 200 mW power output in 40 μM benzene as solvent [7]. Singlet oxygen has a sharp peak in the infrared region. This is used to detect the presence of $^1O_2$ in samples.

4.2 EPR detection of singlet oxygen

EPR is a technique used to detect the presence of unpaired electron (free radicals) using an external magnetic field and a sensitive microwave detection system. Since singlet oxygen is not a free radical, it cannot be directly detected by EPR. However, if made to react with a stable molecule, it can form a relatively long-lived free radical, which can be detected by EPR. The molecule mostly used in the case of singlet oxygen is 2,2,6,6-tetramethyl-4-piperidone (TEMP).

The reaction of TEMP with $^1O_2$ produces the free radical 2,2,6,6-tetramethyl-4-piperidone-N-oxyl (TEMPO) [7] (**Figure 2**).

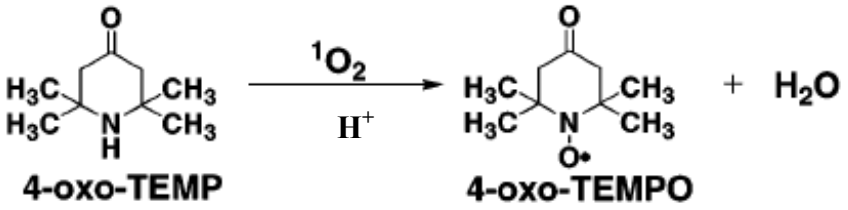

Figure 2: Singlet oxygen converts the stable 4-oxo-TEMP to a free radical (TEMPO) that can be detected by EPR. Thus, EPR can be used to detect singlet oxygen indirectly [7].

Figure 3 shows an example of EPR detection of singlet oxygen generated by the activation of photosensitizer Rose Bengal. Singlet oxygen was detected indirectly by the sharp peaks that correspond to 4-oxo-TEMPO production.

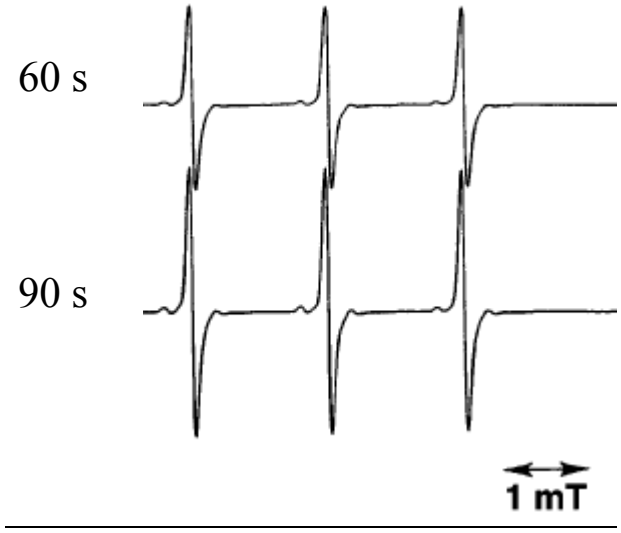

Figure 3: EPR spectrum of 4-oxo-TEMPO in the presence of 0.4% aqueous solution of photosensitizer Rose Bengal. Irradiation time for Rose Bengal was 60 or 90 s with a 300 W photoreflector lamp. EPR spectra was recorded at 296 K using 9.394 GHz microwave frequency, 16 mW microwave power, field modulation 0.1 mT at 100 kHz and scan time of 2 min. The signal produced is directly proportional to irradiation time of the photosensitizer [7].

4.3 Photothermal methods of detection

Photothermal methods detect the heat given out in the production and decay of $^1O_2$ using calorimetry [8]. These methods are used when $^1O_2$ is produced or decayed *via* radiationless processes. Some of the photothermal methods are photoacoustic calorimetry, time-resolved thermal lensing and photothermal beam deflection. The signal intensity in these methods is very weak, although some improvement can be done by varying the solvent. Thus, these methods are less common than spectroscopy based techniques.

5. Quenching of singlet oxygen

Quenching of a molecule refers to a process in which an excited molecule transfers its extra-energy to another molecule and returns to low energy state. Quenching studies help to elucidate typical reactions and reaction mechanisms of the molecule. In the case of $^1O_2$, quenching can be either by energy transfer (physical quenching) or by a chemical reaction (chemical quenching) [9]. It has been shown that physical quenching of $^1O_2$ is more efficient than chemical quenching [9].

Several agents have been used as scavengers to quench singlet oxygen. The most commonly used quenching agents are sodium azide (NaN_3), 9,10-dimethylanthracene, tetramethylethylene, tetraphenylcyclopentadione, carotenoids and amines [10]. In 1979, C.S. Foote, demonstrated the use of 1,4-diazabicyclol [2,2,2] octane (DABCO) as a quenching agent for singlet oxygen. It is one of the most common quenchers currently used [11].

One of the natural quenchers of singlet oxygen in the body is the lipid molecule. Reaction of $^1O_2$ with lipids can either produce LOOH (chemical quenching) or a simple transfer of energy from $^1O_2$ to the lipid molecule (physical quenching). Usually, a combination of both

processes occurs [12]. Thus, singlet oxygen can cause lipid peroxidation. This is one of the ways by which singlet oxygen induces toxicity to cells.

$$^1O_2 + LH \longrightarrow LOOH$$

6. Typical reactions of Singlet oxygen

$^1O_2$ is an elecrophilic species. Thus, it tends to react with any electron rich moiety such as double bonds, π systems, thiols *etc*. This is the basis of nearly all the chemical reactions of singlet oxygen. Some of the common reactions are summarized below:

6.1 "Ene-reaction"*

The ene-reaction of singlet oxygen is a stereospecific reaction forming hydroperoxides. **Figure 4** shows an example.

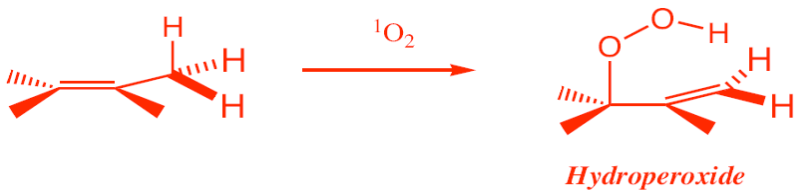

Figure 4*: The "ene"-reaction of singlet oxygen with a double-bond, generating a hydroperoxide. The double bond in the original molecule shifts to a new position in the product.

6.2 Cycloaddition*

Singlet oxygen reacts with the double bonds of aromatic or non-aromatic compounds generating cyclic products. These products are called endoperoxides (**Figure 5**).

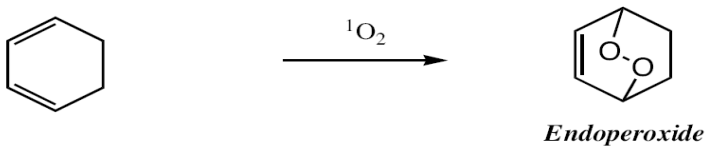

Figure 5*: A: Endoperoxide production by singlet oxygen.

*: http://daecr1.harvard.edu/pdf/smnr_2005_Nagorny_Pavel.pdf, 01/26/05

6.3 Diels-Alder reaction

Another common reaction of $^1O_2$ is the diene-dienophile reaction, commonly known as the Diels-Alder reaction. This reaction is very useful in organic synthesis and is widely used in industry. However, the mechanism of the reaction is not very clear at present. There is a constant debate between the possibilities of a concerted or stepwise mechanism [13]. **Figure 6** shows a possible mechanism of addition of singlet oxygen to a diene.

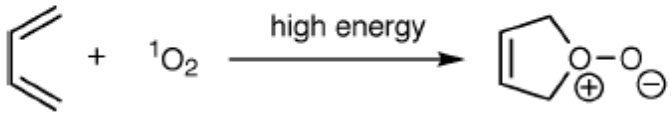

Figure 6: Cheletropic addition of singlet oxygen to butadiene in the classical Diels-Alder reaction: formation of a zwitterionic intermediate.

7. Biological significance of Singlet oxygen

Singlet oxygen production is the basis of photodynamic therapy for cancer. Patients are given a dose of a photosensitizer molecule intravenously and then exposed to LASER light at the specific tumor site. $^1O_2$ can adversely affect various molecules in the cells such as DNA, lipids and protein resulting in cytotoxicity. Thus, $^1O_2$ produced by the activated photosensitizer kills the tumor. It has also been shown that at low concentrations, singlet oxygen can act as signaling molecule with several biological implications [14]. Thus, this extra-energetic form of oxygen can be toxic to us but at the same time can be used to our best advantage as in PDT.

8. References

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