Singlet Oxygen

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Abbreviations:

D$_2$O: deuterium oxide $^1\Delta_g$O$_2$: singlet oxygen

ESR: electron spin resonance $^1\Sigma_g$O$_2$: singlet oxygen

^{3}O$_2$: ground-state dioxygen $^3\Sigma_g$O$_2$: ground-state dioxygen

1O2: singlet oxygen   PDT: photodynamic therapy

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Abstract

Singlet oxygen ($^1O_2$) is a higher energy state molecular oxygen species. It is one of the most active intermediates involved in chemical and biochemical reactions. To fully appreciate the reactivity of $^1O_2$, it is necessary to understand its physical and chemical nature. This paper reviews the generation, quenching and detection of $^1O_2$. Its properties, possible chemical reactions are presented as well as its biological role in Photodynamic therapy (PDT).

Introduction

Singlet oxygen ($^1O_2$) was first observed in 1924 [1] and then defined as a more reactive form of oxygen [2]. The main method of production of $^1O_2$ is by photosensitization reactions [3]. In 1931, Kautsky first proposed that $^1O_2$ might be a reaction intermediate in dye-sensitized photo-oxygenation [4]. Following this suggestion, many studies have showed that $^1O_2$ is an important intermediate species in the detrimental oxidation of biomolecules. It has been demonstrated now that $^1O_2$ can react with many kinds of biological molecules such as DNA, proteins and lipids [5]. Since oxygen is ubiquitous and efficiently quenches electronically excited states, $^1O_2$ is likely to be formed following irradiation in countless situations and involved in various chemical and biological process as well as in several disease processes [2]. Therefore, a better understanding of its chemical and physical nature are important. This paper will discuss the generation, quenching and detection of $^1O_2$. Its properties, reactions are presented as well as its biological effects in PDT.

Electronic structure and the lifetime of singlet oxygen

Molecular orbital theory can explain the electronic structure of molecular oxygen. The lowest electronic state of oxygen is a triplet ground state ($^3\Sigma_g^-$) with two unpaired electrons distributed in the highest occupied orbitals [6]. Rearrangment of the electron spins within these two degenerate orbitals results in two possible singlet excited states (Fig. 1). The $^1\Delta_g$ state has an energy only 23 kcal above that of the ground state; both electrons are paired in a single orbital, leaving the other vacant. This state might be expected to undergo two-electron reactions. The higher singlet state ($^1\Sigma_g^+$) comes from the spin pairing electrons in different orbitals and might be expected to undergo one-electron free-radical reactions. Because there are no unpaired electrons,

neither $^1\Delta_g$ form nor $^1\Sigma_g$ are radicals. In both forms of $^1O_2$, the spin restriction is removed so that

the oxidizing ability is greatly increased [2].

The $^1\Sigma_g^+$ state has a much shorter lifetime than $^1\Delta_g$ state because $^1\Sigma_g^+$ is more reactive than the

$^1\Delta_g$ form. It decays to $^1\Delta_g$ state before chemical reactions can occur. The different energy states

of two excited states of molecular oxygen are summarized in Table 1. The lifetime of $^1O_2$

depends on the nature of the solvent (Table 2).

Table 1. Dfifferent energy states of molecular oxygen [7]

States of the oxygen molecule	Occupancy of highest orbitals	Energy above ground state
Second excited state ($^1\Sigma_g$+)	↑ ↓	37 kcal / mol
First excited state ($^1\Delta_g$)	↑↓	22 kcal / mol
Ground state ($^3\Sigma_g^-$)	↑ ↑	

Table 2. Lifetime of singlet oxygen in various solvents [7]

Solvent	t/μs	Solvent	t/μs
H_2O	2	C_6H_{12}	17
D_2O	20	C_6F_6	600±200
CH_3OH	7	$CHCl_3$	60±15
50% D_2O	11	$CDCl_3$	300±100
50% CH_3OH	11	CCl_4	700±200
C_2H_5OH	12	CS_2	200±60
CH_3COCH_3	26	Freon 11	1000±200

Generation of singlet oxygen

Both physical and chemical methods can generate singlet oxygen.

Physical methods

1. Photosensitization method and biologic effect in PDT: The most popular photosensitizer is

Photofrin. Photofrin absorbs in the UV and in the visible region. Photodynamic Therapy (PDT)

using Photofrin is performed by irradiating at 630 nm regions, because red light most easily

penetrate to tissue. Photofrin, when activated by light, undergoes a chemical reaction, which

produces a cytotoxicity agent, leading to cell death. For most application of PDT, the cytotoxic

agent is produced by one of two different processes called Type I or Type II [8]. See Figure 1.
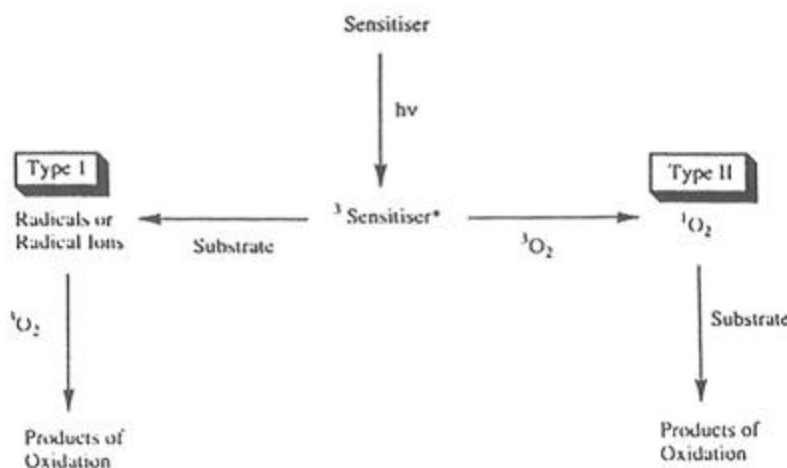

Figure 1. Two pathways in PDT [8]. Type I generates radicals and Type II generates $^1O_2$. Both radicals and $^1O_2$ cause oxidative destruction of tissue.

A Type I mechanism results in hydrogen atom or electron transfer reactions between the

sensitiser and some substrate or the solvent to yield either radicals or radical ions. Radicals and

radical ions formed can then react with oxygen to yield superoxide radical anion. At low pH the

superoxide radical anion can protonate to form the reactive $HO_2^{\bullet}$ radical. The Type II process is

detailed in Figure 2. In this case, Photofrin absorbs the energy of the light that raises the

molecule to an "excited triplet state". The excitation energy can be transferred to an adjacent O_2

molecular, converting it to the singlet state while the photosensitizer molecular returns to the

ground state.

Both singlet oxygen and superoxide are cytotoxic species, causing oxidative destruction of tissue and they consitute the basis for photodynamic cancer therapy.
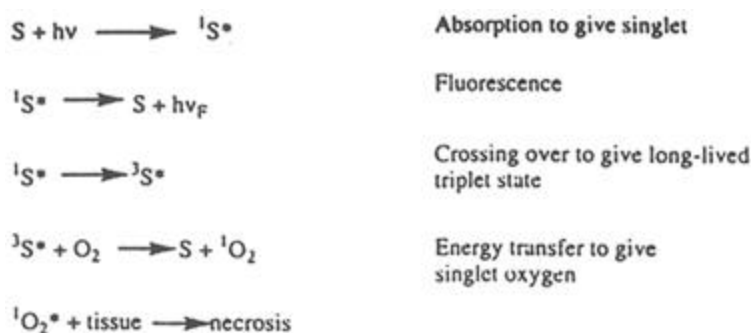
$$S + h\nu \longrightarrow {}^1S\bullet \qquad \text{Absorption to give singlet}$$

$${}^1S\bullet \longrightarrow S + h\nu_F \qquad \text{Fluorescence}$$

$${}^1S\bullet \longrightarrow {}^3S\bullet \qquad \text{Crossing over to give long-lived triplet state}$$

$${}^3S\bullet + O_2 \longrightarrow S + {}^1O_2 \qquad \text{Energy transfer to give singlet oxygen}$$

$${}^1O_2\bullet + \text{tissue} \longrightarrow \text{necrosis}$$

Figure 2. Type II process [8]. Photofrin (S) is excited by the light and transfers the excited energy to O_2 molecular that produces ${}^1O_2$, but the photofrin returns to the ground state.

2. Another physical method is microwave or radiofrequency discharge that generates up to 10% concentration of singlet oxygen (${}^1\Delta_g$) in an oxygen atmosphere.

Chemical methods

Several chemical methods can generate ${}^1O_2$. One of the biologically singnificant reactions is hydrogen peroxide and hypochlorite ions reaction because OCl^- can be formed by the enzyme myeloperoxidase during phagocytosis [2]. This reaction is $OCl^- + H_2O_2 \rightarrow {}^1O_2 + Cl^- + H_2O$. Other chemical methods include the thermolysis of endoperoxides of some aromatic hydrocarbons and some organic ozonides.

Detection of singlet oxygen

1. Scavengers: Scavengers can inhibit a reaction dependent of ${}^1O_2$ [9]. For example, azide acting as a physical scavenger reacts with ${}^1O_2$ to give a reactive azide radical, $N_3^- + {}^1O_2 \rightarrow N_3^\bullet + O_2$ [9]. Other scavengers including carotene, ascorbate, thiols and histindine act as chemical scavengers.

2. D_2O: D_2O can be used to detect ${}^1O_2$ presence because the lifetime of ${}^1O_2$ is 10-fold longer in D_2O than in H_2O [10]. So, if a reaction in aqueous solution is dependant on singlet oxygen, carrying it out in D_2O instead should greatly potentiate the reaction.

3. Luminescence: As $^1O_2$ decays back to the ground state, some of the energy is emitted as light.

The light from singlet oxygen appears in the infrared as 1268 nm [3]. $O_2\ (^1\Delta_g) \rightarrow O_2\ (^3\Sigma^-_g) + h\nu$
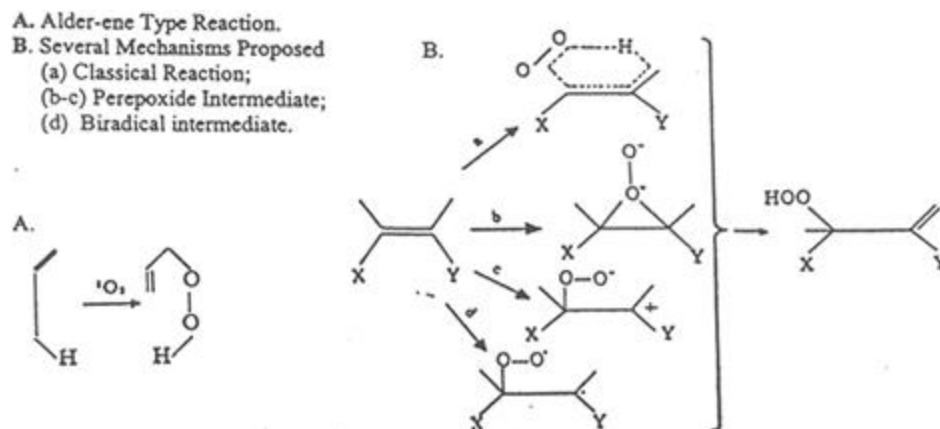
(1268 nm)

Other detection methods include ESR, calorimetry, photo ionization and mass spectroscopy.

Reactions of singlet oxygen

The reactions of $^1O_2$ often involve carbon-carbon double bond, which are present in many

biological molecules, such as carotene, chlorophyll, and unsaturatal fatty acids.

1. Alder-ene reaction (Schenck reaction)

In this reaction, oxygen adds to the alkyl substituted olefins to form allylic hydroperoxides with a

migration of the double bond. The resulting allylic hydroperoxides can be easily converted into

α,β-unsaturated carbonyl compounds and allyl alcohols [7]. A variety of mechanisms have been

proposed to explain the products observed in this reaction. They involve either a six-centered

transition state typical of the classical ene reaction (a), a peroxide of closely related intermediate

(b and c) or a biradical intermediate (d) [2]. See figure 3. While the involvement of peroxide

intermediate is consistent with the experimental observations, the classical ene mechanism is

favored by most authors [7]. Involvement of radical intermediates has been ruled out on the

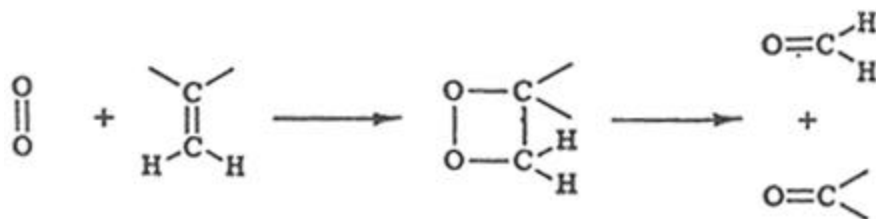basis of various evidences. Figure 3 [7]

A. Alder-ene Type Reaction.
B. Several Mechanisms Proposed
   (a) Classical Reaction;
   (b-c) Perepoxide Intermediate;
   (d) Biradical intermediate.

2. Diels-Alder reaction

With acceptors such as *cis* dienes or aromatic hydrocarbons, $^1O_2$ can react as a good dienophile. This reaction is similar to the well-known Diels-Alder reaction [7]. The initially formed endoperoxides may undergo fragmentation back to reactants or rearrange to products through the homolytic fission of the oxygen-oxygen bond. The concerted character of this addition reaction has been indicated based on experimental observations.

3. Reaction with electron rich systems

Singlet oxygen can also react with electron rich systems [7], in which carbon-carbon double bonds have adjacent electron donating atoms (nitrogen, sulfur). In these reactions, an oxetane type adduct is formed. These dioxetanes may be unstable and decompose to give carbonyl compounds (Reaction below). Decomposition of dioxetane is sometimes accompanied by chemiluminiscence.

Reaction is from [7]

Quenching of singlet oxygen

The quenching of singlet oxygen involves the deactivation of the excited state of molecule. Deactivation can be accomplished by either physical or chemical quenching. Physical quenching leads only to the deactivation of singlet oxygen to its ground state with no oxygen consumption or product formation. In chemical quenching, by contrast, singlet oxygen reacts with quencher R to produce RO_2. Two major mechanisms of $^1O_2$ quenching are known, namely energy transfer and charge transfer quenching.

1. *Energy transfer quenching*

This mechanism of quenching is the reverse of reaction by which singlet oxygen is formed [2].

It involves formation of triplet quencher and ground state oxygen ($^1O_2 + Q \rightarrow {}^3O_2 + Q$).

2. *Charge transfer quenching*

This mechanism involves the interaction between the electron-deficient $^1O_2$ molecule and

electron donors to form a charge transfer complex [2]. Intersystem crossing restrictions are

relaxed in the complex which can then dissociate into donor and ground state oxygen.

Summary

Singlet oxygen is a higher energy state molecular oxygen species. Rearrangement of the electron

spins within the degenerate orbital results in two possible singlet excited states. Several

techniques can detect $^1O_2$ including scavengers and luminescence. The reactions of $^1O_2$ often

involve carbon-carbon double bonds. For example, Alder-ene reaction is $^1O_2$ shifts the double

bonds. Energy transfer and charge transfer quenching are two major mechanisms of $^1O_2$

quenching.

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