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Singlet Oxygen

by

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Abbreviations:

D_2O : deuterium oxide
ESR: electron spin resonance
$^3O_2$: ground-state dioxygen
$^1O_2$: singlet oxygen
$^1\Delta g$: singlet oxygen
$^1\Sigma g$: singlet oxygen

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Abstract

Singlet oxygen($^1O_2$) is the excited state of oxygen with rearrangement of its electrons in its highest occupied molecular orbitals. Two different forms of $^1O_2$ are found. Each has a different energy level and lifetime. They are not free radicals. They can be produced or quenched by various physical and chemical methods. Singlet oxygen mainly reacts with electron- rich compounds. It has damaging effects on biomolecules and exerts genotoxic, virucidal and cytotoxic effects. It also involves in carcinogenesis as well as signaling transduction pathways.

Introduction

Singlet oxygen ($^1O_2$) is the oxygen with extra energy. It was first proposed by Kautsky as a possible reaction intermediate in dye-sensitized photo-oxygenations as early as 1931 [1]. Since then thousands of reactions of $^1O_2$ have been studied. Singlet oxygen has been found to be involved in various physical, chemical and biological processes as well as in many disease processes.

Properties of singlet oxygen

There are two kinds of $^1O_2$ [2]. One is the $^1\Delta g$ O_2 with paired electrons in one molecular orbital. Another is the $^1\sum g^+O_2$ with spin pairing in different orbitals (Figure 1). Both of them are the excited states of molecular oxygen. These two kinds of $^1O_2$ have different energy levels and different lifetimes. Table 1 and 2 show their properties and their lifetimes in different solvents [1]. From table 1 and 2, we see that the lifetime of $^1\sum g$ state is very short; and it decays to $^1\Delta g$ state. Also, $^1\sum gO_2$ is more reactive than $^1\Delta g$ form. However, these two forms are not radicals because there are no unpaired electrons in these two forms.

Figure 1: Electronic state of diatomic oxygen molecules has been explained by molecular orbital theory. From [1].

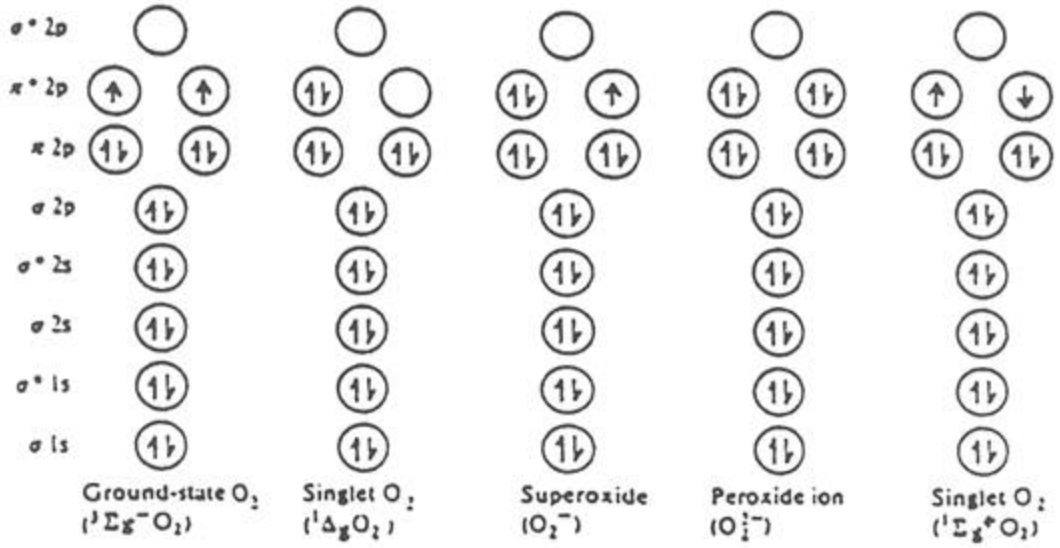


Table 1. Properties of Singlet Molecular Oxygen. From [1]

Properties	$^1\Delta_g$	$^1\Sigma_g$
Energy above ground state	23.5 kcal/mole	37.6 kcal/mole
Light emission	1268.7 nm	761.9 nm
Half life at one atmosphere	5.0×10^{-2} s	—
Solution life time	10^{-5} s	10^{-10} s
Gas collision survival	10^8	10-100
Radiative life time	2,700 s	7 s

Table2. Lifetime of Singlet Oxygen in Various Solvents. From [1]

Solvent	t (μs)	Solvent	t (us)
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

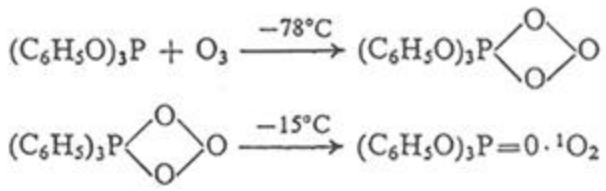
Several methods are available for the generation of $^1O_2$.

Chemical methods:

1. Hydrogen peroxide reacting with sodium hypochlorite can produce $^1O_2$. The biological meaning of this reaction is that hypochlorite can be produced by the enzyme called myeloperoxidase during phagocytosis [2].

$$H_2O_2 + Na^+OCl^- \rightarrow {}^1O_2 + NaCl + H_2O$$

2. Solid adducts formed between the triaryl phosphite and ozone at low temperature decompose to release $^1O_2$ [1].



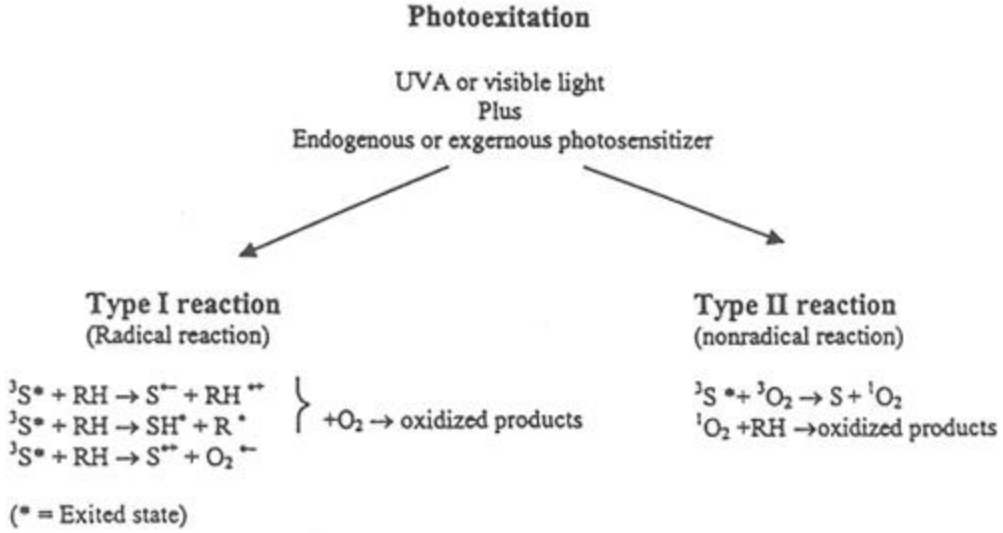
3. Superoxide ion O_2^- is another potential source of $^1O_2$, since loss of an electron of appropriate spin could produce ground state molecular oxygen or $^1O_2$ [1].

$$O_2^- \rightarrow {}^{1,3}O_2 + e^-$$

Physical methods:

One of the most important physical methods for the generation of $^1O_2$ is photosensitization reaction [2]. In this process, photosensitizers are illuminated with light of a given wavelength. They absorb it and the energy raises the photosensitizer to an excited triplet state. The excitation energy can be transferred to the adjacent O_2 molecules, which can be converted to the singlet state.

Figure 2. Scheme Outlines Type I and Type II Photooxygenation Reactions upon Irradiation of a Photosensitizer. From [3].

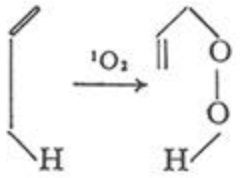


Reactions of singlet oxygen

Singlet oxygen can interact with other molecules in two ways. One is that $^1O_2$ may combine with other molecules chemically Another is that $^1O_2$ may transfer its excitation energy to other molecules.

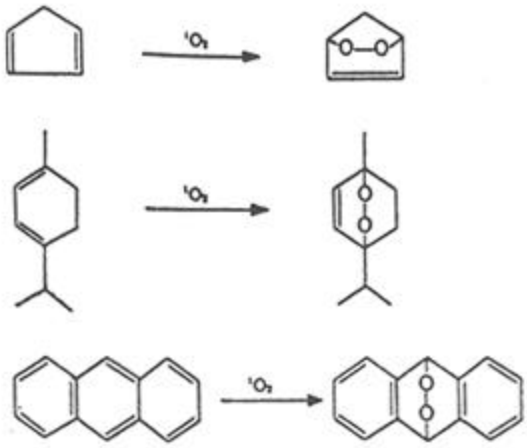
1. Alder-ene type reaction

This reaction occurs when $^1O_2$ reacts with a compound with one double bond. This reaction is also called Schenck reaction or " ene" reaction. They involve either a six-centered transition state typical of the classical reaction, a peroxide of closed related intermediate or a biradical intermediate[1,2].



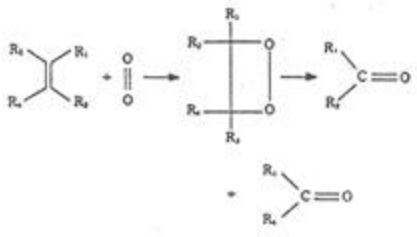
2. Diels-Alder reaction

With acceptors such as *cis* dienes or aromatic hydrocarbons, $^1O_2$ appears to behave as a good dienophile [1].



3. Reaction with electron rich systems

Singlet oxygen may react with double bonds which have electron donating atoms such as nitrogen and sulphur. In this reaction, an oxetane type adduct is formed. These dioxetanes may be unstable and decompose to give carbonyl fragments [1]. Decomposition of dioxetane is sometimes accompanied by chemiluminiscence.



4. Singlet oxygen also can react with DNA, carotene, tryptophan, methionine, cystine, histidine and NADPH [2]. $^1O_2$ can oxidize these amino acids and thus damage proteins. Histidine has the highest second order rate constant for reaction with $^1O_2$. Oxidative destruction of histidine is the major cause of destruction of many enzymes.

Quenching of singlet oxygen

The quenching of $^1O_2$ involves the deactivation of the excited singlet states of oxygen molecule. It may be either physical or chemical quenching [1]. Chemical quenching is that in which $^1O_2$ reacts with quencher (Q) to give product QO_2. Physical quenching leads only to the deactivation of $^1O_2$ to its ground state with no oxygen consumption or product formation. Schafer *et al* reported that $^1O_2$ can be quenched by water, lipids, nucleic acids, polyunsaturated fatty acids and other small molecules [4]. Two major mechanisms of $^1O_2$ are known. They are energy transfer and charge transfer quenching.

1. Energy transfer quenching [1]

This mechanism of quenching is the reverse of reaction by which $^1O_2$ is formed. It involves formation of triplet quencher and ground state oxygen and to be efficient, requires that triplet state of the quencher should be very near or below the energy $^1\Delta g$.

$$^1O_2 + {}^2Q \rightarrow {}^3O_2 + {}^3Q$$

Other quenchers like dyes, metal complex and other compounds with very extensive conjugated systems may also involve this mechanism of quenching.

2. Charge transfer quenching [1]

This mechanism involves the interaction of the electron deficient $^1O_2$ with electron donors.

$$D + {}^1O_2 \leftrightarrow [\ D\text{---}O_2^-\ ]^1 \leftrightarrow [D^+ \text{ --- } O_2^-]^3 \leftrightarrow D + {}^3O_2$$

Since $^1O_2$ acts as an electron acceptor, easily oxidizable compounds prove to be the best quenchers. Compounds quenching $^1O_2$ by the charge transfer mechanism include amines, phenols, metal complexes, sulfides, iodide, azide, superoxide ion and similar electron rich compounds. Recently, Hessler *et al* use humic substances (HSs) to quench $^1O_2$. The efficiency of $^1O_2$ quenching depends on the origin of the HS sample [4].

Detection of singlet oxygen

Various techniques are used for detecting the presence of $^1O_2$ [1].

1. Paramagnetic resonance

This method of detecting $^1O_2$ is specific for $^1\Delta g$ state since $^1\Sigma g$ state is diamagnetic. It is proved that $^1O_2$ concentration constitutes about 10% of the total oxygen concentration.

2. Calorimetric method

An isothermal calorimetric method of detecting $^1\Delta g$ O_2 rests on its efficient deactivation on a cobalt coated platinum wire. The heat released in the deactivation corresponds to the excitation energy. Recently, a lower-cost laser deflection calorimeter (LDC) apparatus is developed to determine $^1O_2$ quantitatively [6]. This method should be specially designed for studying chemical and biochemical processes at interfaces by measuring low amounts of heat released.

3. Emission spectroscopic method

An optical emission from $^1\Delta g$ and $^1\Sigma g$ provides another method by which excited oxygen molecules may be detected in the gas. The emission bands for these two forms appear at 1270 nm and 760 nm respectively.

4. Mass spectroscopic method

The formation of O_2^+ species from $O_2(^1\Delta g)$ occurs at a lower appearance potential than from O_2 ($^3\Sigma g$) since the former have higher energy. Thus mass spectrometry have been helpful in detecting $^1\Delta g$ state. However, the method has limited scope since relatively large concentrations of $O_2(^1\Delta g)$ are required.

Biological significance of singlet oxygen

Singlet oxygen is involved in many biological areas such as dye sensitized photo oxygenation, erythrocyte dysfunction associated with G6DP deficiency, membrane destructive process, phagocytosis, photoaging, metabolic hydroxylation, age related changes of the lens, and carcinogenesis [2]. Recently, Singlet oxygen is also found to be involved in signaling transduction pathway [7]. A novel observation is the activation of transcription factor AP-2 and cellular signaling cascades comprising the activation of

c-Jun-N-terminal kinases (JNK/SAPK) and NF-kappa B system. Singlet oxygen has damaging effects on biomolecules and exerts genotoxic, virucidal and cytotoxic effects [7]. For example, Singlet oxygen can induce apoptosis in HL-60 cells because $^1O_2$ can induce nuclear condensation and DNA fragmentation into nucleosome-size fragments in a dose dependent manner [8]. Singlet oxygen can also induce oxidative DNA base damage [9] and may have dramatic effects on eukaryotic gene expression [10]. The toxicity of $^1O_2$ is cell line-dependent [3]. The greater the protein content of cells the more they are protected against membrane damage. The cell size correlates inversely with ability of

cells to cope with a given flux of $^1O_2$. Human catalase can be oxidized by $^1O_2$ in myeloid leukemia cells [11]. Catalases are oxidized by $^1O_2$ giving rise to more acidic conformers detected in zymograms after electrophoresis in polyacrylamide gels. This shift in catalase mobility can be indicative of $^1O_2$ production in *vivo*. Stief *et al* reported that singlet oxygen can inactivate fibrinogen, factor V, factor VIII, factor X, and platelet aggregation of human blood, suggesting that Singlet oxygen is an anti-thrombotic agent [12]. Singlet oxygen produced by photodynamic action can inactivate mitochondrial permeability transition pore. The most likely targets for $^1O_2$ are critical histidines that undergo degradation [13]. Tatsuzawa *et al* reported that singlet oxygen can inactivate bacterial respiratory chain enzymes [14]. Exposure of wild-type E. coli to $^1O_2$ causes a significant loss of E. coli viability due to inactivation of membrane respiratory chain enzymes by $^1O_2$, suggesting that singlet oxygen produced by phagocytic leukocytes is a major bactericidal oxidant in the phagosome .

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