# This student paper was written as an assignment in the graduate course

## *Free Radicals in Biology and Medicine*

**(77:222, Spring 2001)**

**offered by the**

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**B-180 Med Labs**
**The University of Iowa**
**Iowa City, IA 52242-1181**
**Spring 2001 Term**

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# Superoxide ($O_2^{\cdot -}$): An Introductory Glimpse from the Chemical/ Biochemical Perspective

by

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For 77: 222, Spring 2001
8.February 2001

Abbreviations: $AscH^-$ (ascorbate monoanion), $Asc^{\cdot -}$ (ascorbate radical), $A^{\circ}$ (absorbance), cyt c (cytochrome c), DMPO ( 5,5-dimethylpyrroline-N-oxide), ESR (electron spin resonance), $E^{\circ'}$ (extinction coefficient), $^3\Sigma g\ O_2$ ( ground state oxygen), $HO_2^{\cdot}$ (hydrogen dioxide radical), $O_2^{\cdot -}$ (dioxygen), pH ( -log [A]), pKa ( -log [A]), $L_x$ (stabilizing ligand/ lipid oxidase)

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## Abstract

The superoxide free radical is a powerful oxidant occurring in biological systems where it can cause severe damage to cellular structures. Cells dismute or catabolize this small molecule as rapidly as possible, producing less reactive species which eventually break down further into water and dioxygen. Understanding superoxide's behavior in cells necessitates understanding it's basic properties and reactions.

## Introduction

Although direct toxicity from dioxygen ($O_2$) has been suggested [1, 11], the rates of enzyme inactivation in aerobic cells are too slow to account for oxygen toxicity [10]. The realization that oxidative damage occurs in aerobic organisms as a result of dioxygen exposure [1, 3, 13] meant a more reactive daughter species might be present. The observation of free radicals in biological materials around 1954 [9] contributed to the discovery of superoxide radicals in 1969 [12] followed by the hypothesis that superoxide radicals might be involved in oxidative stress in cells. Since the chemistry of biological systems occurs in aqueous environments, any effective discussion about superoxide ($O_2^{\cdot -}$) must also address its protonated form, the hydrogen dioxide radical ($HO_2^{\cdot}$). A limited selection of physical and chemical properties, reactions, and detection methods for these two reactive oxygen species are presented here.

## Physical and Chemical Properties

The ubiquitous bi-radical molecule commonly known as oxygen composes approximately 20.9% [14] of air and forms the basis for superoxide. Adding an electron to oxygen creates a highly reactive superoxide species in a thermodynamically unfavorable reaction where $E^{\circ'}$ = -330 mV. Where the bond length of ground state oxygen is 1.2074 A°, the solid state bond length of $O_2^{\cdot -}$ is 1.33 A°, and further confirms the relative instability of superoxide [5, 8]. Superoxide generally acts as a reductant/ reducing agent which loses electrons in most chemical reactions, and it also usually acts

as a base. The hydrogen dioxide radical, superoxide's protenated form, generally acts as an oxidant/ oxidizing agent, which takes up electrons; it is a weak acid with a pKa = 4.8 ± 0.1 [5]. The orbital representations of dioxygen and superoxide provide the clearest illustration of the physical difference between the two molecules [10]:

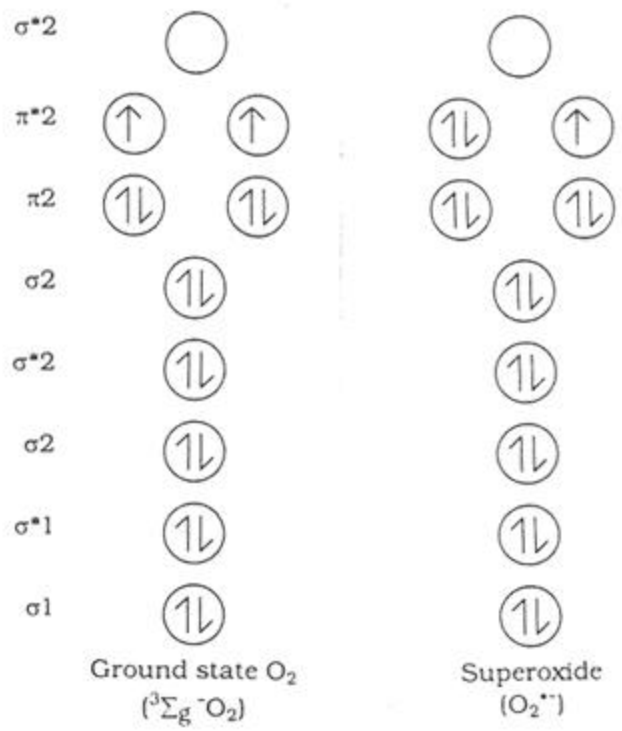


Figure 1. Orbital representation of ground state oxygen and superoxide

## Reactions

Two methods of superoxide production in cells include enzymatic catalysis and autooxidation [10]. Many enzymes have been shown to catalyze the reduction of dioxygen to superoxide [6]. Some of these enzymes include peroxidases (plants, bacteria, some animal tissue), xanthine oxidase (intestine, ischaemic tissues), nitric oxide synthetase or NOS (most mammalian cells), indoleamine 2,3 dioxygenase (most animal tissue), and tryptophan dioxegenase (liver)[6].

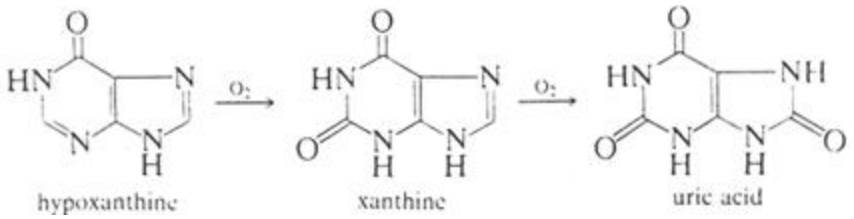


Figure 2. Xanthine oxidase catalyses oxidation of both hypoxanthine and xanthine to uric acid while reducing $O_2$ to both $O_2^{\cdot -}$ and $H_2O_2$ [10].

Autooxidations occur when important biomolecules reduce dioxygen to superoxide with the catalytic prescence of transition metals [10]. These reactions also oxidize the biomolecules involved:

$$FMNH_2 + O_2 \rightarrow FMN + O_2^{\cdot -} \quad (1)$$

$$FADH_2 + O_2 \rightarrow FAD + O_2^{\cdot -} \quad (2)$$

Additional biomolecules which serve this function are glyceraldehydes, hormones, the neurotransmitter dopamine, and thiol compounds such as cysteine [10].

The dismutation or superoxide removal reactions stand out as some of the most significant superoxide reactions occurring in biological systems. There are three major dismutation reactions whose contributions toward the observed rate constant are determined by pH [2]:

$$O_2^{\cdot -} + HO_2^{\cdot} + H^+ \rightarrow O_2 + H_2O_2 \quad k = 9.7 \times 10^7\ M^{-1}s^{-1} \quad (3)$$

$$HO_2^{\cdot} + HO_2^{\cdot} \rightarrow O_2 + H_2O_2 \quad k = 8.3 \times 10^5\ M^{-1}s^{-1} \quad (4)$$

$$O_2^{\cdot -} + O_2^{\cdot -} + H^+ \rightarrow O_2 + H_2O_2 \quad k < 0.3\ M^{-1}s^{-1} \quad (5)$$

Reaction (4) has its greatest effect below pH 2. Reaction (3) dominates the pH range on up to 13 with its maximum rate at the 4.8 pKa of $HO_2^{\cdot}$. Reaction (5) has its greatest effect over a small range near pH 13. Reaction (3)'s domination of the area around

physiological pH due to the exceedingly small reaction constant for reaction (5) which can be considered negligible in comparison to the other two reactions' rates. Reaction (5) only occurs in biological systems when the SOD catalyst is present. The observation of these dismutation reactions as they relate to pH has been verified by multiple and consistent data sets [2]:

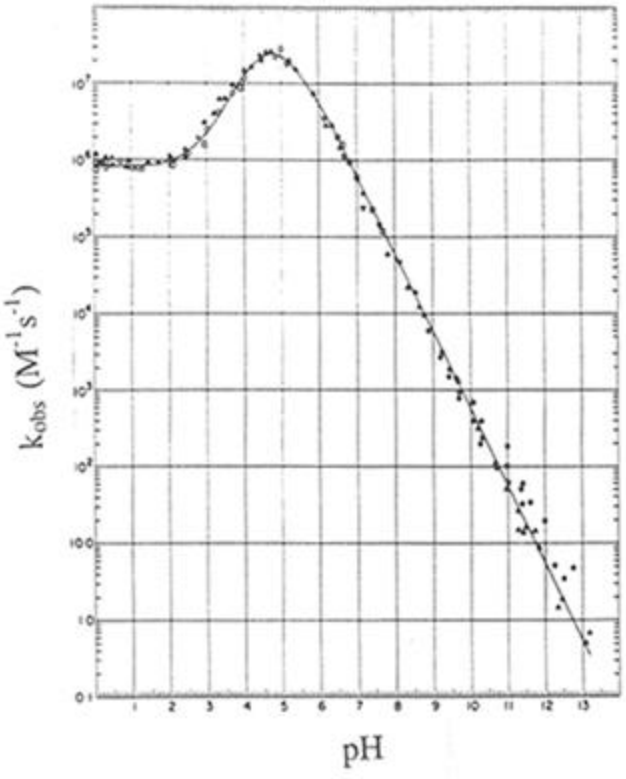


Figure 3. Observed dismutation of $O_2^{\cdot-}/HO_2^{\cdot}$ in aqueous solution, *i.e.* second order rate constant as a function of pH

The following are a few other biologically relevant reactions worth noting. Reaction (6) shows superoxide as an oxidizing agent, although it is probably reacting as the protonated form. Reaction (7) shows superoxide as a reducing agent. The kinetic efficiency described by rate constants is dependant on the ligand environment in each

reaction. Reactions (7) and (8) are also known as the iron catalyzed Haber-Weiss reaction or superoxide driven Fenton reaction [7]. Equation (8) describes the completion of the iron-ligand's role as a catalyst.

$O_2^{\cdot -} + AscH^- + H^+ \rightarrow Asc^{\cdot -} + H_2O_2$ $k = 2.7 \times 10^5\ M^{-1}s^{-1}$ pH = 7.4 [2] (6)

$HO_2^{\cdot} + HO_2^{\cdot} \rightarrow O_2 + H_2O_2$ $k = 1.5 \times 10^8\ M^{-1}s^{-1}$ pH = 7.0 [7, 10] (7)

$O_2^{\cdot -} + O_2^{\cdot -} + H^+ \rightarrow O_2 + H_2O_2$ [7,10] (8)

## Detection

The use of indirect detection methods for $O_2^{\cdot -}/HO_2^{\cdot}$ becomes necessary because of their highly reactive nature. UV/ visible light spectroscopy and ESR, two currently available tools for detection, do not possess the sensitivity to detect biological quantities of $O_2^{\cdot -}/HO_2^{\cdot}$ without additional aide. Spectroscopy utilizes indicator molecules such as Fe(III)-cytochrome c or NBT, which react rapidly with superoxide to produce a very dark, absorbant compound. Molar extinction coefficients are a quantitative indicator of a molecule's detectability as a function of absorbance. Compounds with higher extinction coefficients, therefore, are more readily detectable through spectrophotometry. It is also important to look for evidence of these compounds at their maximal absorbance wavelengths. As might be expected, contaminating particles in solution can alter absorbance readings and create misleading results. The following is an example of spectrophotometric data acquisition using the reduction of NBT chloride to diformazan [7]:

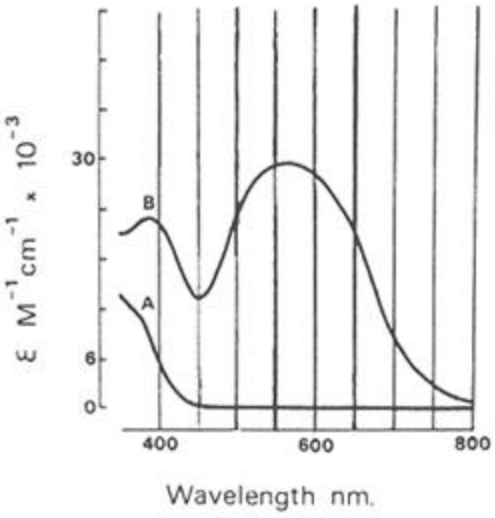


Figure 4. Visible spectra of (A) NBT and (B) biformazan in water.

Spin trapping occurs when a radical, such as $HO_2^{\cdot}$, reacts with a spin trap to create a covalent bond without losing radical character. This creates a more stable species, a spin adduct, that can then be detected by ESR. The reaction of DMPO and a hydrogen dioxide radicals is an example of this type of reaction [ 7, 4]:

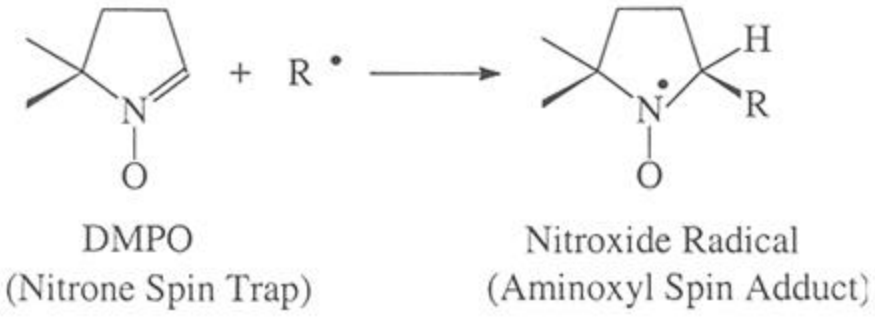


Figure 5. Nitrone (DMPO) spin-trap reaction with superoxide.

The following is an example of data output from an EPR machine [4]:

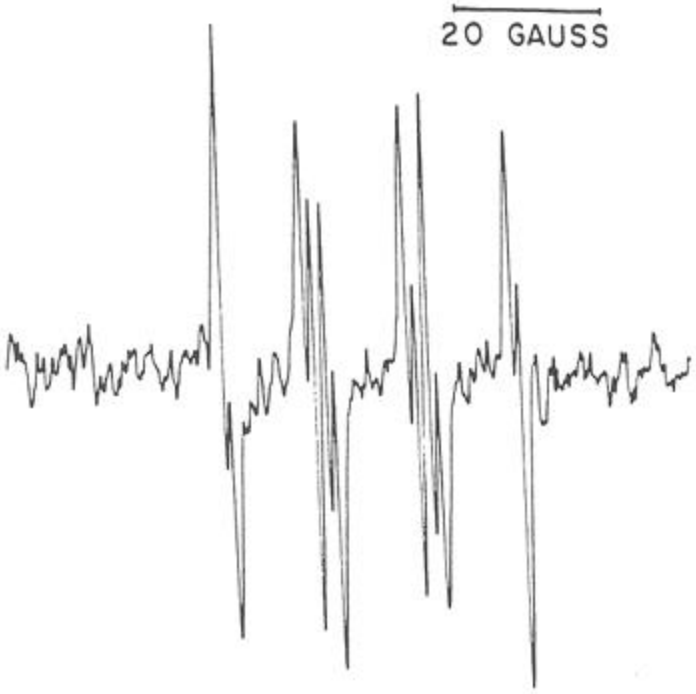


Figure 6. "The superoxide spin adduct spectrum of DMPO produced by a solution of 0.1 mM xanthine, 0.15 mM DETA-PAC, 70mM DMPO, and 0.012 unit/ml of xanthine oxidase in 50mM phosphate buffer, pH 7.8. the spectrum was obtained on a Varian E-109 equipped with the E-238 aqueous solution cavity. Time constant = 0.5 sec, moduation amplitude = 0.8 gauss, gain = 5 x $10^4$, power = 20 mW at room temperature, scan-rate = 25 gauss/min, $a_N$ = 14.2 gauss, $a_\beta^H$ = 11.2 gauss, $a_\gamma^H$ = 1.3 gauss, g = 2.0060 [4]."

## Summary

Our understanding of $O_2^{\cdot -}/HO_2^{\cdot}$ is by no means complete. While superoxide reactions in controlled aqueous environments are now well characterized, *in vitro* versions of these reactions are less understood. The foundation of basic chemical knowledge concerning

superoxide reactions is sound and as we continue to explore, important advances in our understanding of this vital biomolecule's biochemistry will continually be discovered.

## Acknowledgements

Thank you to Dr. Buettner, Dr. Schafer, and Sean Martin for their patience.

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