MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A
# Toxicity, Mutagenesis and Aging due to Endogenous Oxygen Radicals

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**Abstract:**

We have been examining the oxidation of ammonia to nitrite by oxygen radicals generated by the xanthine oxidase reaction. This oxidation (\(\text{NH}_3 \rightarrow \text{NO}_2^-\)), which can easily be demonstrated, is inhibited by superoxide dismutase, or by catalase, or by scavengers of the hydroxyl radical. We conclude that the iron-catalyzed reduction of \(\text{H}_2\text{O}_2\) to \(\text{OH}^- + \text{OH}^-\) by \(\text{O}_2^-\) is...
20. Abstract (continued)

involved and that OH· is the first oxidant of NH3. When NH3 is replaced by NH2OH we see NO2⁻ production which is inhibited by superoxide dismutase but not by catalase. In this case we conclude that O2⁻, per se, can oxidize NH2OH to NO2⁻. We have proposed a mechanism which includes the following intermediates:

\[
\text{H}_2\text{N}^+, \text{H}_2\text{NOH}, \text{HONO}OH \text{ and finally } \text{NO}_2^-
\]

This is of interest because O2⁻, H2O2 and OH· are known to be generated in cells and our mechanism provides a route which can explain the endogenous production of NO2⁻, which has previously been noted.

We have been reinvestigating the killing of E. coli by paraquat. Our earlier studies showed that the lethality of paraquat was dependent upon O2⁻ and an electron source and was decreased by elevated intracellular levels of superoxide dismutase. All of this, plus measurements of cyanide-resistant respiration, showed that O2⁻ was essential for expression of the lethality of paraquat. This work was done in a nutrient broth medium. We now see that paraquat is much more lethal in the nutrient broth medium than it is in a simpler Vogel/Bonner medium. Indeed there is a heat stable and dialyzable factor in nutrient broth which appears essential for expression of paraquat lethality. This factor, once identified, should greatly increase our understanding of the mechanism of cell killing by O2⁻. We have begun to isolate this factor.
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TOXICITY, MUTAGENESIS AND AGING DUE TO ENDOGENOUS OXYGEN RADICALS

Final Report

Irwin Fridovich

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C. List of All Publications during Tenure of Contract


Participating Personnel

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Janice Blum, research assistant, received Ph.D., and research assistant
Wayne Beyer, Postdoctoral fellow
Debbie Clare, Postdoctoral fellow
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Effects of Molecular Oxygen on Detection of Superoxide Radical with Nitroblue Tetrazolium and on Activity Stains for Catalase

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The usual method of staining polyacrylamide gel electropherograms for superoxide dismutase activity utilizes a photochemical flux of $O_2^-$ to reduce nitroblue tetrazolium. Superoxide dismutases intercept $O_2$, preventing formazan production and thus causing achromatic bands. In the presence of $H_2O_2$, catalases also yield achromatic bands during this staining procedure. This is due to local elevation of $pO_2$ by the catalatic decomposition of $H_2O_2$, in turn, inhibiting the reduction of the tetrazolium by $O_2$. This phenomenon provides a new activity stain for catalase. A previously described activity stain for catalase has also been reexamined and significantly improved.

Increased Superoxide Radical Production Evokes Inducible DNA Repair in Escherichia coli

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Paraquat induced the SOS response in Escherichia coli. This was measured in terms of acquired resistance towards UV lethality in a wild-type strain and in terms of appearance of $\beta$-galactosidase activity in a din::Md(Ap lac) fusion strain. However measured, the induction of the SOS response by paraquat was entirely dioxygen-dependent; whereas induction of the SOS response by mitomycin C was independent of the presence of dioxygen. As expected, recA(Def) and lexA(Ind) isogenic strains did not show the SOS response. It appears likely that $O_2^-$, whose intracellular production is increased by paraquat, leads to DNA damage which in turn induces the SOS response.

Abstract

1. Growth of Chlorella sorokiniana in the presence of 7.5 mM sulfite, which halved the growth rate while doubling the superoxide dismutase EC 1.15.1.1 content per cell, rendered the cells resistant to the toxic effects of 30 μM paraquat.

2. While increasing total superoxide dismutase content, sulfite increased the relative amount of the $\text{H}_2\text{O}_2$-resistant manganese-containing superoxide dismutase.

3. It appears that $\text{O}_2^-$ may be involved in mediating the toxicity of $\text{SO}_2$ in this green alga.

Key Words: Chlorella - Chlorophyta, paraquat, sulfite, superoxide dismutase
The superoxide radical (O$_2^-$) is a frequently encountered intermediate of the reduction of dioxygen and it poses a threat to living cells, much as does H$_2$O$_2$. Metalloenzymes, called superoxide dismutases (SODs), provide a defense against O$_2^-$ and are found in virtually all organisms. These enzymes, properly called superoxide/superoxide oxidoreductases, catalyze the conversion of O$_2^-$ to H$_2$O$_2$ + O$_2$ and operate close to the diffusion limit. A decade has passed since the last review on SODs appeared in ADVANCES IN ENZYMOLOGY (1). Interest in these enzymes has grown steadily and rapidly. We will now survey some of the work fueled by this interest.
Diaminobenzidine (DAB) has repeatedly been used as a chromogenic substrate for peroxidases and for the peroxidatic activity of glutaraldehyde-treated catalase (Graham and Karnovsky, 1966; Fahimi, 1968; Novikoff and Goldfischer, 1969; Herzog and Fahimi, 1974). DAB plus horseradish peroxidase (HRP) has also been used to provide a negative stain for catalase activity on polyacrylamide gels (Gregory and Fridovich, 1974). In this method, gel electropherograms were soaked first in DAB plus HRP and then in $H_2O_2$. Zones containing catalase would become depleted of $H_2O_2$ and thus not show the chromogenic peroxidation of DAB by HRP, leaving achromatic zones against a uniformly stained background. It was subsequently noticed that changing the order of application of these reagents, such that DAB was the last reagent applied, markedly increased the sensitivity of this negative stain for catalase activity (Clare et al., 1984). This result was explained on the basis of the inhibition of catalase by DAB. Since the inhibition of catalase by DAB had not previously been reported, we undertook an investigation of this inhibition and also examined the effects of related compounds. The results of this study, which indicated that DAB inhibits catalase, both reversibly and irreversibly, and a mechanism consistent with these results, are presented below.