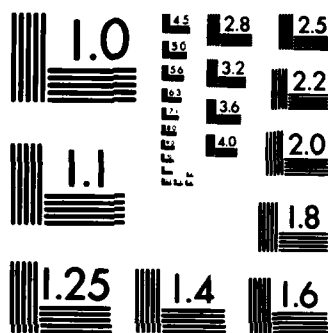


AD-A150 121 TOXICITY MUTAGENESIS AND AGING DUE TO ENDOGENOUS OXYGEN 1/1
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19. KEY WORDS (Continue on reverse side if necessary and identify by block number) superoxide radical, hydroxyl radical, hydrogen peroxide, superoxide dismutases, paraquat, nitrite, catalase, sulfur dioxide.
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) 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 H_2O_2 to $\text{OH}^- + \text{OH}^\cdot$ by O_2^- is

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20. Abstract (continued)

involved and that $\text{OH}\cdot$ is the first oxidant of NH_3 . When NH_3 is replaced by NH_2OH we see NO_2^- production which is inhibited by superoxide dismutase but not by catalase. In this case we conclude that $\text{O}_2^{\cdot-}$, per se, can oxidize NH_2OH to NO_2^- . We have proposed a mechanism which includes the following intermediates:



This is of interest because $\text{O}_2^{\cdot-}$, H_2O_2 and $\text{OH}\cdot$ are known to be generated in cells and our mechanism provides a route which can explain the endogenous production of NO_2^- , 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 $\text{O}_2^{\cdot-}$ 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 $\text{O}_2^{\cdot-}$ 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 $\text{O}_2^{\cdot-}$. We have begun to isolate this factor.

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Growth of Chlorella sorokiniana in the Presence of Sulfite Elevates Cell Content of Superoxide Dismutase and Imparts Resistance Towards Paraquat. *Planta*, in press (1984).

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TOXICITY, MUTAGENESIS AND AGING DUE TO ENDOGENOUS
OXYGEN RADICALS

Final Report

Irwin Fridovich

December 14, 1984

U. S. ARMY RESEARCH OFFICE

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Effects of Molecular Oxygen on Detection of Superoxide Radical with Nitroblue Tetrazolium and on Activity Stains for Catalase¹

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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 . O_2 , in turn, inhibits 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.

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Increased Superoxide Radical Production Evokes Inducible DNA Repair in *Escherichia coli**

(Received for publication, June 6, 1984)

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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 β -galactosidase activity in a *din::Mu d(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 μ M paraquat.

2. While increasing total superoxide dismutase content, sulfite increased the relative amount of the H_2O_2 -resistant manganese-containing superoxide dismutase.

3. It appears that O_2^- may be involved in mediating the toxicity of SO_2 in this green alga.

Key Words: Chlorella - Chlorophyta, paraquat, sulfite, superoxide dismutase

Darr, D., and Fridovich, I., Inhibition of catalase by 3,3'-diaminobenzidine. Biochem. J., in press, 1984.

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_2O_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 $H_2O_2 + O_2$ 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.

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