Reversal of the Superoxide Dismutase Reaction*

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Received July 26,1973

Summary

The superoxide dismutase from bovine erythrocytes has been shown to catalyze the reduction of oxygen by H_2O_2. This reversal of the spontaneous disproportionation of superoxide radicals was made feasible through the use of tetranitromethane as an effective scavenger for the superoxide radicals.

Introduction

SOD‡ has been shown to catalyze the disproportionation of O_2^- and thus to serve as a defense against the cytotoxicity of this radical (1-4). Thermodynamics requires that a catalyst accelerate a given reaction equally in both forward and reverse directions. Since the rate constant for the reaction of O_2^- with SOD [$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$] is $2 \times 10^9 M^{-1} sec^{-1}$ (5,6), catalysis of the reverse reaction might be demonstrable even in the face of an equilibrium constant which greatly favors the forward reaction. SOD has now been shown to catalyze the production of O_2^- from $H_2O_2 + O_2$. O_2^- was detected through its reaction with TNM‡ (7).

Materials and Methods

SOD was prepared from bovine erythrocytes (8). TNM was obtained from

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*This work was supported in full by Research Grant GM-10287 from the National Institutes of Health, Bethesda, Maryland.

‡ Abbreviations: SOD, superoxide dismutase; TNM, tetranitromethane.

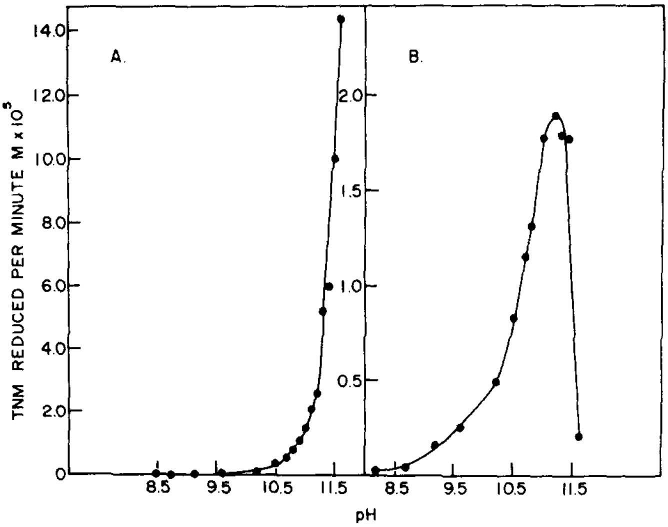
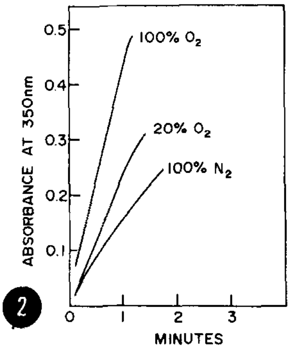


Figure 1 - The Effects of pH on the Reduction of TNM by H_2O_2 in the Absence and in the Presence of SOD - In 1A reaction mixtures contained 6.6 x 10^{-5}M H_2O_2, 5.56 x 10^{-5}M TNM, 2.4 x 10^{-4}M O_2, 1 x 10^{-4}M EDTA and 0.05M sodium carbonate at 10° and at the indicated pH. In 1B conditions were indentical except that 1 x 10^{-6}M SOD was also present and the rate measured at each pH in the absence of SOD was subtracted from that measured in the presence of SOD. In all cases initial rates of nitroform production were monitored at 350 nm.

Sigma and was extracted with water to remove colored impurities. Stock solutions of 4.17 x 10^{-2}M TNM in ethanol were prepared each day. Reactions under controlled atmospheres were performed in cuvettes similar to those described by Lazarow and Cooperstein (9). To minimize losses by evaporation, TNM was added after equilibration with the gas mixtures. The reduction of TMN was followed at 350 nm where E = 14,800$M^{-1}cm^{-1}$ for the nitroform anion (10).

Results and Discussion

TNM was reduced by H_2O_2, as shown in figure 1A. These results are in accord with the assumption that HO_2^- was much more reactive than H_2O_2 and with a pKa of 11.6 for H_2O_2 (11). When SOD was added to reaction mixtures containing TNM, H_2O_2 and O_2 it caused an enhancement of the rate of production of nitroform. Figure 1B presents this enhancement as a function of pH. The rate seen in the presence of 1 x 10^{-6}M SOD was four times greater than that seen in its absence at pH 10.2 and was 2.5 times greater at pH 11.0. Since TNM is very rapidly



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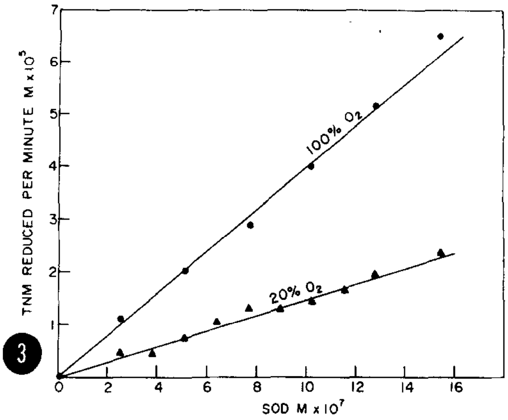

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Figure 2 - The Effect of Oxygen on the Enzymatic Reduction of TNM by H_2O_2 - Reaction mixtures contained 6.6 x 10^{-5}M H_2O_2, 5.56 x 10^{-5}M TNM, 1 x 10^{-4}M EDTA, 0.05M sodium carbonate and 5 x 10^{-7}M SOD at 22° and at pH 10.2. Reaction mixtures were equilibrated with the indicated gases prior to initiating the reactions by tipping in the H_2O_2.

Figure 3 - The Effect of Enzyme Concentration on the Reduction of TNM by H_2O_2 - Reaction mixtures contained 6.6 x 10^{-5}M H_2O_2, 5.6 x 10^{-5}M TNM, 1 x 10^{-4}M EDTA, the indicated concentrations of SOD and 0.05M sodium carbonate at 22° and at pH 10.2. Reactions were performed under equilibration with 20% O_2 or with 100% O_2, as indicated. In all cases the rates shown have been corrected for the non-enzymatic and oxygen-independent reduction of TNM by H_2O_2.

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reduced by O_2^- it appeared probable that this rate enhancement was due to the SOD-catalyzed reduction of O_2 by H_2O_2.

If the enhancement of the reduction of TNM by SOD was due to a reversal of the superoxide dismutase reaction then it must be oxygen-dependent. Figure 2 demonstrates that it was. The rate seen in the absence of O_2 was the same as that seen in the absence of SOD.

Figure 3 demonstrates that the enzymatic enhancement of the reduction of TNM by H_2O_2 was proportional to the concentration of SOD whether observed under 20% O_2 or under 100% O_2. We conclude that the O_2 and SOD dependent reduction of TNM by H_2O_2 was due to a reversal of the superoxide dismutase reaction and to the subsequent reduction of TNM by O_2^- (7).

The rate of the non-enzymatic reduction of TNM by H_2O_2 was proportional to [TNM] and to [H_2O_2] as expected for a second order reaction. In contrast the enzymatic reaction was independent of [TNM] in the range 1.4 ⟶ 8.4 x 10^{-5}M and was saturateable with respect to H_2O_2. The latter aspect of the enzymatic reaction is shown in figure 4. These results support the following mechanism:

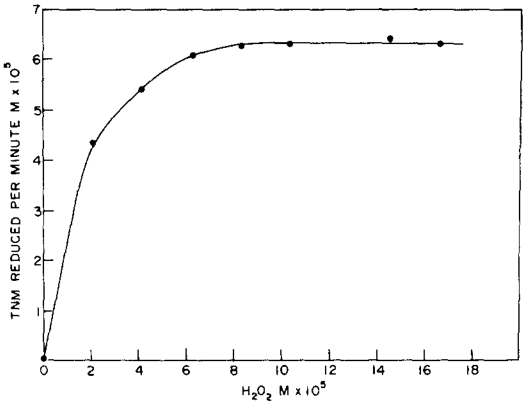

Figure 4 - The Effect of H_2O_2 on the SOD-Dependent Reduction of TNM - Reaction mixtures contained 3×10^{-5}M TNM, 5×10^{-7}M SOD, 1×10^{-4}M EDTA, 2.4×10^{-4}M O_2, the indicated concentrations of H_2O_2 and 0.05M sodium carbonate at pH 10.2 and 22°. The rates shown have been corrected for the non-enzymatic rate.

(A) $\text{E-Cu}^{++} + HO_2^- \xrightarrow{ka} \text{E-Cu}^{+}\text{-}O_2H$

(B) $\text{E-Cu}^{+}\text{-}O_2H \xrightarrow{kb} \text{E-Cu}^{+} + H^+ + O_2^-$

(C) $\text{E-Cu}^{+} + O_2 \xrightarrow{kc} \text{E-Cu}^{++} + O_2^-$

If ka were faster than kb or kc then the overall process could easily be saturated with respect to H_2O_2. Since the rate of reaction of TNM with O_2^- is very rapid (7) we should expect that the enzymatic reduction of TNM should be limited by the enzymatic processes and should thus be independent of [TNM] over a wide range of concentration of TNM.

It must be emphasized that reactions A, B and C do not proceed readily, as written. It is the great reactivity of TNM with O_2^- (7) which permitted it to compete effectively with SOD for O_2^- and thus to pull the reactions in the direction written.

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