THE DECAY OF PEROXY RADICALS OF METHANOL
AND ISOPROPAOL IN THE PRESENCE OF COPPER
IONS AND SUPEROXIDE DISMUTASE

PROGRESS REPORT

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Work carried out during the period:
December 1st 1978 to November 30 1979

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THE DECAY OF PEROXY RADICALS OF METHANOL AND ISOPROPA NOL IN THE PRESENCE OF COPPER IONS AND SUPEROXIDE DISMUTASE

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SUMMARY

The decay of the peroxy radicals produced from methanol and isopropanol was followed in the presence and in the absence of Cu$^{2+}$ ions and the enzyme Superoxide Dismutase. The results indicate that both Cu$^{2+}$ and Superoxide do not affect the decay of the alcohol peroxy radicals. They catalyze the decay of O$_2$ radicals which are formed from the alcoh olic peroxy radicals, and which absorb light at the same wavelengths region as these radicals. This catalysis enables the resolution of the decay of the alcoholic peroxy radicals, without the interference of absorption changes originating in the decay of HO$_2$ and O$_2^-$ radicals.

INTRODUCTION

Among the radicals which are produced radiolytically in aerated aqueous solutions, mainly OH· radicals were shown to cause biological damage (1). Neither e$_{aq}$, nor O$_2^-$ cause significant damage to the living cell. Superoxide dismutase (S.O.D) catalyzes the dismutation of O$_2^-$( 2). It was suggested that the biological role of S.O.D. is to protect the living tissue from the superoxide radicals. Since O$_2^-$ does not damage the living cell, this seems not to be the case. OH· radicals react with many components of the living cell: proteins, lipids, carbohydrates etc., to form radicals. In the presence of O$_2^-$, the radiation damage in biological systems is approximately doubled. It was suggested that peroxy radicals are formed, and thus oxygen induces fixation of the damage caused by OH· radicals, and prevents repair of this damage. ( 3).

On these grounds, it is possible that the role of S.O.D. is to catalyze the dismutation of biological peroxy radicals produced from the components of the cell.

In order to check this assumption, we have undertaken the study of the decay of some peroxy radicals in the presence of S.O.D.. We have chosen the simple molecules of methanol and isopropanol as models of the more complex biological molecules. We have also studied the decay of these peroxy radicals in the presence of Cu$^{2+}$ ions, which serve as a model for S.O.D.

EXPERIMENTAL

The Varian 7715 linear accelerator of the Hebrew University was used for pulse radiolysis experiments. Solutions were subjected to 0.1–1.5 μsec pulses of 200 mA current of 5 MeV. The total concentration of radicals was about 20 Spectrosil irradiation cells, 4cm long (for experiments in the presence of Cu$^{2+}$), or 1cm long (for experiments in the presence of S.O.D.), with optical paths of 12.3cm, or 3.1cm respectively, were used. A IP-28 photomultiplier in conjunction with a Hilger-Watts grating monochromator constituted the detection system.
Appropriate light filters were used to the purest available grade, and were used without further purification. S.O.D. was purchased from Sigma (type I). Solutions were saturated by bubbling a mixture of 9.6% O₂ and 90.35% N₂O through the solutions, using the syringe technique.

The concentration of radicals was determined by pulsing an argon-saturated solution of 0.1M methanol at pH 9.5, assuming $\varepsilon = 1.06 \times 10^4 \text{m}^{-1}\text{cm}^{-1}$, and $G(e^-_{aq}) = 2.7 \text{ molecules/100eV}$.

RESULTS

a. Effect of Cu²⁺ on the decay of RO₂ at pH=3

The irradiated solutions contained 0.1M methanol or 0.5M isopropanol, and 1mM HClO₄ (pH=3.0-3.1), and were saturated with N₂O/O₂ mixtures of ∼10/1. The hydrated electrons produced by the pulse, reacted mainly with N₂O, producing OH· radicals within the pulse duration. The OH· and H· radicals reacted with the alcohol molecules, abstracting H atoms. The alcohol radicals thus formed, reacted with O₂ to produce their peroxy radicals: O₂CH₂OH or O₂(CH₃)₂COH. These reactions were completed within 1µsec after the pulse. The decay of RO₂ radicals was followed at 245-260nm.

In solutions containing methanol, the absorption decay in the presence of 4µM - 1mM CuSO₄ was identical, within the experimental error, to the decay in the absence of copper ions. The decay showed some deviations from pure second order, as expected for the simultaneous decay of HO₂ and O₂CH₂OH (4,5).

The observed first half life time was: √0.25 msec.

In solutions containing isopropanol, in the absence of Cu²⁺ ions, the absorption decay was similar to the decay of HO₂ radicals in a blank solution containing formate and no alcohol. In the presence of Cu²⁺ (4-30µM), the decay of the absorption was enhanced and became a pure first order process with a rate constant of 660sec⁻¹. It was independent on the concentration of Cu²⁺.

b. Effect of S.O.D. on the decay of RO₂ at pH=5.7

At this pH we have studied the influence of S.O.D. on the decay of the peroxy radicals of methanol and isopropanol. The concentrations of S.O.D. were 4 - 20µM. Solutions contained 0.1M methanol, or 0.5M isopropanol, 3mM phosphate buffer and S.O.D. enhanced the rate of the absorption decay, as compared to solutions which did not contain S.O.D. Yet, the rate of decay was independent on the concentration of S.O.D.

DISCUSSION

The peroxy radicals produced from methanol and isopropanol decay in a mixture of first and second order reactions (5). The first order decay results in the formation of HO₂ or O₂⁻ radicals (depending on the pH). These peroxy radicals and HO₂ and O₂⁻ radicals, all absorb light at the same region - around
250nm. Therefore, the observed absorption decay is a superposition of the RO₂ decay and HO₂ or O₂⁻ formation and decay.

When Cu²⁺ ions or S.O.D. are present, the absorption of HO₂ or O₂⁻ radicals decays very fast - practically immediately after its formation. Under these conditions, the decay of RO₂ becomes rate determining, and the observed decay reflects the decay of RO₂ absorption only.

The enhancement of the absorption decay in the presence of Cu²⁺ or S.O.D. is caused by their influence on the decay of HO₂ or O₂⁻ radicals, and not by a catalysis of the decay of RO₂ radicals, which would have resulted that the decay rate would be proportional to the concentration of the catalyst. Proportionality of this kind was not found, as the concentration of Cu²⁺ or S.O.D. had no influence on the absorption decay.

The results obtained in our experiments are of two kinds: a) In methanol containing solutions at pH=3, no effect of Cu²⁺ on the absorption decay was observed. b) In methanol containing solutions at pH=5.7, and in isopropanol containing solutions, both at pH=3 and at pH=5.7, the absorption decay was enhanced by the presence of Cu²⁺ or S.O.D., but the concentrations of Cu²⁺ or S.O.D. had no effect on this enhancement. O₂CH₂OH radicals decay both by first and second order processes:

\[
\begin{align*}
(1) & \quad O₂CH₂OH \rightarrow HO₂ + CH₂O \quad k₁ = 500s^{-1} \quad (pH=3) \quad (5) \\
(2) & \quad 2O₂CH₂OH \rightarrow \text{products} \quad 2k₂ = 3 \times 10^8 \text{M}^{-2} \cdot \text{s}^{-1} \quad (pH=3) \quad (5)
\end{align*}
\]

Under our experimental conditions, the radicals concentration was high enough (\(N 2 \times 10^{-5} \text{M}\)), to let the second order decay (reaction (2)) predominated at pH=3. HO₂ is formed only to a negligible extend, and the enhancement of its disappearance by Cu²⁺ had almost no effect on the absorption decay. In this system it is straightforward that Cu²⁺ does not catalyze the decay of O₂CH₂OH radicals.

The rate constants for reactions (1) and (2) at pH=5.7 are not known, (O₂⁻ is formed in reaction (1), instead of HO₂), but their ratio is probably different than that at pH=3, since the absorption decay in the absence of S.O.D. was a mixture of a first and a second order process, and S.O.D. enhanced significantly this decay. The enhancement of the absorption decay by S.O.D. was however independent on the concentration of S.O.D. This indicates that S.O.D. catalyzes the decay of O₂⁻, but not the decay of O₂CH₂OH radicals.

O₂(CH₃)₂COH radicals also decay in two reactions:

\[
\begin{align*}
(3) & \quad O₂(CH₃)₂COH \rightarrow HO₂ + (CH₃)₂CO \quad k₃ = 700s^{-1} \quad (pH=3) \quad (5) \\
(4) & \quad 2O₂(CH₃)COH \rightarrow \text{products} \quad 2k₄ = 1.1 \times 10^7 \text{M}^{-1} \cdot \text{s}^{-1} \quad (pH=3) \quad (5)
\end{align*}
\]

Under our experimental conditions, the first order decay (reaction (3)) predominates at pH=3. In this reaction, HO₂ is formed, which decays later in a second order process. Therefore the absorption decay in the absence of Cu²⁺ ions was a mixture of the decay of the absorption of O₂(CH₃)₂COH...
radicals, and of the formation and decay of the absorption of HO$_2$ radicals.

When Cu$^{2+}$ ions were present, HO$_2$ radicals disappeared immediately, and the rate determining step was the decay of O$_2$(CH$_3$)$_2$COH radicals. Therefore, we could resolve in the presence of Cu$^{2+}$, the kinetics of reaction (3), and obtain its rate constant directly. Our results give $k_3=660$sec$^{-1}$, in agreement with earlier determination ($k_3=700$sec$^{-1}$ (5,6) ). This method, of resolving the kinetics of the decay of RO$_2$ radicals when HO$_2$ are simultaneously present, is also applicable to other systems.

At pH=5.7, S.O.D. also enhanced the absorption decay, but now influence of its concentration on the rate of absorption decay was observed. The decay in the presence of S.O.D. was a mixture of first and second order processes, probably because of a different ratio of $k_3$ and $k_4$ at this pH.

Since an enhancement of the half life time of 20% would have been easily resolved, we can calculate the following upper limits for catalysis of RO$_2$ decay by Cu$^{2+}$ ions and S.O.D.: Cu$^{2+}$ ions at pH=3: $k < 10^6$M$^{-1}$s$^{-1}$ for CH$_2$OH radicals and $k < 3 \times 10^6$M$^{-1}$s$^{-1}$ for (CH$_3$)$_2$COH radicals. S.O.D. at pH=5.7: $k < 2.5 \times 10^8$M$^{-1}$s$^{-1}$ for both radicals.

In conclusion, the results indicate that Cu$^{2+}$ and S.O.D. do not catalyze the decay of peroxy radicals produced from methanol and isopropanol. As S.O.D. apparently does not catalyze the decay of RO$_2$, and as O$_2^-$ is not attacking directly biological compounds, it seems that S.O.D. must have some other biological role than protecting the living cell from O$_2^-$ and RO$_2$. It is possible however, as suggested earlier, that O$_2^-$ is indirectly toxic, as it may form in secondary reactions, toxic OH$^-$ radicals (7).

REFERENCES