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

PROGRESS REPORT

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The Hebrew University of Jerusalem,
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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
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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 alcoholic 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 $\text{OH}^\cdot$ 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. $\text{OH}^\cdot$ 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 $\text{OH}^\cdot$ 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 5MeV. The total concentration of radicals was about 2×10^{-6} M. 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% O_2 and 90.35% N_2O 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 $\epsilon = 1.06 \times 10^4 \text{ m}^{-1} \text{ cm}^{-1}$, and $G(e^-_{aq}) = 2.7 \text{ molecules/100eV}$.

RESULTS

a. Effect of Cu^{2+} on the decay of RO_2 at pH=3

The irradiated solutions contained 0.1M methanol or 0.5M isopropanol, and 1mM $HClO_4$ (pH=3.0-3.1), and were saturated with N_2O/O_2 mixtures of $\sim 10/1$. The hydrated electrons produced by the pulse, reacted mainly with N_2O , producing $OH^\cdot$ radicals within the pulse duration. The $OH^\cdot$ and $H^\cdot$ radicals reacted with the alcohol molecules, abstracting H atoms. The alcohol radicals thus formed, reacted with O_2 to produce their peroxy radicals: O_2CH_2OH or $O_2(CH_3)_2COH$. These reactions were completed within 1 μsec after the pulse. The decay of RO_2 radicals was followed at 245-260nm.

In solutions containing methanol, the absorption decay in the presence of 4 μM - 1mM $CuSO_4$ 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 HO_2 and O_2CH_2OH (4,5).

The observed first half life time was: $\sim 0.25 \text{ msec}$.

In solutions containing isopropanol, in the absence of Cu^{2+} ions, the absorption decay was similar to the decay of HO_2 radicals in a blank solution containing formate and no alcohol. In the presence of Cu^{2+} (4 - 30 μM), the decay of the absorption was enhanced and became a pure first order process with a rate constant of 660 sec^{-1} . It was independent on the concentration of Cu^{2+} .

b. Effect of S.O.D. on the decay of RO_2 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 - 20 μM . 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 HO_2 or O_2^- radicals (depending on the pH). These peroxy radicals and HO_2 and O_2^- radicals, all absorb light at the same region - around

250nm. Therefore, the observed absorption decay is a superposition of the RO_2 decay and HO_2 or O_2^- formation and decay.

When Cu^{2+} ions or S.O.D. are present, the absorption of HO_2 or O_2^- radicals decays very fast - practically immediately after its formation. Under these conditions, the decay of RO_2 becomes rate determining, and the observed decay reflects the decay of RO_2 absorption only.

The enhancement of the absorption decay in the presence of Cu^{2+} or S.O.D. is caused by their influence on the decay of HO_2 or O_2^- radicals, and not by a catalysis of the decay of RO_2 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 Cu^{2+} 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 Cu^{2+} 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 Cu^{2+} or S.O.D., but the concentrations of Cu^{2+} or S.O.D. had no effect on this enhancement. O_2CH_2OH radicals decay both by first and second order processes:

Under our experimental conditions, the radicals concentration was high enough ($\approx 2 \times 10^{-5} M$), to let the second order decay (reaction (2)) predominated at pH=3. HO_2 is formed only to a negligible extent, and the enhancement of its disappearance by Cu^{2+} had almost no effect on the absorption decay. In this system it is straight forward that Cu^{2+} does not catalyze the decay of O_2CH_2OH radicals.

The rate constants for reactions (1) and (2) at pH=5.7 are not known, (O_2^- is formed in reaction (1), instead of HO_2), 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 O_2^- , but not the decay of O_2CH_2OH radicals.

$O_2(CH_3)_2COH$ radicals also decay in two reactions:

Under our experimental conditions, the first order decay (reaction (3)) predominates at pH=3. In this reaction, HO_2 is formed, which decays later in a second order process. Therefore the absorption decay in the absence of Cu^{2+} ions was a mixture of the decay of the absorption of $O_2(CH_3)_2COH$

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 $\text{O}_2(\text{CH}_3)_2\text{COH}$ 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 $k_3 = 660 \text{ sec}^{-1}$, in agreement with earlier determination ($k_3 = 700 \text{ s}^{-1}$ (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 $\text{pH} = 5.7$, 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 $\text{pH} = 3$: $k < 10^6 \text{ M}^{-1} \text{ s}^{-1}$ for CH_2OH radicals and $k < 3 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ for $(\text{CH}_3)_2\text{COH}$ radicals. S.O.D. at $\text{pH} = 5.7$: $k < 2.5 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ 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 $\text{OH}^\cdot$ radicals (7).

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