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REACTIVITY OF CHEMICALLY GENERATED SUPEROXIDE RADICAL ANION WITH PEROXIDES AS DETERMINED BY COMPETITION KINETICS

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ABSTRACT

A steady-state competition system has been developed to investigate the reactions of the superoxide radical anion ($O_2^{\cdot-}$) with various peroxides, including the so-called Haber-Weiss reaction. Potassium superoxide dissolved in an oxygen-free solution of DMSO containing 18-dicyclohexyl-6-crown, is the source of $O_2^{\cdot-}$. High pressure liquid chromatography is used as an assay system for $O_2^{\cdot-}$ reactivity, to detect and quantitate the yield of anthracene, formed as a major product in the reaction between $O_2^{\cdot-}$ and 9,10-dihydroanthrancene. Decrease in anthracene yields, in the presence of peroxide, may be used to indicate a possible competing reaction between $O_2^{\cdot-}$ and added peroxide. Complications involving peroxide-stimulated formation of anthraquinone derivatives are discussed. No evidence for a competing reaction between $O_2^{\cdot-}$ and peroxide can be detected up to a 10-fold excess of peroxide over 9,10-dihydroanthracene.

INTRODUCTION

The Haber-Weiss reaction (1)

$$O_2^{\cdot-} + H_2O_2 \rightarrow {}^{\cdot}OH + OH^- + O_2 \qquad (1)$$

has been suggested as the source of $^{\cdot}OH$ radicals in toxic processes in the cell (2,3), but there is no present evidence of a direct interaction of $O_2^{\cdot-}$ and peroxides, in the absence of metal ions or other catalysts (4,5).

We have developed a steady-state chemical competition system to investigate this interaction. It is based on the reaction of $O_2^{\cdot-}$ (KO_2) with 9,10-dihydroanthracene in oxygen-free DMSO (6). The major product of this reaction is anthracene, with minor products consisting of anthrone, anthra-

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quinone and bianthronyl. The reaction was studied using high performance liquid chromatography. The inhibition of anthracene formation was used as a measure of the competitive reaction of peroxides with $O_2^{\cdot-}$.

Peroxides were found not to inhibit anthracene formation but did increase the production of secondary products anthrone, anthraquinone and bianthronyl. The concentration of these quinone derivatives increased with increasing peroxide concentration. There was no direct evidence of an $O_2^{\cdot-}$-ROOH interaction in this system.

MATERIALS AND METHODS

Benzoyl peroxide, anthracene, 9,10-dihydroanthracene, anthrone, anthraquinone and bianthronyl were obtained from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin. Spectro-analyzed grade dimethyl sulfoxide and 30% hydrogen peroxide were obtained from Fisher Scientific, New Jersey. The dimethyl sulfoxide was stored over a molecular sieve (Linde 5A). Potassium superoxide was obtained from Alfa Inorganics, Beverly, MA. Ascaridole was obtained from Pfaltz and Bauer, Inc., Stamford, CN. Ethyl hydroperoxide and acetyl peroxide were obtained from Poly Science Inc., Warrington, PA. Tert-butyl peroxide was obtained from Koch-Light Laboratories Limited, Colnbrook, England. Purified 18-dicyclohexyl-6-crown was gift from Dr. Paterson, Dupont Laboratories.

A sample of potassium superoxide was ground in a mortar and pestle under dry conditions, weighed and added to a degassed solution of 18-dicyclohexyl-6-crown in DMSO. The solution was stirred under N_2 until used. This stock was 20 mM KO_2 and 20 mM 18-dicyclohexyl-6-crown and was not used after 1-5 hours, because of the instability of KO_2 under these conditions.

The reaction of 3×10^{-3}M 9,10-dihydroanthracene and 8×10^{-3} M KO_2 was studied under N_2. The time from injection of degassed KO_2-crown stock into 9,10-dihydroanthracene solution to injection on the column was three minutes. The peroxides of varying concentrations were added just prior to injection of KO_2-crown stock.

The reaction was carried out in the absence of light and the solutions were transferred directly from the reaction chamber to the column injection port in a 1 mL syringe, to prevent oxygen contamination. 9,10-dihydroanthracene, anthracene, anthrone, anthraquinone and bianthronyl were analysed directly by chromatography on a Partisil 10 ODS column (Whatman, Inc. Clifton, NJ) which was maintained at 70°C. The eluent was 70% aqueous acetonitrile at a pressure of 1500 psi. The detector used was a Chromatronix model 230, dual channel absorbance detector set at 280 nm.

Figure 1 shows a liquid chromatogram of reactants and products of the reaction. Peak B is a mixture of anthrone and anthraquinone, as attempts to separate these compounds were unsuccessful.

RESULTS

Peroxides were first tested for their reactivity with 9,10-dihydroanthracene. Controls were run to test if any peroxides caused interference in

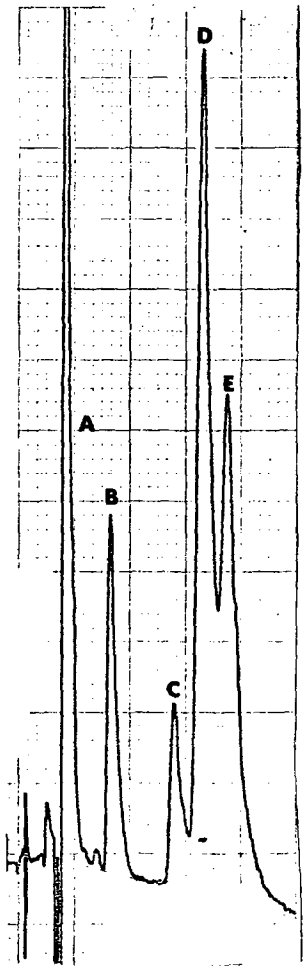


FIGURE 1. Chromatogram of the reactants and possible reaction products. Peak A, DMSO; Peak B, anthrone and anthraquinone, Peak C, 9,10-dihydroanthracene; Peak D, anthracene, and Peak E, bianthronyl.

the analysis of the reaction products and reactants. Peroxides in concentrations up to 2.5 x 10^{-2}M were added to 3 x 10^{-3}M 9,10-dihydroanthracene under N_2 and reacted for 3 minutes. None of the peroxides and hydroperoxides tested had any detectable effect on the 9,10-dihydroanthracene control. Ethyl hydroperoxide, tert-butyl hydroperoxide, ascaridole and hydrogen peroxide, unlike the other peroxides, ran at the solvent front, which proved to be convenient for chromatographic analysis.

Ascaridole, ethyl hydroperoxide and tert-butyl hydroperoxide were reacted in the 9,10-dihydroanthracene-$O_2^{\cdot-}$ system over a concentration range

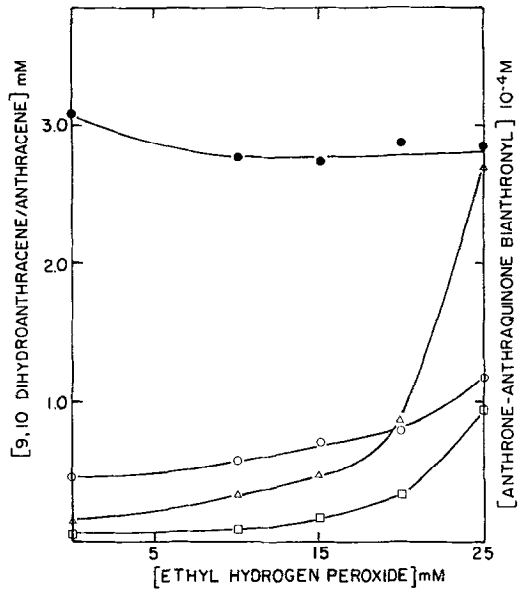


FIGURE 2. Variation in the yields of 9,10-dihydroanthracene (●), anthracene (O), anthrone/anthraquinone (Δ) and bianthronyl (□) in DMSO solutions of KO_2, as a function of the ethyl hydroperoxide concentration.

1.0×10^{-2} M to 2.5×10^{-2}M. Ascaridole was found to have little effect on either anthracene inhibition or quinone derivative production. Figure 2 shows the result for ethyl hydroperoxide in the presence of 9,10-dihydroanthracene and $O_2^{\cdot-}$. Anthracene production was not inhibited and 9,10-dihydroanthracene loss did not substantially change. As the peroxide concentration increased the production of anthrone, anthraquinone and bianthronyl increased. It was also observed that at longer reaction time, the concentration of bianthronyl increased. This would indicate that anthrone is formed first and then converted to bianthronyl.

The results for tert-butyl hydroperoxide (Figure 3) were similar to those for ethyl hydroperoxide. The 9,10-dihydroanthracene loss and anthracene formation were not greatly affected.

Hydrogen peroxide was found to be more reactive than the other peroxides, producing similar results to those for t-butyl hydroperoxide and ethyl hydroperoxide, but at an order of magnitude lower concentration range.

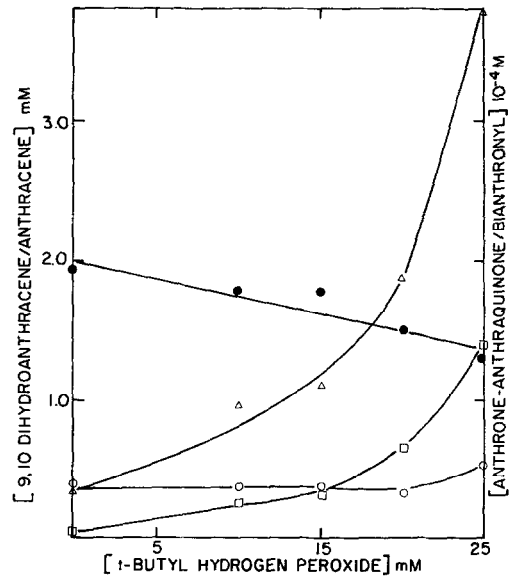


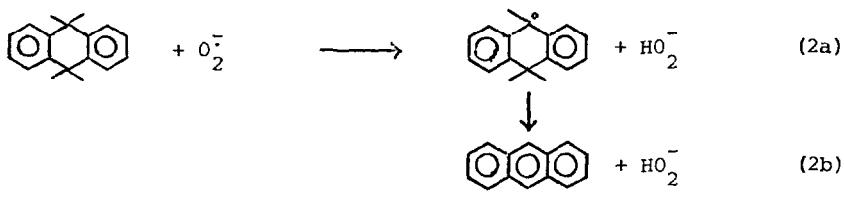
FIGURE 3. Variation in the yields of 9,10-dihydroanthracene (●), anthracene (○), anthrone/anthraquinone (Δ) and bianthronyl (□) in DMSO solutions of KO_2 as a function of t-butyl hydroperoxide concentration.

In all cases, in the absence of oxygen, there is a general increase in concentration of quinonoid products with increasing peroxide concentration.

DISCUSSION

In our system, we did not find evidence of a direct interaction between $O_2^{\cdot-}$ and peroxides to inhibit anthracene formation from 9,10-dihydroanthracene (reactions 2 and 3). However, in the presence of $O_2^{\cdot-}$ (and only in the presence of $O_2^{\cdot-}$) the peroxides did produce quinone derivatives.

A mechanism for anthracene formation has been proposed by Tezuka (7).



$$ROOH + O_2^{\cdot-} \longrightarrow \text{Products} \qquad (3)$$

Small amounts of anthraquinones are formed by $O_2^{\cdot-}$ in the absence of peroxides which might be accounted for by ^{-}OOH formation. This would be consistent with the greatly increased yield of quinones when peroxides were added into the $O_2^{\cdot-}$-9,10-dihydroanthracene system.

The possible cytotoxicity of $O_2^{\cdot-}$ towards living cells may arise from two different chemical damaging mechanisms. It can react directly with substrates having weak or labile bonds (6) forming primary products which are toxic. Alternatively, it may react indirectly with molecules other than target molecules, such as with peroxides formed by irradiation or autoxidation or in normal or aberrant cellular metabolism. These secondary reactions of $O_2^{\cdot-}$, capable of producing damage at a distance, may be analogous to that producing quinones from 9,10-dihydroanthracene in the presence of peroxides. This simple chemical model may be useful in the study of other secondary reactions of $O_2^{\cdot-}$, possibly involved in chain reactions, such as lipid peroxidation, post-irradiation effects, or damage at a distance.

The conversion of 9,10-dihydroanthracene to anthracene by KO_2/crown appears to be solvent dependent. Anthracene is formed rapidly in DMSO, DMF and propyl sulfoxide, but is not detectable over short reaction times (up to 1 h) in benzene, acetonitrile or tetrahydrofuran. Satisfactory solvents to provide stable KO_2 solutions for the chemical production and reaction of $O_2^{\cdot-}$ include DMSO, DMF, DME and diethyl ether (8).

In addition to the solvent effect, we have found that the addition of potassium hydroxide under conditions of air saturation produced similar results to the addition of KO_2-crown. The presence of O_2 is essential for this reaction. A similar observation has been reported (9), for luminol chemiluminescence. A chemiluminescent reaction was observed with luminol in DMSO in the presence of O_2 and base (10) or t-butoxide which may be attributed to $O_2^{\cdot-}$. This hypothesis is supported by the finding that the chemiluminescence of luminol is inhibited by superoxide dismutase (11). We

have confirmed that the addition of KO_2 as a source of $O_2^{\cdot-}$, to a solution of luminol, produced chemiluminescence.

Further studies are being undertaken to further investigate the possibility that the reaction of O_2 and OH^- in DMSO does produce $O_2^{\cdot-}$, thereby accounting for the oxidation of 9,10-dihydroanthracene to anthracene.

The chemistry of superoxide is complex. The products of both primary and secondary reactions may be responsible for $O_2^{\cdot-}$ cytotoxicity in the cell. Peroxides do not seem to react directly with $O_2^{\cdot-}$ as in the Haber-Weiss reaction. A secondary reaction involving $O_2^{\cdot-}$ and ROOH or some reactive intermediate, and leading to quinone formation from DHA, could be important in understanding the chemical reactivity and possible toxicity of $O_2^{\cdot-}$ formed in irradiated or metabolizing biological systems.

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