

SINGLET OXYGEN PRODUCTION FROM THE REACTIONS OF SUPEROXIDE ION IN APROTIC SOLVENTS: IMPLICATIONS FOR HYDROPHOBIC BIOCHEMISTRY

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A number of reactions of superoxide ion in aprotic solvents have been reported to produce singlet oxygen. There is strong evidence for singlet oxygen generation from the reactions of superoxide ion with chlorine-containing halocarbons, bromine-containing halocarbons, hexafluorobenzene, diacylperoxides, lead tetraacetate, iodobenzene diacetate, cerium (IV) cation and tetranitromethane. Earlier studies reporting singlet oxygen generation from the reactions of superoxide ion with iodine, ferricenium ion, thianthrene cation radical, tris (N,N'-dioxobipyridyl) manganese (III) cation and di- μ -oxo-bis[phenanthroline] manganese (IV) cation need to be repeated because of the methods used to detect singlet oxygen had low specificity.

The results of studies of superoxide ion chemistry in aprotic solvents should not be uncritically extrapolated to hydrophobic biological microenvironments, such as the cell membrane.

KEY WORDS: Singlet oxygen, superoxide ion, aprotic solvent, halocarbons, diacylperoxides, cell membrane.

INTRODUCTION

Singlet oxygen ($^1\Delta_g$) is a highly reactive species with a lifetime of about 1 μ s in the biological environment. It oxidizes a wide variety of biological molecules, inactivates plasmid DNA and kills bacteria.¹⁻³ While superoxide ion is a well-established biochemical intermediate, the existence of significant biochemical routes to singlet oxygen remains controversial.⁴ Within the last decade, a number of reactions of superoxide in aprotic solvents have been reported to generate singlet oxygen.⁵⁻¹³ This chemistry is of potential biological interest because the reactions of superoxide ion in aprotic solvents may be useful models for the reactions of superoxide ion in certain hydrophobic biological microenvironments, such as the cell membrane.¹⁴ The chemistry of superoxide ion in hydrophobic biological microenvironments is likely to be very different from its chemistry in hydrophilic regions, since the reactivity of superoxide ion is much greater in aprotic solvents than in aqueous solution.¹⁴⁻¹⁷

This paper will discuss (1) the difficulties of making quantitative measurements of singlet oxygen production in systems containing large concentrations of superoxide ion, (2) the various superoxide ion reactions reported to produce singlet oxygen and

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(3) the limitations of extrapolating results from aprotic solvents to hydrophobic biological microenvironments.

RESULTS AND DISCUSSION

Quantitative Measurements of Singlet Oxygen Production in Systems Containing Large Concentrations of Superoxide Ion

Measurements of 1270 nm chemiluminescence have proven to be a reliable method for quantitatively determining singlet oxygen production in complex systems.⁴ Since the intensity of the 1270 nm emission is proportional to the product of the rate of production of singlet oxygen and the singlet oxygen lifetime, a knowledge of the singlet oxygen lifetime is required for quantitative measurements of singlet oxygen production. In many systems, the singlet oxygen lifetime is simply the singlet oxygen lifetime of the solvent. Superoxide ion is a powerful singlet oxygen quencher, however, with $k = 1.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$.¹⁸ When significant concentrations of superoxide ion are present, the singlet oxygen lifetime will be given by the Stern-Volmer equation.

$$\frac{1}{\tau} = \frac{1}{\tau_s} + k[\text{O}_2^-] \quad (1)$$

where τ is the singlet oxygen lifetime, τ_s is the singlet oxygen lifetime in the pure solvent, $[\text{O}_2^-]$ is the superoxide ion concentration and k is the superoxide ion quenching constant. In experiments where superoxide ion is present in large excess, the singlet oxygen lifetime will be approximately constant throughout the reaction and quantitative measurements of singlet oxygen can be made using the calculated singlet oxygen lifetime. A major problem is the low intensity of the singlet oxygen emission, however. A 1 mM concentration of superoxide ion will reduce the singlet oxygen lifetime in acetonitrile from 60 μs to 0.6 μs and consequently reduce the intensity of the singlet oxygen emission by a factor of 100.

Systems in which superoxide ion is the limiting reagent present a more complicated problem because the singlet oxygen lifetime will greatly increase during the course of the reaction. We can calculate a "weighted" singlet oxygen lifetime to use for quantitative measurements of singlet oxygen production, if we assume that (1) the stoichiometry for singlet oxygen generation is constant throughout the reaction and (2) that no significant concentration of intermediates are generated (i.e., superoxide ion is not consumed before the singlet oxygen is generated).

$$\tau_w = \frac{1}{C_0} \int_0^0 \frac{-d[\text{O}_2^-]}{\frac{1}{\tau_s} + k[\text{O}_2^-]} \quad (2)$$

where τ_w is the "weighted" singlet oxygen lifetime, τ_s is the solvent singlet oxygen lifetime, k is the singlet oxygen quenching constant for superoxide ion and C_0 is the initial superoxide ion concentration. Evaluation of Equation (2) gives

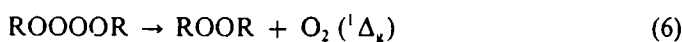
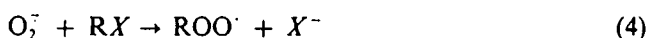
$$\tau_w = [\ln(1 + kC_0\tau_s)]/C_0k \quad (3)$$

Unfortunately, the assumptions needed to derive Equation (3) are not valid for many complex systems.

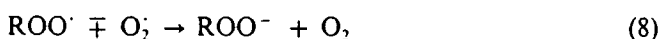
Problems similar to those described above for the measurement of singlet oxygen production using 1270 nm chemiluminescence must also be addressed in order to use chemical traps for the quantitative measurement of singlet oxygen production in systems containing superoxide ion.

Halocarbons

Superoxide ion reacts with a number of bromine-containing and chlorine-containing halocarbons to produce singlet oxygen.^{10,12,19} Strong evidence for singlet oxygen formation has been obtained from chemical trapping studies and from the detection of the characteristic singlet oxygen emission at 1270 nm.^{10,12,19} Figure 1 shows the near-infrared singlet oxygen emission from the reactions of superoxide ion with several representative halocarbons. The mechanism for singlet oxygen generation is shown by Equations (4–7).



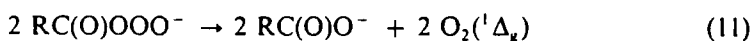
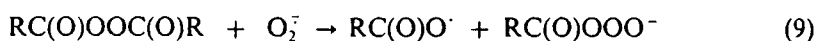
Nucleophilic substitution by superoxide ion gives rise to a peroxy radical.^{16,19} Production of peroxy radicals has been confirmed by ESR spectroscopy.²⁰ Two peroxy radicals can combine to form a tetraoxo intermediate, which can decompose via Reaction 6 to give singlet oxygen.²¹ If α hydrogens are present, the tetraoxo intermediate may assume a cyclic conformation and decompose via Reaction 7.^{22,23} This decomposition route, called the Russell mechanism, yields electronically excited products, either singlet oxygen or an excited carbonyl compound. Excess halocarbon favors the formation of singlet oxygen, since Reaction 5 must compete with reduction of the peroxy radical by a second superoxide ion.



The reaction of superoxide ion with hexafluorobenzene also generates singlet oxygen by an analogous mechanism.¹³

Diacyl Peroxides

Evidence from both chemical trapping and 1270 nm chemiluminescence support the formation of singlet oxygen from the reaction of superoxide ion with diacyl peroxides.^{6,10} Equations (9) through (11) provide a plausible mechanism that is consistent with both the reaction stoichiometry and the reaction products.



The species, RC(O)OOO^- can be viewed as an analog of the chloroperoxy anion, ClOO^- , that is believed to be the precursor of singlet oxygen in the well studied hydrogen peroxide-hypochlorous acid reaction. An alternative mechanism to account

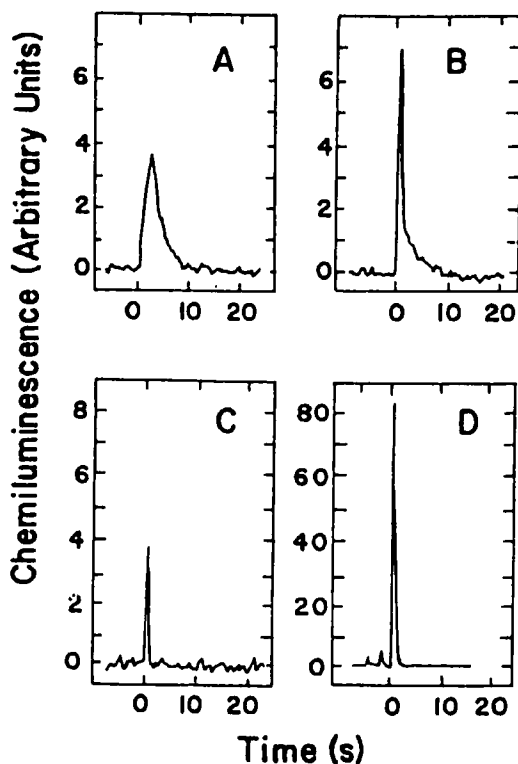
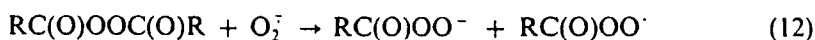


FIGURE 1 Time course for the 1268 nm emission from the reactions of O_2^- with halocarbons in acetonitrile (A) 0.4 mM O_2^- , 1.3 mM CCl_4 ; (B) 0.4 mM O_2^- , 35 mM α,α,α -trichlorotoluene; (C) 1 mM O_2^- , 350 mM 1-bromobutane; (D) 3 mM O_2^- , 3.8 mM CBr_4 . (Reprinted from J.R. Kanofsky, H. Sugimoto, D.T. Sawyer (1988) *Journal of the American Chemical Society*, 110, 3698–3699, with permission.)

for the singlet oxygen generation involves nucleophilic attack by superoxide ion on the carbonyl carbon to yield a peracid radical and a peracid ion.



Two peracid radicals could then react to form singlet oxygen via a tetraoxo intermediate. In the presence of trace amounts of hydrogen ion, the peracid ion can also generate singlet oxygen.²⁴

Oxidants

Corey *et al.* have detected 1270 nm singlet oxygen emission from the reactions of superoxide ion with four potent oxidants (cerium (IV) ammonium nitrate, lead tetraacetate, iodobenzene diacetate and tetranitromethane).¹¹ The singlet oxygen presumably results from the decomposition of a complex formed between superoxide ion and the oxidant. It is important to recognize, however, that singlet oxygen is also generated by the reaction hydrogen peroxide anion with a number of oxidants.²⁵ Thus, hydrogen peroxide formation catalyzed by trace amounts of water is an alternative mechanism for the singlet oxygen formation detected by Corey *et al.*

Several other oxidants (ferricenium ion,^{5,9} iodine,⁷ the thianthrene cation radical,⁸ *tris*(N,N'-dioxypyridyl) manganese (III) cation,⁹ di- μ -oxo-bis-[phenanthrolinato manganese (IV)] cation⁹) have been reported to react with superoxide ion to produce singlet oxygen. The methods used to identify singlet oxygen in these studies (e.g., furan traps, measurement of visible chemiluminescence) are now felt to have relatively low specificity for singlet oxygen.⁴ In view of this, these reactions should be studied with more specific methods (e.g., 1270 nm chemiluminescence, cholesterol trapping) before any conclusions about singlet oxygen can be made.

Implications for Hydrophobic biochemistry

Extrapolation of data from simple chemical systems to complex biological systems always has hazards. It is not clear that substantial concentrations of superoxide ion exist in hydrophobic biological environments. Even within the hydrophobic membrane core, allylic hydrogen atoms may be sufficiently acidic to convert most superoxide ions into perhydroxyl radicals. Further, many of the singlet-oxygen-generating reactions discussed in the prior sections of this paper have no biochemical analogs. This includes many of the reactions of superoxide ion with powerful oxidants such as tetranitromethane.

The reaction of superoxide ion with halocarbons may be biologically significant. Carbon tetrachloride as well as several other halocarbons are potent liver toxins. The reaction of superoxide ion with these compounds may well constitute one mechanism for the production of highly reactive and highly toxic intermediates. Unfortunately, it is necessary for most investigators to use millimolar concentrations of reactants to study these reactions. Using high reactant concentrations and an excess of halocarbon, singlet oxygen and perhaps the tetraoxo intermediate are the principal reactive species generated.¹⁹ Since both of these species have a quadratic dependence on the reactant concentrations, at the submicromolar concentrations that may be present biologically, peroxy radicals are likely to be the most important reactive species.

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