The Organic Chemistry of Superoxide

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1 Introduction

The importance of oxygen in sustaining life forms directly or indirectly is unquestionable and has been the subject of intensive studies by biologists and chemists over the past two centuries. Although reactions of oxygen with organic compounds are in general highly exothermic, such reactions are usually slow at physiological temperatures. The fact that the electronic spins of oxygen (triplet) and most stable organic substrates (singlet) are incongruent is probably the most important reason why oxygen does not behave as a random oxidizing agent capable of destroying complex organic material.

A number of mechanisms and intermediates for the interaction of molecular oxygen with specific biological substrates have been suggested.[1] These include singlet oxygen, metal–oxygen complexes, and various reduced states of molecular oxygen either free or bound to an enzyme. Recent evidence indicates that the superoxide anion is present in all aerobic organisms and is believed to be involved in several enzyme oxidation processes.[2] However, its presence does not necessarily imply that it is the reactive form, and it may be either the metabolite present in the terminal electron transfer to molecular oxygen during the oxidation–reduction process or a precursor to other reactive species directly involved in oxygen addition. The involvement of O_2^- in biological oxidations can be investigated by studying its reactions with simple substrates with functionalities similar to those encountered in the more complex living system. Until recently the chemistry of superoxide has been limited to physicochemical studies, detection in enzymes and related systems, and upper-atmosphere studies in the gas phase. With the discovery of new superoxide reagents and the preparation of stable solutions of O_2^- in aprotic organic solvents, the chemistry of superoxide can be extended to include a wide range of simple organic substrates which may serve as models for the biological counterparts. This review summarizes these recent chemical investigations and relates the findings to certain biological oxidation mechanisms.

2 Occurrence and Preparation of Superoxide

Superoxide can result from reduction of molecular oxygen [equation (1)] and from oxidation of peroxide [equation (2)]. The reduction potential of the O_2/O_2^-

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[1] G. A. Hamilton, in 'Chemical Models and Mechanisms for Oxygenases in Molecular Mechanisms of Oxygen Activation', ed. O. Hayaishi, Academic Press, New York, 1975, p. 405.

[2] I. Fridovich, *Adv. Enzymol.*, 1974, **41**, 35.

$$O_2 + e \rightarrow O_2^- \tag{1}$$

$$O_2^{2-} \rightarrow O_2^- + e \tag{2}$$

couple is highly dependent on the nature of the medium and ranges from -0.2 to -0.5 V [*vs.* normal hydrogen electrode (n.h.e.)].[3–5] The electron affinity of oxygen, determined as $+0.42$ to $+0.44$ eV,[6,7] indicates that one-electron reduction of oxygen is an exothermic process in the gas phase. The reduction potential of the O_2^-/O_2^{2-} couple and electron affinity of O_2^- are -1.8 and $+1$ V, respectively;[4,8] however, attempted electrolytic production of O_2^- from peroxide yields molecular oxygen.[9] The generation of O_2^- from peroxide can be accomplished by non-electrolytic oxidative methods (see below).

A number of electroreducing methods for the generation of O_2^- in both aprotic solvents and water have been described.[3–5,9,10] Many of these procedures call for the use of tetra-alkylammonium perchlorate as the supporting electrolyte; in an atmosphere of oxygen, tetra-alkylammonium superoxides are formed in appreciable concentrations. Other methods for generating O_2^- in aqueous solutions include reduction of oxygenated solutions by hydrated electrons or by hydrogen atoms generated during photolysis in the far ultraviolet region,[11–14] and radiolysis[10,15] or ultrasonication of water.[16] The short lifetime of O_2^- (k_2 decay $= 10^7$ l mol^{-1} s^{-1}) in aqueous solutions preclude studies of substrates oxidized relatively slowly by O_2^-.

In principle, any substance with an oxidation potential greater than $+0.5$ V (n.h.e.) should be capable of reducing molecular oxygen to O_2^-. Thus a number of alkali metals and transition-metal ions react in this way. The combustion of potassium to yield 'oxides of potassium' was described by Gay Lussac in 1811, and is presently used in the commercial preparation of KO_2.[17,18] Both sodium and potassium superoxides can be prepared by direct oxygenation of the metals in diglyme with catalytic quantities of xanthone.[19] In the case of lithium, only peroxide is obtained under these conditions. Superoxides have also been found

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on a number of transition-metal surfaces, *e.g.* Pt, Au, Co, Rh, W, and Mo, when these are exposed to oxygen.[20] Silver dispersed on silica gel is oxidized by oxygen to AgO_2.[21] A number of superoxo-complexes of transition metals have been prepared from direct oxygenation of lower oxidation state metal complexes such as cobalt(II),[22,34] iron(II),[23] iridium,[24] rhodium(II),[25] and chromium(II) although the superoxide nature in the iridium and rhodium complexes is questionable.[26] In many of these systems superoxide is present as a tightly bound ligand with little dissociation to free superoxide. These systems have been studied as possible models for biological oxygen carriers, oxygenases and oxidases. Some μ-superoxobiscobalt(III) complexes have been prepared by oxidation of the corresponding μ-peroxobiscobalt 2:1 adducts.[27,28] The superoxide nature of dioxygen in these complexes has been inferred from Raman,[29] u.v.-visible,[30] and electron spin resonance (e.s.r.) spectroscopy,[31] X-ray crystallographic analysis,[32] and molecular orbital calculations of the O—O bond distance.[33] For example the O—O bond distance in μ-superoxobis(penta-aminocobalt) was determined as 1.31 Å.[32,33] This compares with the O—O bond distance of 1.20 Å for molecular oxygen, 1.28 Å for alkali-metal superoxides, and 1.35—1.45 Å for peroxides.[34] E.s.r. studies have shown that ferrocene forms an Fe^{III} superoxide on porous Vycor glass rods.[35]

The ability to reduce oxygen is not restricted to metals and metallic ions. Certain organic anions, radicals, and neutral species have been found to undergo efficient one-electron transfer to O_2. These include flavins (mono- and di-nucleotides),[36,37] reduced dyes such as methylene blue,[38] fluorescein,[39] saponified chlorophyll,[40] and various aromatics (tryptophan,[39] perylene,[41] and catechols[42]

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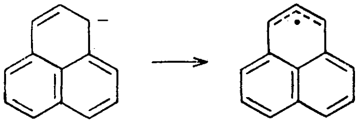
and their semiquinones[43]). In some cases[36,39,40] the reduction of O_2 is promoted by irradiation with u.v. or visible light. In an earlier report[44] on the oxidation of perinaphthenide (1) to perinaphthyl radical (2) by molecular oxygen, the reduced product was not identified but it was proposed to be peroxide. It is likely that this oxidation, as well as other autoxidations of carbanions with oxygen, proceeds with concomitant production of O_2^-.


(1) (2)

Formation of O_2^- from oxidation by peroxide is less common although a number of such preparations have been reported. Hydrogen peroxide can be oxidized by ceric(IV)[45] and periodate[46] ions. Certain metal peroxides can be oxidized by ozone to a mixture of superoxides and ozonides [equation (3)] at low temperatures.[47]

$$M(O_2^{2-})_n + O_3 \rightarrow M(O_2^-)_n + M(O_3^-) \quad M = \text{Li, Mg, Ba, or Sr} \qquad (3)$$

A number of superoxides have been prepared by ligand substitution with O_2^-. Thus rare earth superoxides can be synthesized from their perchlorate salts by direct substitution with sodium superoxide [equation (4)].[48]

$$NaO_2 + M(ClO_4)_n \xrightarrow{NH_3} M(O_2^-)_n \qquad (4)$$

Tetramethylammonium superoxide can be conveniently made by heterogenous reaction of either its fluoride or hydroxide salt with potassium superoxide and subsequent extraction with liquid ammonia.[49] Other ammonium or tetra-alkylammonium superoxides can be prepared by electrolysis in an aprotic medium with a suitable ammonium salt as the supporting electrolyte.[3–5,9,10] The greater solubility of tetra-alkylammonium superoxides in organic solvents make these more suitable reagents than the metal salts in the study of the organic chemistry of superoxide.

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[47] (*a*) I. I. Volnov, S. A. Tokareva, V. N. Belevskii, E. I. Latysheva, V. I. Klimanov, and G. P. Pilipenko, *Neorg. Perekisyne Soedinenii, Doklady Vses. Soveshch.*, 1973, 110; (*b*) S. A. Tokareva, G. P. Pilipenko, V. I. Klimanov, and I. I. Volnov, *Tezisy Doklady Vses. Soveshch. Khim. Neorg. Perekisnye Soedinenii*, 1973, 14.

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Both iron(III) and cobalt(III) superoxo-complexes have been obtained by nucleophilic displacement by O_2^-. Aquocobalamin reacts with O_2^- to give superoxocobalamin,[50] and perchlorate ion is displaced from iron protoporphyrin dimethyl ester perchlorate.[51]

A number of oxyanions can expel O_2^- under different conditions. γ-Irradiation of chromate ion in alkaline solution,[52] *X*-radiation of solid potassium chlorate,[53] and decomposition of potassium peroxochromate[54] have been reported to generate superoxide.

3 Detection of Superoxide

A number of physical methods have been developed for the detection of O_2^-, including conductimetry,[55] and u.v., i.r., Raman, and e.s.r. spectroscopy. Transient O_2^- in aqueous solution exhibits a λ_{max} at 240 nm ($\epsilon = 1060$),[56] although an absorption at 443 nm has been reported in pyridine[57] and attributed to a pyridine superoxide complex. The i.r. spectrum of O_2^- shows no characteristic sharp bands[58] as would be expected for homopolar molecules. On the other hand, the Raman spectrum of KO_2 shows a $\Delta\nu$ at 1145 cm^{-1} attributed to the O—O stretch,[58] which is in close agreement with results obtained from electron scattering experiments with oxygen ($\Delta\nu = 1089$ cm^{-1}).[59] The i.r. absorption at 1100 cm^{-1} of potassium superoxopentacyanocobaltate(III) and μ-superoxopentaminocobalt(III) has been assigned to the O—O stretch of superoxide rather than that of peroxide, which would exhibit an absorption at 800 cm^{-1}.[60]

The free radical nature of O_2^- permits its detection by e.s.r. spectroscopy. Pyridine solutions of O_2^- gives an e.s.r. signal at $g = 2.020$,[57] just slightly above the free electron values. This compares with values of $g_{\parallel} = 2.175$ and $g_{\perp} = 2.002$ for solid KO_2 and NaO_2. E.s.r. detection of O_2^- in enzyme systems indicates somewhat lower g values ($g_{\parallel} = 2.08$ and $g_{\perp} = 2.00$);[61] however, further evidence that the paramagnetic species was O_2^- was obtained by the use of ^{17}O-enriched oxygen and observation of the resultant hyperfine splitting.[62] Under conditions where O_2^- cannot be detected by direct e.s.r. measurements a method of spin trapping has been developed.[63] 5,5-Dimethyl-1-pyrroline-1-oxide (3) is used to trap O_2^- and produce the stable paramagnetic *N*-oxide radical (4).

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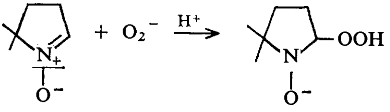


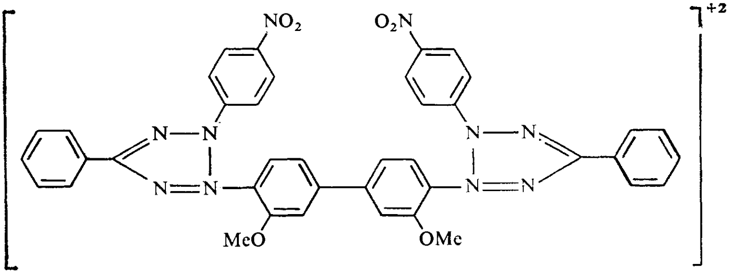
(3) (4)

Chemical tests for O_2^- rely on its oxidative and reducing properties. A widely used method [equation (5)] is the reduction of tetranitromethane to nitroform anion, which absorbs in the u.v. at 350 nm (ϵ = 15 000).[64] Although the test is

$$O_2^- + C(NO_2)_4 \rightarrow C(NO_2)_3^- + NO_2 + O_2 \qquad (5)$$

efficient with bimolecular rate constants approaching diffusion control values, the reduction is not specific to O_2^- and other reducing species such as hydrogen atom, hydroxyl, and solvated electrons also reduce tetranitromethane.

Another commonly used assay for O_2^- is reduction of nitrotetrazolium blue (5) to its diformazan (6) (λ_{max} = 560, $\epsilon = 30 \times 10^3$), which can be monitored



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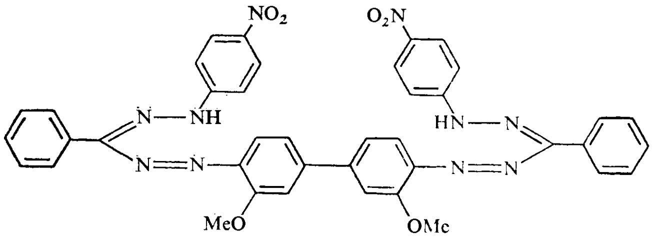

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by visible spectroscopy.[65] However, it is conceivable that in this case other reducing radicals may also give a positive test.

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[64] B. H. J. Bielski and A. O. Allen, *J. Phys. Chem.*, 1967, **71**, 4544.

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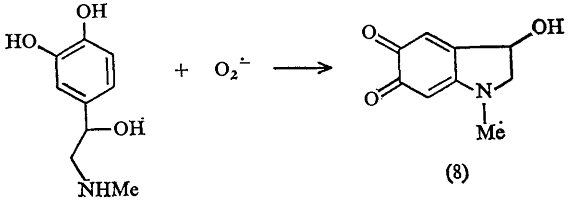
A less efficient method for O_2^- detection involves reduction of ferricytochrome derivatives to ferrocytochromes, which can be monitored by visible spectroscopy (λ_{max} = 550 nm).[66–68] Again, this method is not specific for O_2^- and furthermore, the bimolecular rate constant for ferrocytochrome reduction is only 10^6 l mol^{-1} s^{-1}.[67]

Epinephrine (adrenalin) (7) is oxidized to adrenochrome (8) by O_2^-, and



(7) (8)

adrenochrome production (λ = 480 nm, ϵ = 4020) can be monitored as a method of assay.[68] Lengfelder and co-workers have found that this oxidation involves several reactive intermediates, not all of which lead to adrenochrome[69] and also that adrenochrome, once formed, can be reduced to a semiquinone. Caution, therefore, has to be exercised in interpreting results of this test on a quantitative basis.

Another oxidative method for detection of O_2^- involves observation of chemiluminescence from luminol oxidation with O_2^-.[70] However, the background luminescence must be evaluated concurrently, since the same phenomenon with ground-state triplet oxygen has been observed in alkaline solutions of luminol.[71]

Seyb and Klemberg[72] have described a volumetric analysis of oxygen evolution upon quenching of alkali-metal superoxides with acetic acid [equation (6)].

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \qquad (6)$$

In water this dismutation is quite efficient ($k \simeq 10^7$ l mol^{-1} s^{-1}) and follows second-order kinetics in O_2^-.[73] It is catalysed ($k > 10^9$ l mol^{-1} s^{-1}) by a number of specific metalloenzymes, called superoxide dismutases,[2] which can be used in combination with any of the above-mentioned chemical tests to provide unambiguous evidence for the presence of O_2^-. However, there has been some recent controversy over the specificity of these enzymes which is discussed in Section 5.

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4 Reactivity of Superoxide

A. O_2^- as a Reducing Agent.—The relatively low reduction potential of oxygen is consistent with the ease with which O_2^- can act as a reducing agent, and reactions involving electron transfer from O_2^- to metallic ions in their higher oxidation states as well as organic substrates have been mentioned as assays for O_2^-. The reduction of cytochrome *c* (Fe^{III}) by O_2^- deserves some comment since the cytochromes are found in cells of all aerobic organisms and play an important role in the electron-transport chain and oxidase activity. The reaction is described by equation (7), which appears to be the reverse of the reaction for electron

$$\text{cyt } c\,(Fe^{3+}) + O_2^- \rightarrow \text{cyt } c\,(Fe^{2+}) + O_2 \tag{7}$$

transport from ferrocytochrome *c* to molecular oxygen and its conversion into water and oxidized organic substrate. The oxidation potential for the Fe^{2+}/Fe^{3+} couple is reported to be -0.771 V.[74] If the reduction potential for the O_2/O_2^- couple is taken as -0.29 V, E_0 for reaction (8) would be $+1.06$ V. Rayo and

$$Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2 \tag{8}$$

Hayon[75] measured an E_0 of $+0.27$ V for cytochrome *c* reduction by O_2^-, indicating that ferrocytochrome *c* is a stronger reducing agent than uncomplexed ferrous ions. It is therefore conceivable that in a different medium, the oxidation potential of the Fe^{2+}/Fe^{3+} cytochrome couple may become more positive thus shifting the equilibrium to the left in equation (7). Such reversal has been observed[76] in the formation of O_2^- by oxygenation of haemoglobin [equation (9)].

$$Hb(Fe^{2+}) + O_2 \rightleftharpoons Hb(Fe^{2+})O_2 \rightarrow Hb(Fe^{3+}) + O_2^- \tag{9}$$

The ability of edta-complexed iron(III) to catalyse the O_2^- dismutation reaction[129] (see Section 5), in which the mechanism involves alternate reduction and reoxidation of Fe^{3+}, points to the ease with which iron can either oxidize O_2^- or reduce O_2. Valentine and Quinn[77] recently reported the reduction of a Mn^{III} tetraphenylporphyrin (TPP) salt to Mn^{II}TPP, and showed that the reverse oxygenation of Mn^{II}TPP to $Mn^{III}TPP^+$ does not lead to superoxide formation.

Transfer of an electron by O_2^- to *ortho*- or *para*-quinones leads to semiquinone radicals [equations (10) and (11)].[75,78-84] The reaction is reversible[82] and the balance of equilibrium depends on the one-electron reduction potential of the

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[74] 'Handbook of Chemistry and Physics', 44th Edn. Chem. Rubber Co., 1962-63, p. 1744.

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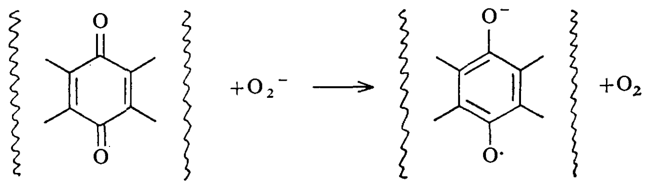

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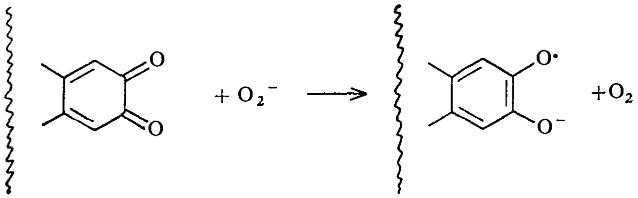

(11)

quinone. Willson and Patel[82] obtained one-electron reduction potentials of a number of quinones by determining equilibrium concentrations. Whereas O_2^- transfers an electron to ubiquinone, 1,4-naphthoquinone, *p*-benzoquinone, and methylated *p*-benzoquinones, semiquinones of vitamin K, 2,3-dimethylnaphthoquinone, and anthroquinone-2,6-disulphonate will reduce dioxygen to superoxide. Since semiquinones are involved in electron transport in cells, dioxygen reduction by these radicals may be one possible source of O_2^-.

In addition to quinones, a number of other organic compounds will oxidize O_2^-. These include tetranitromethane and nitrotetrazolium blue, which have already been mentioned as characteristic tests for O_2^-. Studies of the chemistry of O_2^- in the gas phase have revealed that ozone, nitrogen dioxide, and oxygen itself can act as oxidizing agents, as shown in equations (12)—(14).[85-88] These

$$O_2^- + O_3 \rightarrow O_3^- + O_2 \tag{12}$$

$$O_2^- + NO_2 \rightarrow NO_2^- + O_2 \tag{13}$$

$$O_2^- + 2O_2 \rightarrow O_4^- + O_2 \tag{14}$$

processes represent the major decomposition pathways for O_2^- decay in the ionospheric regions.

Studies of Fenton's reagent led Haber and Weiss[89] to propose reaction (15),

$$O_2^- + H_2O_2 \rightarrow OH^- + \cdot OH + O_2 \tag{15}$$

in which an extremely reactive oxidant, the hydroxyl radical, is generated. This reaction seems to occur readily in buffered aqueous systems and is believed to be responsible for a number of aromatic hydroxylations occurring in living organisms

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[85] D. A. Parkes, *J.C.S. Faraday I*, 1972, **68**, 2103.
[86] F. C. Fehsenfeld and E. E. Furguson, *Planet Space Sci.*, 1968, **16**, 701.
[87] E. E. Furguson, *Accounts. Chem. Res.*, 1970, **3**, 402.
[88] J. D. Payzant and P. Kebarle, *J. Chem. Phys.*, 1972, **56**, 3482.
[89] F. Haber and J. Weiss, *Proc. Roy. Soc.*, 1934, **A147**, 332.

(see Section 4B). Organic peroxides and hydroperoxides are reduced by O_2^- according to equations (16) and (17).[90,91] In the reduction of hydroperoxides,

$$ROOR + O_2^- \rightarrow RO^- + RO\cdot + O_2^- \qquad (16)$$

$$ROOH + O_2^- \rightarrow RO\cdot + OH^- \qquad (17)$$

alkoxyl radical formation appears to be dominant to hydroxyl radical formation,[91] as would be expected from electronegativity considerations.

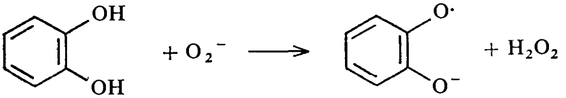
B. Superoxide as an Oxidizing Agent.—The reduction potential of the O_2^-/O_2^{2-} couple has been determined as -2.02 V (*vs.* standard calomel electrode) in dimethyl sulphoxide;[9] however, the potential for the transformations written in the direction shown in equations (18) and (19) are $+1.51$ and $+0.94$ V,

$$HO_2 + H^+ + e \rightarrow H_2O_2 \qquad (18)$$

$$O_2^- + 2H^+ + e \rightarrow H_2O_2 \qquad (19)$$

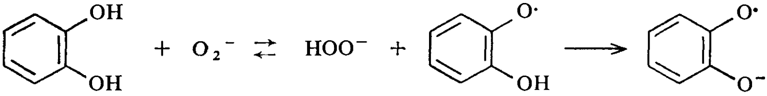
respectively (n.h.e.).[92,93] It is somewhat surprising that the latter potential is lower as the pK_a of O_2^- is 4.8[10] and protonation of O_2^- would be expected to be exothermic. In any case, O_2^- would be expected to react as a powerful oxidant.

Hydrogen abstraction from diphenols to produce semiquinones [equation (20)]



(20)

has been reported for a number of catechols,[78,94-95] hydroquinones,[78,96] and ene-1,2-diols such as ascorbic acid.[97] It is not known whether the process is concerted or involves a sequence of H· and H^+ abstractions. However, it appears unlikely that the concerted mechanism would operate in the case of the *p*-hydroquinones owing to the large inter-hydrogen distance. Two possible schemes can be envisaged for two-step elimination: hydrogen-atom abstraction by O_2^- followed by proton transfer to the resultant HOO^- [equation (21)] or



(21)

the reverse order [equation (22)]. If the pK value for HO_2 of 4.8[10] is reliable, the equilibrium constant for the first step of equation (21) would be 10^{-4} (taking

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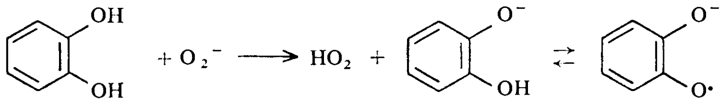
[90] A. LeBerre and Y. Berguer, *Bull. Soc. chim. France*, 1966, 2363.
[91] J. W. Peters and C. S. Foote, *J. Amer. Chem. Soc.*, 1976, **98**, 873.
[92] D. H. Busch, H. Shull, and R. T. Conley, 'Chemistry, Allyn and Bacon, 1973, p. 447.
[93] P. M. Wood, *F.E.B.S. Letters*, 1974, **44**, 22.
[94] R. W. Miller and U. Rapp, *J. Biol. Chem.*, 1973, **248**, 6084.
[95] Y. Moro-Oka and C. S. Foote, *J. Amer. Chem. Soc.*, 1976, **96**, 1510.
[96] P. S. Rao and E. Hayon, *J. Phys. Chem.*, 1973, **77**, 2274.
[97] M. Nishikimi, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 463.


(22)

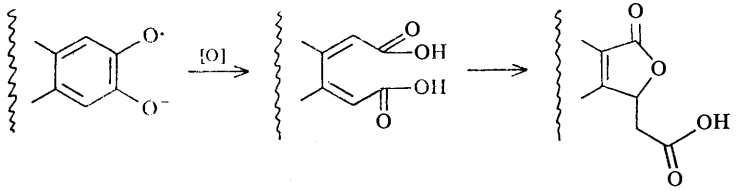
$\mathrm{p}K = 9$ for phenols). It has also been found that phenol will not transfer a proton to O_2^- in the gas phase.[98] On the other hand, the decomposition of O_2^- in water ($\mathrm{p}K = 15$) indicates the intermediacy of hydroperoxyl (HO_2) radicals, which subsequently disproportionate according to equation (23),[10] so that

$$HO_2 + HO_2 \rightarrow H_2O_2 + O_2 \qquad (23)$$

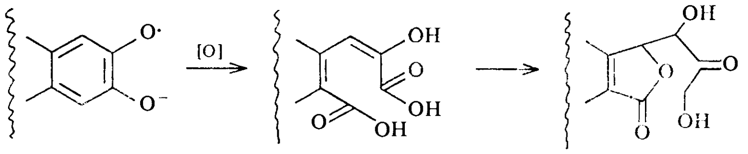
although the acid–base equilibrium between O_2^- and catechol may favour the reactant side, subsequent oxidation by hydroperoxyl radicals may be efficient enough for the overall transformation to occur by this mechanism. No visible evolution of oxygen accompanied catechol oxidation, as would be expected if perhydroxyl radical were present (in sufficient quantities for bimolecular decay). In some cases subsequent oxidation of the semiquinones results in the production of quinones, as in the case of adrenalin,[69] *p*-hydroquinone,[78] and ascorbic acid.[97] The production of quinones could also arise from bimolecular redox reaction between two semiquinones according to equation (24).[69] In the case of catechol

$$Q^{\bullet-} + Q^{\bullet-} \rightarrow Q + Q^{2-} \qquad (24)$$

semiquinones, oxidative ring-opening takes place to yield muconic acid derivatives [equation (25)][78,99] or *o*-hydroxymuconic semialdehydes [equation (26)].[95] This mode of oxidative decomposition is similar to the oxidation of catechols by pyrocatechase and metapyrocatechase.[100,101]



(25)



(26)

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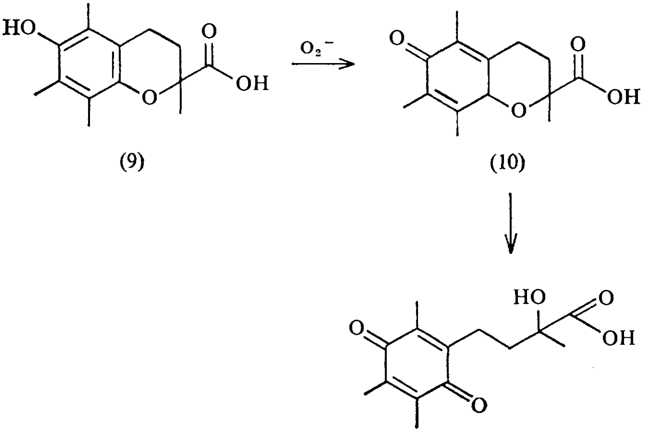
[98] I. Dzidic, D. I. Carrol, R. N. Stillwell, and E. C. Horning, *J. Amer. Chem. Soc.*, 1974, **96**, 5258.

[99] M. Tezuka, Y. Ohkatsu, and T. Osa, *Bull. Chem. Soc. Japan*, 1975, **48**, 1471.

[100] O. Hayaishi and K. Hashimoto, *J. Biochem. Japan*, 1950, **37**, 371.

[101] S. Dagley and D. A. Stopher, *Biochem. J.*, 1959, **73**, 16.

Vitamin E (α-tocopherol) acts as an antioxidant in the inhibition of peroxidation of lipids, a chain reaction believed to be initiated by O_2^- and singlet oxygen.[102] Nishikimi[103] investigated the interaction of superoxide and a chromanol derivative (9), a model for tocopherol, and reported the formation of quinone (11) *via* the ketonized chromanone (10). Quinone (11) or a similar derivative from α-tocopherol could subsequently act as a free radical scavenger and inhibit the peroxidation chain reaction.



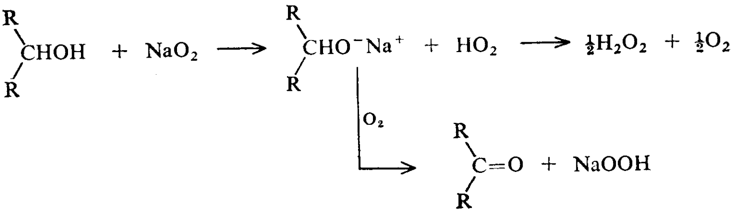
(9) (10) (11)

With simple primary and tertiary alcohols, O_2^- behaves in much the same fashion as its dismutation in aqueous solution [equation (27)].[90] With secondary alcohols, auto-oxidation of the metal alkoxide with oxygen generated *in situ*

$$2\,ROH + 2O_2^- \rightarrow 2RO^- + H_2O_2 + O_2 \qquad (27)$$

is observed, with ketones being the end products [equation (28)].

Aldehydes are susceptible to oxidation by O_2^- to give the corresponding carboxylic acids in yields of 59—72%, according to the stoicheiometry given in



$$\begin{array}{l} R_2CHOH + NaO_2 \longrightarrow R_2CHO^-Na^+ + HO_2 \longrightarrow \tfrac{1}{2}H_2O_2 + \tfrac{1}{2}O_2 \\ \qquad\qquad\qquad\qquad \xrightarrow{O_2} R_2C{=}O + NaOOH \end{array} \qquad (28)$$

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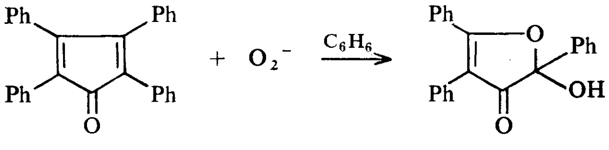
[102] D. D. Tyler, *F.E.B.S. Letters*, 1975, **51**, 180.
[103] M. Nishikimi and L. J. Machlin, *Arch. Biochem. Biophys.*, 1975, **170**, 684.

equation (29).[104] Reaction conditions are mild so this constitutes an efficient

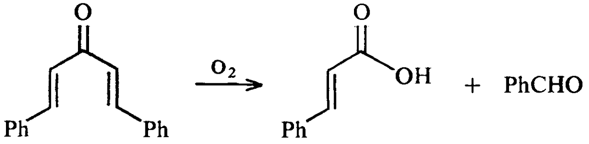
$$2NaO_2 + RCHO \rightarrow RCO_2Na + O_2 + NaOH \qquad (29)$$

method for alkaline oxidation of aldehydes. Ketones are inert to oxidation by O_2^-, although enolizable ketones undergo self condensation in the presence of O_2^-.[105] A novel oxidative ring-opening transformation of tetracyclone (12) to give a ketohemiketal (13) has been reported by Rosenthal.[106] In a related study[105]



(12) (13)

dibenzal acetone (14) reacted with O_2^- to yield cinnamic acid and benzaldehyde, which resisted further oxidation. Although the mechanism of these trans-



(14)

formations is not clear it would appear that the initial step may involve a Michael addition of O_2^- to the enone. Oxidative cleavage of α-diketones by O_2^- has been reported by Le Berre and Berguer.[104] Phenanthraquinone (15) and 1,2-naphthaquinone (17) are transformed into carboxylic acids (16) and (18)+(19), respectively. Benzil is converted by O_2^- into a mixture of benzoic and benzilic acids. San-Filippo and co-workers made similar observations in their study of O_2^- oxidations of α-keto-, α-hydroxy-, and α-halogeno-ketones, esters, and carboxylic acids;[107] in all cases carboxylic acids are produced.

A number of superoxide-mediated systems that are capable of hydroxylating aromatic compounds have been discovered. Horseradish peroxidase with dihydroxyfumarate and molecular oxygen can hydroxylate *p*-coumaric, *p*-hydroxybenzoic, and salicylic acids.[108] These reactions are inhibited by catalase

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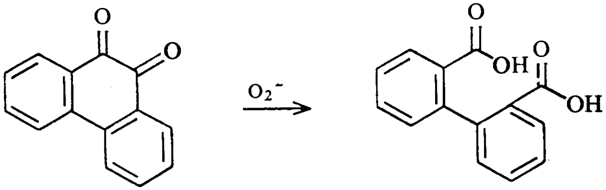
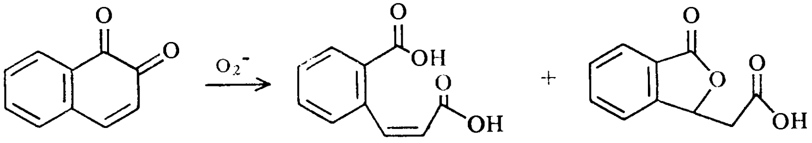
[104] A. LeBerre and Y. Berguer, *Bull. Soc. chim. France*, 1966, 2368.
[105] E. Lee-Ruff, unpublished results.
[106] I. Rosenthal and A. Frimer, *Tetrahedron Letters*, 1975, 3731.
[107] J. S. San-Filippo, C. I. Chern, and J. S. Valentine, *J. Org. Chem.*, 1976, **41**, 1077.
[108] B. Halliwell and S. Ahluwalia, *Biochem. J.*, 1976, **153**, 513.



(15) (16)



(17) (18) (19)

and superoxide dismutase, indicating that both hydrogen peroxide and O_2^- are necessary for hydroxylation. This in turn suggests that the hydroxyl radical produced by the Haber–Weiss reaction is the reactive species in these processes. Another O_2^- mediated oxidation involving hydroxyl radical is the oxidation of methional (20) to ethylene. Methional sulphoxides are also detected in the

$$\underset{(20)}{MeSCH_2CH_2CHO} \rightarrow CH_2CH_2 + CO$$

reaction mixture.[109]

Peroxidation of lipids is initiated by O_2^- generating systems.[110] Competitive inhibition tests have shown that both superoxide dismutase and catalase will inhibit such oxidations, again implicating hydroxyl radical as the initiator in the chain reaction.[111] However, trapping experiments[112] have shown that hydroxyl radicals are not directly involved in peroxidations, and moreover, in the absence of hydrogen peroxide, O_2^- adsorbed on silica gel will react with olefins to produce alkylperoxyl radicals.[113] Oxidative cleavage of olefins and formation of epoxides with O_2^- have been reported in the case of benzalfluorene (21) and cyclohexanone (22).[114]

Oxidation of benzilic carbon by O_2^- with production of hydroperoxide has

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[109] W. Bors, E. Lengfelder, M. Saran, C. Fuchs, and C. Michel, *Biochem. Biophys. Res. Comm.*, 1976, **70**, 81.

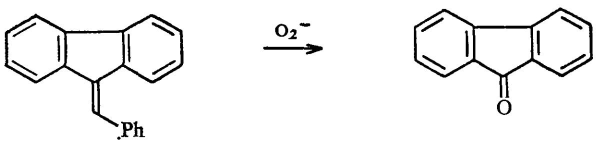
[110] P. B. McCay, K. L. Fong, M. King, E. Lai, C. Weddle, L. Poyer, and K. R. Hornbrook, *Lipids*, 1974, **1**, 157.

[111] R. Zimmerman, L. Flahe, U. Weser, and H. J. Hartman, *F.E.B.S. Letters*, 1973, **29**, 117.

[112] D. D. Tyler, *F.E.B.S. Letters*, 1975, **51**, 180.

[113] O. I. Lyubimova, A. G. Kotov, and L. Y. Karpova, *Kinetika i Kataliz*, 1972, **13**, 1603 (*Chem. Abs.*, 1973, **78**, 71 040).

[114] R. Dietz, A. E. J. Forno, B. E. Larcombe, and M. E. Peover, *J. Chem. Soc.* (*B*), 1970, 816.



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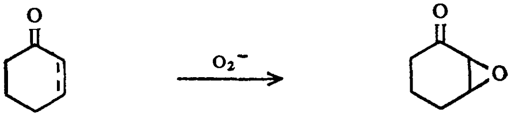

(22)

been observed in the transformation cumene into cumene hydroperoxide.[99] Hydroperoxides may also intervene in the conversion of fluorene into fluorenone and diphenylmethane into benzophenone. Efficient dehydrogenation of 1,9-dihydroanthracene to anthracene has been reported.[99] It is conceivable that in lipid autoxidations mediated by O_2^--producing systems, formation of hydroperoxides as initiators of the chain reaction may be the result of allylic hydrogen abstraction by O_2^- in a typical free radical process.

C. Superoxide as a Base, Nucleophile, and Ligand.—The pK of the hydroperoxyl radical (conjugate acid of O_2^-) has been reported as 4.8.[10] This implies that under physiological conditions superoxide exists in the non-protonated form and may be expected to exhibit weakly basic and nucleophilic properties. Decay of the hydroperoxyl radical follows second-order kinetics ($k \sim 10^7$ l mol^{-1} s^{-1}), giving oxygen and hydrogen peroxide.[10] Superoxide will abstract a proton in the gas phase from a number of oxygen and carbon acids including benzoic acid, malononitrile, *p*-nitrophenol, trinitrotoluene, picric acid, and acetic acid.[98] On the basis of the solution pK reported for O_2^-, the acidity of HO_2 should be comparable with that for acetic acid. In the gas phase, however, acetic acid is a stronger acid than HO_2. Such reversal in trends of acidities on going from the condensed to the gas phase have been observed for other acids.[115]

Superoxide acts as a nucleophile in displacement reactions on alkyl halides, tosylates, acyl chlorides, esters, and anhydrides. One of the earliest such reports[116] was of reactive chlorinated derivatives such as benzoyl and triphenylmenthyl chloride giving the corresponding peroxides in the presence of water. Trimethylchlorosilane (23) reacts with potassium superoxide to yield disilyl ether (24).

$$\underset{(23)}{Me_3SiCl} + KO_2 \rightarrow \underset{(24)}{Me_3SiOSiMe_3}$$

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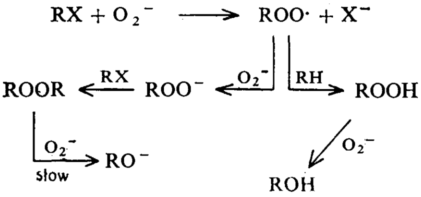
[115] D. K. Bohme, E. Lee-Ruff, and L. B. Young, *J. Amer. Chem. Soc.*, 1972, **94**, 5153.
[116] M. Schmidt and H. Bipp, *Z. anorg. Chem.*, 1960, **303**, 190.

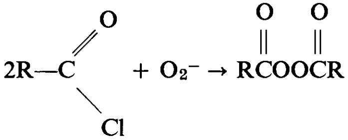
Less efficient reactions with O_2^- were observed for saturated alkyl halides and sulphates,[104] and in certain cases alcohols are obtained, presumably from the further reduction of the peroxides initially formed. The inefficiency of these reactions is probably due to the limited solubility of NaO_2 and KO_2 in organic solvents since in all cases suspensions of NaO_2 were used. However, effective nucleophilic substitutions were observed in electrogenerated superoxide solutions containing saturated alkyl halides, resulting in initial alkylperoxyl radical formation (Scheme 1).[114,117] Once formed, the peroxyl radical is reduced either by hydrogen-donating reagents giving hydroperoxides or by O_2^- to give peroxide anion which can undergo nucleophilic substitution with another alkyl halide to yield peroxides. Peroxides and hydroperoxides are reduced by O_2^- to give alcohols.[90]



Scheme 1

With the use of crown ethers to solubilize superoxide in organic solvents,[118] a number of preparative methods for the syntheses of alkyl peroxides,[119] acyl peroxides,[120] and alcohols[121,122] have been developed using O_2^- as an oxygen nucleophile [equations (30)—(32)].

$$2RX + O_2^- \rightarrow ROOR \ (X = Cl, Br, \text{ or } I) \qquad (30)$$



$$2R{-}C(=O)Cl + O_2^- \rightarrow RC(=O)OOC(=O)R \qquad (31)$$

$$2RX + O_2^- \rightarrow 2RO^- + 3O_2 + 2X^- \qquad (32)$$

Alkyl peroxides and alcohols are formed with net inversion of stereochemistry of the alkyl group,[122] indicating a bimolecular mechanism for O_2^- substitution in these systems. Cyclic peroxides can be obtained in fair yields from substrates possessing two reactive leaving groups. Thus dimesylate (25) and di-iodides (27)

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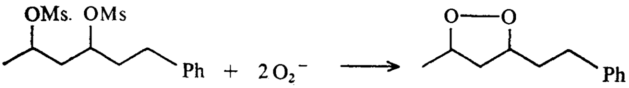
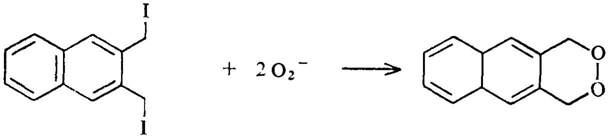
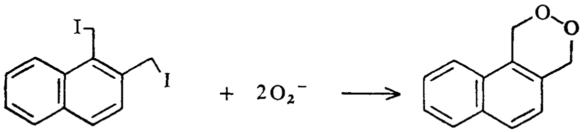
[117] M. V. Merritt and D. T. Sawyer, *J. Org. Chem.*, 1970, **35**, 2157.
[118] J. S. Valentine and A. B. Curtis, *J. Amer. Chem. Soc.*, 1975, **97**, 224.
[119] R. A. Johnson and E. G. Nidy, *J. Org. Chem.*, 1975, **40**, 1680.
[120] R. A. Johnson, *Tetrahedron Letters*, 1976, 331.
[121] J. S. San-Filippo, C. I. Chern, and J. S. Valentine, *J. Org. Chem.*, 1975, **40**, 1678.
[122] E. J. Corey, K. C. Nicolaou, M. Shibasak, Y. Macluda, and C. S. Shiner, *Tetrahedron Letters*, 1975, 3183.

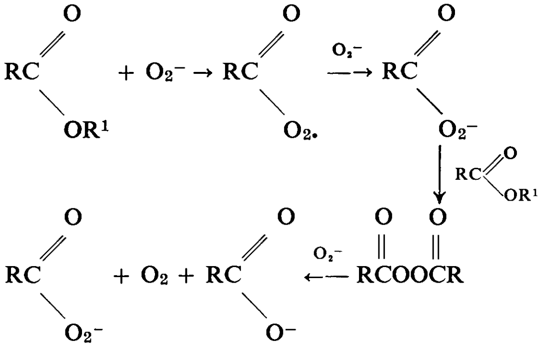

(25) (26)


(27) (28)


(29) (30)

and (29) react with O_2^- to give cyclic peroxides (26), (28) and (30), respectively.[122,123]

Alkyl and aryl carboxylic acid esters and phosphate esters are sensitive to superoxide hydrolysis reactions,[124,125] whereas amides and nitriles are inert. The mechanism of these hydrolyses is summarized by Scheme 2. It was shown by the use of a chiral group (R^1) that 99% retention occurred, indicating that


Scheme 2

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[123] E. Lee-Ruff and J. Rigaudy, unpublished results.

[124] J. S. San-Filippo, L.J. Romano, C. I. Chern, and J. S. Valentine, *J. Org. Chem.*, 1976, **41**, 586.

[125] F. Magno and G. Bontempelli, *J. Electroanalyt. Chem.*, 1976, **68**, 337.

acyl oxygen bond cleavage had taken place. Also acyl peroxides and acyl peroxy-esters are reduced under these conditions to carboxylic acids.

Superoxide can act as a ligand in transition-metal complexes, as is shown by the nature of dioxygen in oxygenated complexes of cobalt.[126] Theoretical molecular orbital calculations and other physical data indicate the superoxide character of dioxygen. However, the majority of these complexes are formed by addition of ground-state dioxygen and only a few have been made by direct ligand exchange or attachment with O_2^-. One such case reported is the synthesis of superoxocobalamin by addition of O_2^- to the cobalt(III) aquocobalamin complex (vitamin B_{12}).[50] The same oxygenated complex can be obtained by direct oxygenation of the reduced cobalt(II) aquocobalamin complex. An example of ligand exchange by O_2^- has been reported by Hill and co-workers[51] in which iron(III) protoporphyrin dimethyl ester perchlorate, in the presence of O_2^-, gives iron(III) protoporphyrin dimethyl ester superoxide. Liberation of O_2^- by ligand exchange of chloride ion in oxyhaemoglobin has been reported [equation (33)].[127] Since chloride ion is a naturally occurring nucleophile it is conceivable that

$$HbO_2 + Cl^- \rightarrow HbCl + O_2^- \tag{33}$$

superoxide production in haemoglobin oxidation may arise in part from such a mechanism.

4 Superoxide Dismutase and Singlet Oxygen

It is not the purpose of this review to dwell on the details of superoxide dismutases since numerous review articles already exist. However, some mention as to the mechanism of catalysis should be made in light of the chemical properties of O_2^- discussed above. These enzymes catalyse reaction (34). The active site is a

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \tag{34}$$

histidine-complexed Cu^{2+} and the mechanism involves alternate reduction and reoxidation of Cu^{2+} during successive interactions with O_2^- [equations (35) and (36)].[128] A number of simple copper complexes and uncomplexed copper salts

$$E\text{—}Cu^{2+} + O_2^- \rightarrow E\text{—}Cu^+ + O_2 \tag{35}$$

$$E\text{—}Cu^+ + O_2^- + 2H^+ \rightarrow E\text{—}Cu^{2+} + H_2O_2 \tag{36}$$

catalyse this reaction with rate enhancements of the same order of magnitude as superoxide dismutase. However, complexing of copper with NH_3, Cl^-, SO_4^-, and certain amino-acids leads to decreased catalytic activity attributed to changes

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[126] A. Dedieu, M. M. Rohmer, and A. Veillard, *J. Amer. Chem. Soc.*, 1976, **98**, 5789, and references therein.

[127] W. J. Wallace, J. C. Maxwell, and W. S. Caughey, *Biochem. Biophys. Res. Comm.*, 1974, **57**, 1104.

[128] D. Klug-Roth, I. Fridovich, and J. Rabani, *J. Amer. Chem. Soc.*, 1973, **95**, 2786.

in the ionization potential of copper.[128–132] Metals other than copper, *e.g.* iron in the iron(II)–edta complex, are equally effective in dismuting O_2^- by a redox cycle involving two equivalents of O_2^- similar to the superoxide dismutase mechanism.[133]

Oxygen can exist in excited states that are exceedingly reactive, and thermodynamic data suggest that singlet oxygen is produced in the dismutation of O_2^-.[134] Since superoxide dismutase can inhibit chemiluminescence associated with O_2^--producing systems,[135,136] and the chemiluminescence attributed to singlet oxygen dimeric emission,[137] it has been proposed that singlet oxygen can be catalytically quenched by superoxide dismutase.[136] Other observations that solid potassium superoxide generates singlet oxygen[138] and that decomposition of peroxochromate, a known singlet oxygen source,[139] results in luminol chemiluminescence which is inhibited by superoxide dismutase, led to suspicions that this enzyme is not selective to O_2^- catalysis. However, subsequent investigations of the peroxochromate system clearly showed that along with singlet oxygen, O_2^- is also produced[54,140] resulting in the earlier misinterpretation of the superoxide dismutase quenching results. Furthermore, direct measurements of the quenching effects of superoxide dismutase in inhibiting singlet oxygen reaction gave negative results, indicating that those enzymes do not act as catalysts in singlet oxygen decay.[141] However, one cannot rule out the possibility of singlet oxygen production in the O_2^- dismutation although the chemiluminescence attributed to singlet oxygen dimer emission, which is quenched by superoxide dismutase, may be due to other species produced from O_2^-. A CO_2 dimer has been suggested as the light-emitting species in these systems.[142] Attempts to trap singlet oxygen with selective scavengers in the O_2^- dismutation reaction were unsuccessful.[143] Recently it has been shown that O_2^- is an effective photochemical quencher of singlet oxygen with rate constants of quenching approaching

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diffusion control values ($k = 2 \times 10^9$ l mol^{-1} s^{-1});[144,145] thus any singlet oxygen generated would have to be scavenged for detection at least as efficiently, as it is quenched by O_2^-, and failure to detect singlet oxygen is probably due to the O_2^- quenching process.

5 Summary

Superoxide has been shown to be present in all oxygen-metabolizing organisms and its toxicity has been known for the last quarter century. However, there is a dearth of information on its reactivity with simple organic compounds. Only recently has the organic chemistry of superoxide been reopened for study. Some general trends of reactivity can be summarized. Superoxide undergoes efficient oxidation and reduction with organic and inorganic substrates having reduction or oxidation potentials exceeding that of O_2^-. It is a weakly reactive radical and base. It can undergo nucleophilic reactions with a number of substrates possessing reactive leaving groups and can act as a ligand in a number of metal complexes. Under certain conditions it can lead to production of highly reactive radicals such as hydroxyl which are capable of indiscriminate oxidation of most organic compounds. Its stability in aqueous solution appears to be minimal, and its decomposition to hydrogen peroxide and oxygen is catalysed by a family of enzymes called superoxide dismutases which are found in all aerobic cells. Its general oxidation and reduction properties may be responsible for its important function in biological oxidation and oxygenation processes. It is clear that before any studies of the interactions of O_2^- with proteins, lipids, polysacharide, nucleic acids, and other biochemicals are undertaken in order to elucidate biochemical oxidation mechanisms involving O_2^- and its toxicity, further studies on simpler molecules with mono- and multi-functional groups have to be carried out. Unfortunately this is an area in which serious inquiries have only just begun and it is to be hoped that the basic mechanisms of interaction of O_2^- with thiols, thioethers, disulphides, phosphates, monosocharides, and polyunsaturated fatty acids will be investigated. More quantitative data are required for the evaluation of relative free radical and nucleophilic reactivities of O_2^- in order to assess its importance and its interactions with more complex systems. However, due restraint has to be exercised in correlating data from reactions of free superoxide with those involving (superoxo) metal complex reactions and more complex enzymes. The effect of metal in the latter plays an important role in such phenomena as binding of the substrate and altering the nature of dioxygen. As a result such systems may be quite different from free superoxide, as found in crown-ether-complexed solutions of its alkali-metal salts.

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