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D. T. Sawyer, J. L. Roberts, T. S. Calderwood, H. Sugimoto, M. S. McDowell and P. Wardman

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Reactivity and activation of dioxygen-derived species in aprotic media (a model matrix for biomembranes)

By D. T. Sawyer, J. L. Roberts, Jr, T. S. Calderwood, H. Sugimoto and M. S. McDowell

Department of Chemistry, University of California, Riverside, California 92521, *U.S.A.*

In aprotic media the electrochemical reduction of dioxygen yields superoxide ion (O_2^-), which is an effective Brønsted base, nucleophile, one-electron reductant, and one-electron oxidant of reduced transition metal ions. With electrophilic substrates (organic halides and carbonyl carbons) O_2^- displaces a leaving group to form a peroxy radical ($ROO^\cdot$) in the primary process. Superoxide ion oxidizes the activated hydrogen atoms of ascorbic acid, catechols, hydrophenazines and hydroflavins. Combination of O_2^- with 1,2-diphenylhydrazine yields the anion radical of azobenzene, which reacts with O_2 to give azobenzene and O_2^- (an example of O_2^--induced autoxidation). With phenylhydrazine, O_2^- produces phenyl radicals. The *in situ* formation of $HO_2^\cdot$ (O_2^- plus a proton source) results in H-atom abstraction from allylic and other groups with weak heteroatom—H bonds (binding energy (b.e.) less than 335 kJ). This is a competitive process with the facile second-order disproportionation of $HO_2^\cdot$ to H_2O_2 and O_2 ($k_{bi} \approx 10^4$ mol^{-1} s^{-1} in Me_2SO).

Addition of $[Fe^{II}(MeCN)_4]$ $(ClO_4)_2$ to solutions of hydrogen peroxide in dry acetonitrile catalyses a rapid disproportionation of H_2O_2 via the initial formation of an adduct $[Fe^{II}(H_2O_2)^{2+} \leftrightarrow Fe(O)(H_2O)^{2+}]$, which oxidizes a second H_2O_2 to oxygen. In the presence of organic substrates such as 1,4-cyclohexadiene, 1,2-diphenylhydrazine, catechols and thiols the Fe^{II}–H_2O_2/MeCN system yields dehydrogenated products; with alcohols, aldehydes, methylstyrene, thioethers, sulphoxides, and phosphines, the $Fe^{II}(H_2O_2)^{2+}$ adduct promotes their monoxygenation. The product from the FeO^{2+}–H_2O_2 reaction, $[Fe^{II}(H_2O_2)_2^{2+}]$, exhibits chemistry that is closely similar to that for singlet oxygen ($^1O_2$), which has been confirmed by the stoichiometric dioxygenation of diphenylisobenzofuran, 9,10-diphenylanthracene, rubrene and electron-rich unsaturated carbon–carbon bonds ($Ph_2C{=}CPh_2$, $PhC{\equiv}CPh$ and *cis*-$PhCH{=}CHPh$). In dry ligand-free acetonitrile (MeCN), anhydrous ferric chloride ($Fe^{III}Cl_3$) activates hydrogen peroxide for the efficient epoxidation of alkenes. The $Fe^{III}Cl_3$ further catalyses the dimerization of the resulting epoxides to dioxanes. These observations indicate that strong Lewis acids that are coordinatively unsaturated, $[Fe^{II}(MeCN)_4]^{2+}$ and $[Fe^{III}Cl_3]$, activate H_2O_2 to form an effective oxygenation and dehydrogenation agent.

When catalytic quantities of superoxide ion are introduced into a dry acetonitrile solution that contains excess substrate (Ph_2SO or $PhCH_2OH$), ambient air, 1,2-diphenylhydrazine and iron$^{(II)}$, the substrate is rapidly and efficiently monoxygenated, the combination provides a catalytic system for the autoxidation of organic substrates via reaction cycles that closely mimic cytochrome P_{450} monoxygenase enzymes.

1. Introduction

(*a*) *Aprotic solvents as models for the chemical environment in biological membranes*

The elucidation of the detailed reaction mechanisms for chemical reactions in biological systems is difficult. An organism or an intact organelle is the biological equivalent of a black box with known inputs and outputs, but whose inner processes are complicated and not understood.

Thus, analysis of the internal reactions of a biological system generally must proceed by isolating and studying each hypothetical reaction inside the black box.

A question then arises as to whether these biochemical reactions take place in an essentially aqueous or in a non-aqueous hydrophobic environment. Without a definitive answer to this question the reactions have been studied in both aqueous and aprotic media. A fundamental justification for the use of aprotic solvents is that they allow the study of species, particularly anion radicals, that are too reactive to study in solvents that have labile protons.

In addition, the properties of oxy ions and metal ions may be significantly different in aprotic solvents and water. Water is such a strong ligand that it displaces weaker ligands, and is both a moderate acid and a weak base. In an aprotic solvent the redox properties of ions, especially anions, are significantly affected and the delicate balance of ligand–metal interactions that is associated with metal ion catalysis is dramatically changed. Thus, studies in aprotic solvents may reveal reaction pathways that are not observed in water and reaction rates that are enhanced by several orders of magnitude. Another important factor is the enhanced solubility of O_2 in aprotic media (at a partial pressure of 1 atm† its concentration is 1 mM in H_2O, 5 mM in dimethylformamide and 8 mM in acetonitrile (Sawyer *et al.* 1982*a*).

A further justification for studies in aprotic solvents is that they provide an environment that closely parallels that of a lipid bilayer membrane with embedded proteins (low proton availability with a polar hydrophilic character). Even the solution in the interior of the cell (the cytosol) may have less protic character than pure water because most of the water in the concentrated cytosol is bound to inorganic ions, proteins, other biomolecules and the membrane surface. If this reasoning is valid, then the chemistry for dioxygen species (O_2^-, $HO_2^{\cdot}$, H_2O_2, HO_2^-) in aprotic solvents is likely to be analogous to that in cells, on the surface of cell membranes, and within biomembranes.

For several reasons both aqueous or aprotic solutions are inadequate model matrices for biological processes. Reaction volumes in biological systems are small and contain many solid organelles surrounded by membranes of large surface area. Thus, the interior of a cell has many solid–solution interfaces with charged double layers that present substantial voltage gradients. Cellular membranes also act as barriers for species whose transport is limited by their size, shape and net charge, and thereby create large concentration gradients. Moreover, the identity and concentration of the reactive intermediates that exist inside a cell are difficult to determine. These factors contribute considerable uncertainty as to the relevance of the reaction pathways in homogeneous aqueous and non-aqueous systems to biological systems.

(*b*) *Previous studies of* O_2*-activation via one-electron reduction*

The role of oxygen in aerobic metabolism has occupied the attention of chemists since the time of Lavoisier. Triplet ground-state dioxygen, $^3O_2$, is a diradical and reacts slowly with most spin-paired organic compounds because the direct reaction of a triplet molecule to give singlet products is a spin-forbidden process (Taube 1965; Hamilton 1974). Therefore, much attention has been focused on how $^3O_2$ is able to react with singlet-state organic compounds in the presence of catalysts and cofactors, in other words, the problem of oxygen activation. A number of modes of activation have been discovered. These include (*a*) the introduction of free-radical

† 1 atm = 101325 Pa.

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initiators that promote reactions with triplet dioxygen by a radical chain mechanism such as lipid peroxidation (Simic & Karel 1980; Pryor 1976); (*b*) the excitation of $^3O_2$ to a singlet state (Krinsky 1979; Foote 1976); (*c*) the binding of triplet-state dioxygen to a transition metal ion that has unpaired electron spins (Spiro 1980) and (*d*) the successive addition of electrons to $^3O_2$ to form active intermediates, the first being the superoxide ion, O_2^- (Frimer 1983; Roberts & Sawyer 1983).

The discovery that superoxide ion is produced in biological systems, albeit as a by-product, and the further discovery of a family of enzymes that catalyse superoxide ion dismutation has provided an important stimulus for research on superoxide ion chemistry (Fridovich 1982).

In aqueous solution at pH 7, superoxide ion reacts rapidly in three characteristic modes. First, as a weak base, for example protonation followed by rapid disproportionation (Bielski 1978); second, as a one-electron reductant of easily reducible substrates, for example Fe^{III} cytochrome *c*, quinones, and oxidized transition metal complexes of Fe^{III}, Mn^{III}, Cu^{II}, Ru^{III}, and Mo^{VI} (Sawyer & Valentine 1981); and third, as a one-electron oxidant, for example, oxidative addition to Fe^{II} EDTA to form a ferric–peroxo complex, Fe^{III} (O_2^{2-}) (Bull *et al.* 1983). Without the stabilization of the peroxide ion afforded by a metal cation or proton, superoxide cannot act as a one-electron oxidant (Sawyer *et al.* 1978).

In aprotic solvents, reduction of dioxygen in the presence of $Zn^{II}(bipy)_3^{2+}$, $Cu^{I}(MeCN)_4^+$ and $Fe^{II}TPP$ produces, respectively $Zn^{II}(bipy)_3O_2$, $Cu^{II}(O_2)$, and the side-on bonded peroxo complex, $O_2Fe^{III}(TPP)^-$, where bipy represents 2,2′-bipyridine and TPP represents tetrapheylporphyrin (Sawyer *et al.* 1984*a*). The latter complex also is formed by the reaction of O_2^- with $Fe^{II}TPP$ (McCandlish *et al.* 1980). Similarly, the addition of four moles of O_2^- per mole of molybdenum(VI)3,5-di-t-butylcatecholate dimer, $[Mo^{VI}(O)(DTBC)_2]_2$, yields two moles of the peroxide adduct, $[Mo^{VI}(O)(O_2)(DTBC)_2]^{2-}$ plus two moles of dioxygen. This represents a catalysed disproportionation, $4O_2^- \rightarrow 2O_2^{2-} + 2O_2$ (Lim & Sawyer 1982). These net reactions resemble the oxidative additions observed in aqueous solution. To date, these complexes are essentially inert as reactants or catalysts for the oxygenation of organic substrates, but their chemistry has not been fully explored.

Bielski (1978) has reported refined values for the acid–base, spectral and kinetic properties of O_2^- and its conjugate acid, $HO_2^{\cdot}$ (the perhydroxyl radical), in aqueous solutions. Superoxide ion is a weak base (the pK_a for $HO_2^{\cdot}$ is 4.9) such that at pH 7, *ca.* 1% of the total superoxide exists as $HO_2^{\cdot}$. Both $HO_2^{\cdot}$ and O_2^- spontaneously disproportionate via a series of reactions.

$$HO_2^{\cdot} + HO_2^{\cdot} \xrightarrow{k = 8.6 \times 10^5\ \mathrm{mol^{-1}\ s^{-1}}} H_2O_2 + O_2; \quad (1)$$

$$HO_2^{\cdot} + O_2^- \xrightarrow{k = 1 \times 10^8\ \mathrm{mol^{-1}\ s^{-1}}} HO_2^- + O_2; \quad (2)$$

$$O_2^- + O_2^- \xrightarrow{k < 0.35\ \mathrm{mol^{-1}\ s^{-1}}} O_2^{2-} + O_2. \quad (3)$$

The tendency of superoxide ion to disproportionate enhances its effective basicity.

$$2O_2^- + H_2O \rightleftharpoons O_2 + HO_2^- + OH^-; \quad (4)$$

$$2O_2^- + HB \rightarrow O_2 + HO_2^- + B^-. \quad (5)$$

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This effect is most clearly seen in aprotic solvents, where O_2^- is long-lived (Chin *et al.* 1982). Sawyer & Gibian (1979) estimated an equilibrium constant of 2.5×10^8 for reaction (4) in water. Thus, even acids much weaker than water (up to $pK_a \approx 23$) can be deprotonated by O_2^- when proton dissociation is controlled by thermodynamic rather than kinetic factors; for example, protons on oxygen and nitrogen acids are generally more labile than those on carbon acids (Crooks 1975).

There are significant differences in the acid–base, redox, and nucleophilic properties of O_2^- in water and in dipolar aprotic solvents such as dimethyl sulphoxide (Me_2SO), dimethylformamide (DMF), and acetonitrile (MeCN). Superoxide ion in water is a weaker base, a weaker one-electron reducing agent and a weaker nucleophile than it is in aprotic solvents (Roberts & Sawyer 1983). These effects are mainly attributable to the strong solvation of O_2^- by water (Koppenol 1983) in contrast to that by aprotic solvents. In water, O_2^- displays a notable lack of reactivity with molecules of biological interest (Bielski 1983; Gebicki & Bielski 1981), except for those that contain acidic protons (ascorbic acid, thiols) or compounds that are easily reducible by single-electron transfer (quinones, Fe^{III} cytochrome *c*). However, $HO_2^{\bullet}$ is an effective one-electron oxidant and can initiate lipid autoxidation (Gebicki & Bielski 1981); i.e. $HO_2^{\bullet}$ is a 'hotter' radical and a more effective hydrogen-atom abstractor than O_2^- (Valentine 1979; Frimer 1983; Sawyer & Roberts 1983). This has shifted the focus of attention from O_2^- to $HO_2^{\bullet}$ in the search for species responsible for the cytotoxicity that accompanies the generation of superoxide ion in biological systems. A significant aspect of this cytotoxicity is the destruction of membranes by autoxidation of their lipid components; this has prompted a re-examination of diene fatty acid autoxidation (Porter 1984).

The initiator of lipid autoxidation most often invoked is the hydroxyl radical ($OH^{\bullet}$), which can be derived from hydrogen peroxide (produced by disproportionation of superoxide) via several routes. The first is the well studied Fenton reaction and its analogues (Green & Hill 1984; Walling 1975).

$$H_2O_2 + M^{n+} \rightarrow M^{(n+1)+} + OH^- + OH^{\bullet} \qquad (6)$$

$$O_2^- + M^{(n+1)+} \rightarrow O_2 + M^{n+} \qquad (7)$$

Another route, demonstrated in aprotic solvents, is the base-induced decomposition of hydrogen peroxide (Roberts *et al.* 1978):

$$2H_2O_2 + OH^- \rightarrow O_2^- + 2H_2O + OH^{\bullet}. \qquad (8)$$

In aprotic solvents the uncatalysed disproportionation of superoxide ion is slow and, in the absence of strong solvation, the 'naked' anion is a much better nucleophile. This behaviour parallels that of another small non-polarizable anion, fluoride ion, which is a weak base and poor nucleophile in water, but a strong base and good nucleophile in aprotic solvents (Valentine 1979; Clark 1980).

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2. Results and discussion

(a) *New aspects of* O_2^- *reactivity in aprotic solvents*

This section summarizes recent studies of superoxide ion reactions with polyhalogenated hydrocarbons and halogenated alkenes, carbonyl compounds and substrates with activated secondary amine functions (substituted hydrazines, hydrophenazines and hydroflavins). The reactions of O_2^- with organic substrates in aprotic solvents yield activated oxygen intermediates (peroxy radicals, peroxides and H_2O_2) that represent a significant biological hazard, especially if they are formed within biomembranes.

(i) *Oxygenation of polyhalogenated hydrocarbons*

The stoichiometries and kinetics for the reaction of O_2^- with polyhalogenated alkanes and alkenes are summarized in table 1 (Roberts *et al.* 1983; Calderwood *et al.* 1983; Calderwood & Sawyer 1984). The normalized first-order rate constants, $k_1/[S]$, that are reported in tables 1–4 were determined by the rotated ring-disk electrode method (Roberts *et al.* 1983) under pseudo-first-order conditions ([substrate] $\gg$ $[O_2^-]$).

The nucleophilicity of O_2^- toward primary alkyl halides (scheme 1) results in an S_N2 displacement of halide ion from the carbon centre. The normal reactivity order, primary

Table 1. Stoichiometries and kinetics for the reaction of 0.1–5 mM O_2^- with polyhalogenated hydrocarbons in dimethylformamide (0.1 M tetraethylammonium perchlorate) at 25 °C[a]

substrate concentration, [S] = 1–10 mM	O_2^-/S	products/S	$k_1/[S]$ / (mol^{-1} s^{-1})[b]
CCl_4	5	$HOC(O)O^-$, $4Cl^-$, $3.3O_2$	3800.0
$FCCl_4$	5	$HOC(O)O^-$, $3Cl^-$, $2.5O_2$	4.0
$HCCl_3$	4	$HOC(O)O^-$, $3Cl^-$, $2O_2$	0.4
CF_3CCl_3	4	$CF_3C(O)O^-$, $3Cl^-$, $2.4O_2$	400.0
$PhCCl_3$	4	$PhC(O)O^-$ (70%), $PhC(O)OO^-$ (30%) $3Cl^-$, $2.4O_2$	50.0
$MeCCl_3$	—		< 0.1
$HOCH_2CCl_3$	4	$HOCH_2C(O)O^-$, $3Cl^-$	47.0
(*p*-ClPh)$_2$CHCCl$_3$ (DDT)	1	(*p*-ClPh)$_2$C=CCl$_2$, Cl^-	100.0
(*p*-MeOPh)$_2$CHCCl$_3$ (Methoxychlor)	1	(*p*-MeOPh)$_2$C=CCl$_2$, Cl^-	10.0
(*p*-ClPh)$_2$CFCCl$_3$ (F-DDT)	1	(*p*-ClPh)$_2$C=CCl$_2$, Cl^-	170.0
PhCHBrCHBrPh	2	2PhCH(O), $2Br^-$, O_2	1000.0
MeCHBrCHBrMe	2	2MeCH(O), $2Br^-$, O_2	160.0
CH_2BrCH_2Br (EDB)	2	$2CH_2(O)$, $2Br^-$, O_2	2000.0
$CH_2BrCHBrCH_2Cl$ (DBCP)	5	$2CH_2(O)$, $HOC(O)O^-$, $2Br^-$, Cl^-, $2O_2$	4000.0
n-BuBr	1	Br^-	960.0
CH_2ClCH_2Cl	2	$2CH_2(O)$, $2Cl^-$, O_2	24.0
cis-CHCl=CHCl	4	$2HOC(O)O^-$, $2Cl^-$	10.0
$CH_2=CCl_2$	3	$HOC(O)O^-$, $2Cl^-$, O_2	2.0
$CHCl=CCl_2$	5	$2HOC(O)O^-$, $3Cl^-$, $1.5O_2$	9.0
$CCl_2=CCl_2$	6	$2HOC(O)O^-$, $4Cl^-$, $3O_2$	15.0
(*p*-ClPh)$_2$C=CCl$_2$ (DDE)	3	$HOC(O)O^-$, (*p*-ClPh)$_2$C=O, $2Cl^-$, O_2	2.0

[a] Stoichiometries determined for O_2^--substrate reactions by titration of excess $(Me_4N)O_2$ (with voltammetric detection); for released Br^- and Cl^- by titration with $AgNO_3$; for released O_2 by negative-scan voltammetry; and for organic products by ether extraction and capillary-column gas chromatography.

[b] Pseudo-first-order rate constants, k_1 (normalized to unit substrate concentration [S]), were determined from measurements with a glassy carbon–glassy carbon ring-disc electrode that was rotated at 900 rev min^{-1}.

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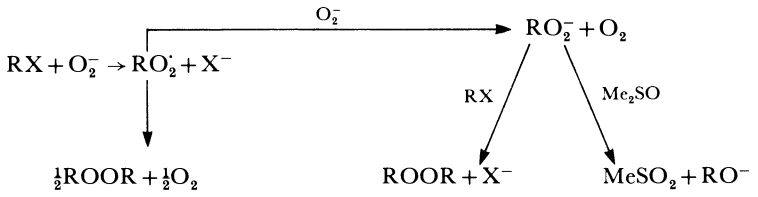


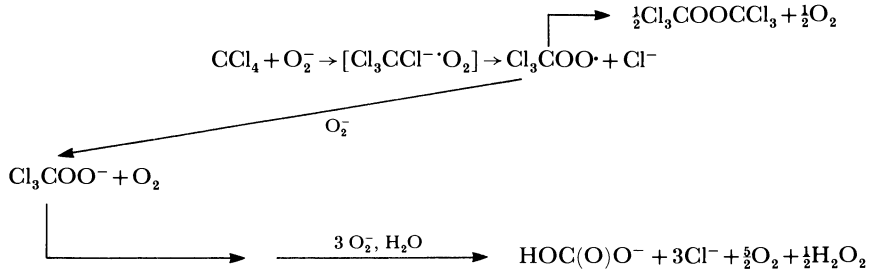
SCHEME 1

> secondary > tertiary and leaving-group order I > Br > OTs > Cl are observed, as are the expected stereoselectivity and inversion at the carbon centre. In dimethylformamide the final product is the dialkyl peroxide. The peroxy radical ($ROO^{\cdot}$), which is produced in the primary step and has been detected by spin trapping (Merritt & Johnson 1977), is an oxidant that is readily reduced by O_2^- to form the peroxy anion (ROO^-). Because the latter can oxygenate Me_2SO to its sulphone, the main product in this solvent is the alcohol (ROH) rather than the dialkylperoxide.

Although formation of the dialkyl peroxide is shown in the prototype reaction (scheme 1), hydroperoxides, alcohols, aldehydes and acids have also been isolated. The extent of these secondary paths depends on the choice of solvent and reaction conditions. Secondary and tertiary halides also give substantial quantities of alkene elimination products.

The reaction of O_2^- with CCl_4 and $RCCl_3$ compounds almost certainly cannot occur via an S_N2 mechanism because the carbon-atom centre is inaccessible. Rather, superoxide ion appears to attack a chlorine atom with a net result that is equivalent to an electron transfer from O_2^- to chlorine (scheme 2). This step is analogous to the 'single-electron transfer' (s.e.t.) mechanism that has been proposed for many nucleophilic reactions; an initial transfer of an electron followed by collapse of a radical pair (Eberson 1982).

$$E^+ + Nu^- \rightarrow [E^{\cdot}\ {}^{\cdot}Nu] \rightarrow E{-}Nu \qquad (9)$$



SCHEME 2

The initiation step for the O_2^-–CCl_4 reaction must be followed by rapid combination in the solvent cage of $^{\cdot}O_2^{\cdot}$ and $Cl_3C^{\cdot}$ to form the $Cl_3COO^{\cdot}$ radical. This radical is thought to initiate lipid peroxidation (Mason 1982), which would account for the hepatotoxicity of CCl_4 (Slater 1982).

The rates of reaction for O_2^- with $RCCl_3$ compounds are proportional to their reduction potentials, which is consistent with the s.e.t. mechanism (Roberts *et al.* 1983). A plot of $\lg k_1/[S]$ (table 1) against the reduction potentials of $RCCl_3$ compounds is approximately linear with

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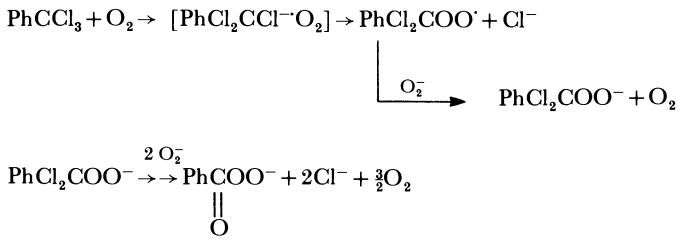
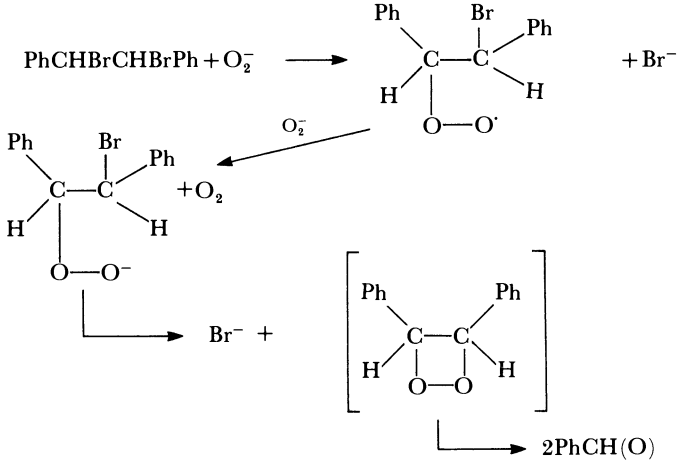
a slope of −4.9 decade per volt. Such behaviour is consistent with a mechanism that occurs via simultaneous electron transfer and nuclear motion (Perrin 1974). This correlation indicates that in water, where the O_2/O_2^- redox potential is about 0.44 V more positive, the rate of the reaction for O_2^- with CCl_4 would be about 100–200 times slower than in aprotic solvents.

The stoichiometric data in table 1 for CCl_4 and $FCCl_3$ are consistent with a net chemical reaction that yields bicarbonate ion and four halide ions in the final aqueous workup of the reaction products. When O_2^- reacts with $RCCl_3$ compounds, the R—C bond is not cleaved (scheme 3). About 30 % of the product from the $PhCCl_3$–O_2^- reaction is perbenzoate ion. Hence, this may be the active intermediate responsible for the epoxidation of olefins by O_2^- in the presence of benzoyl chloride (Oae & Takata 1980).



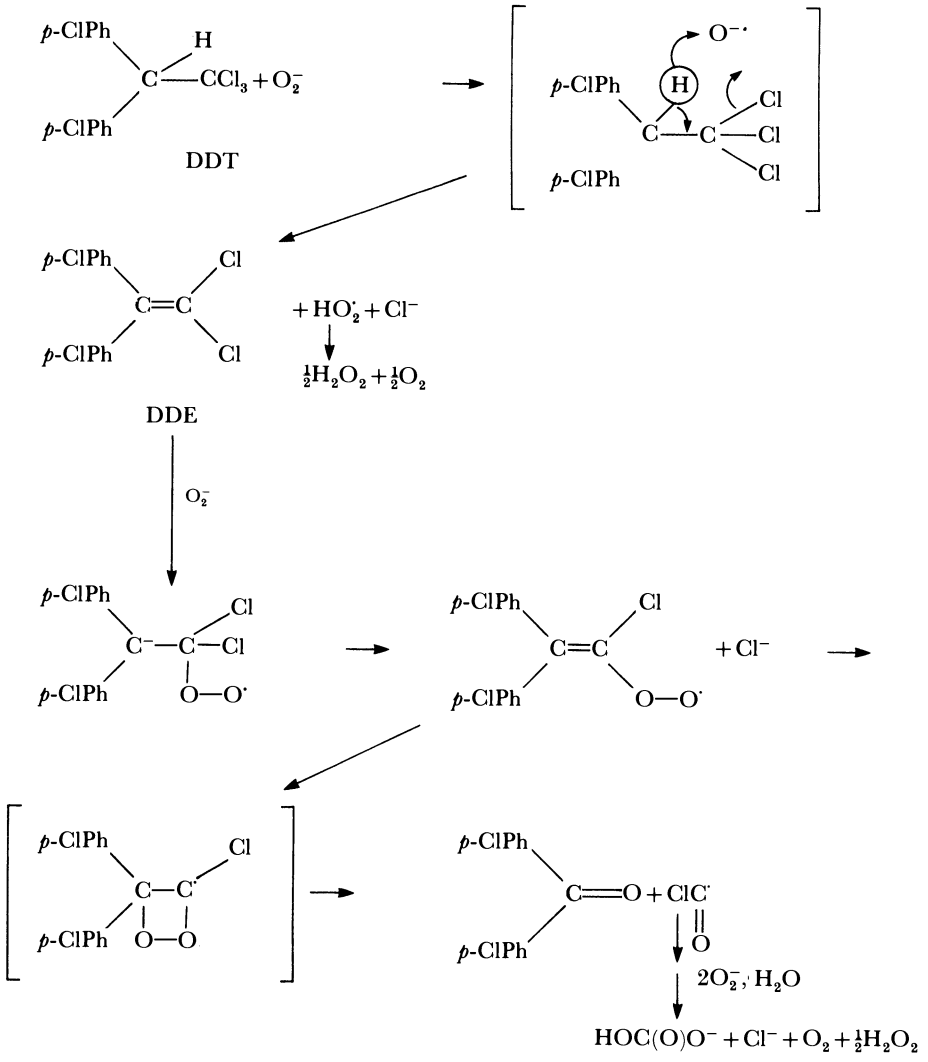
Scheme 3

Superoxide ion reacts with vicinal dibromoalkanes to form aldehydes (see table 1). The mechanism proposed for these reactions (scheme 4) is a nucleophilic attack on carbon, followed by a one-electron reduction of the peroxy radical and nucleophilic displacement on the adjacent carbon to form a dioxetane that subsequently cleaves to form two moles of aldehyde (Calderwood & Sawyer 1984).



Scheme 4

Both *p*,*p*′-DDT and Methoxychlor are rapidly deprotonated by OH^- in aprotic solvents with subsequent elimination of Cl^- to form the dehydrochlorination products, i.e. DDT forms DDE (scheme 5). The same products are formed in their reactions with O_2^-. Because the reaction rates that are measured by the rotated ring-disk electrode method are fairly rapid, the primary step must be a direct reaction with O_2^- and not with OH^- from the reaction of O_2^- with trace



Scheme 5

water in the solvent (see equation (4)). Hence the initial reaction with O_2^- is deprotonation followed by elimination of Cl^- to form DDE.

Although normal alkenes are not reactive with O_2^-, their chlorinated derivatives, including DDE, are readily oxidized (see table 1). Scheme 5 outlines a proposed mechanism for DDE that postulates nucleophilic addition to the activated olefin followed by ring closure of the peroxy radical and cleavage of the dioxetane-like radical to form products. After aqueous workup, the terminal olefinic carbon atom with a halogen is recovered as bicarbonate ion and halide ion. The peroxy radical (and chloracyl radical) intermediates should be effective initiators for the peroxidation of unsaturated lipids, as has been proposed for the $Cl_3COO\cdot$ radical (Slater 1982).

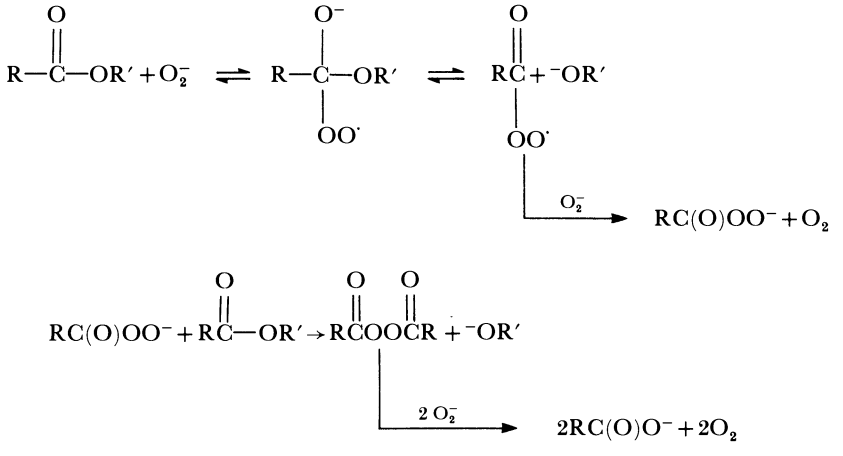
(ii) *Nucleophilic addition to carbonyls*

Table 2 summarizes the available kinetic data for the reaction of O_2^- with esters, diketones and carbon dioxide (Gibian *et al.* 1979, Magno & Bontempelli 1976; Sawyer *et al.* 1983; Roberts *et al.* 1984). Esters react with superoxide ion to form diacyl peroxides or the carboxylate and the alcohol. Initial reaction is proposed to occur via a reversible addition–elimination reaction at the carbonyl carbon (scheme 6). This idea is supported by the products that are observed in the gas-phase reaction of O_2^- with phenyl acetate and phenyl benzoate, studied by Fourier-transform mass spectrometry (Johlman *et al.* 1983). In effect, there is a competition between loss of O_2^- and loss of the leaving group. Carbanions are poor leaving groups so that simple ketones without acidic α-hydrogen atoms are unreactive. The $RC(O)OO^{\cdot}$ radical and the $RC(O)OO^-$ anion should be reactive intermediates for the initiation of autoxidations of allylic hydrogens and the epoxidation of olefins, respectively.

TABLE 2. PRODUCTS AND KINETICS FOR THE REACTION OF 1–5 mM O_2^- WITH CARBONYL COMPOUNDS AT 25 °C

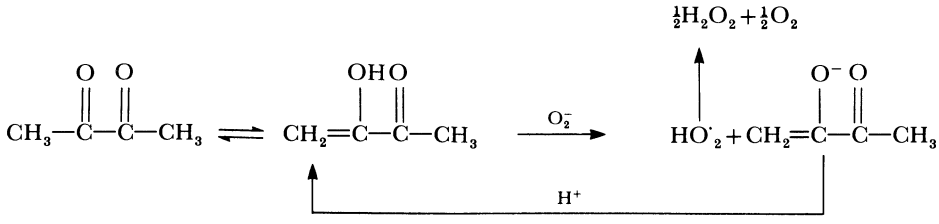
substrate, S	solvent[a]	products/S	k_2 (mol^{-1} s^{-1})
MeC(O)OEt	Py/0.1 M TEAP	—	0.01
MeC(O)OPh	Py/0.1 M TEAP	—	160.0
PhC(O)OPh	Py/0.1 M TEAP	—	5.0
PhCH(O)	Py/0.1 M TEAP	no reaction	—
PhC(O)C(O)Ph	DMF/0.1 M TEAP	$2PhC(O)O^-$	2000.0[b]
MeC(O)C(O)Me	DMF/0.1 M TEAP	$\frac{1}{2}H_2O_2$, enolate	4000.0[b]
MeC(O)C(O)OEt	DMF/0.1 M TEAP	$\frac{1}{2}H_2O_2$, enolate	4000.0[b]
CO_2	Me_2SO/0.1 M TEAP	$\frac{1}{2}\ ^-OC(O)OC(O)OO^-$	1400.0[b]

[a] Py, pyridine; DMF, dimethylformamide; TEAP, tetraethylammonium perchlorate.
[b] Pseudo-first-order rate constants divided by substrate concentration, $k_1/[S]$, determined from measurements with a glassy carbon–glassy carbon ring-disk electrode that was rotated at 900 rev min^{-1}.

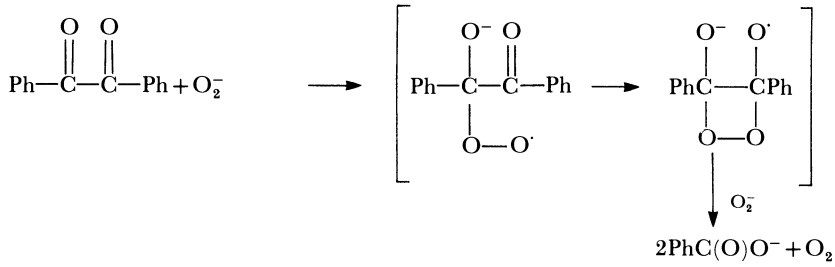


SCHEME 6

Simple diketones such as 2,3-butanedione are rapidly deprotonated by O_2^-, but the original diketone is recovered upon acidification (scheme 7). However, benzil (PhC(O)C(O)Ph) cannot enolize and is oxygenated by O_2^- to give two benzoate ions. Scheme 8 outlines a proposed mechanism that is initiated by nucleophilic attack. Frimer (1983) has discussed an alternative



SCHEME 7



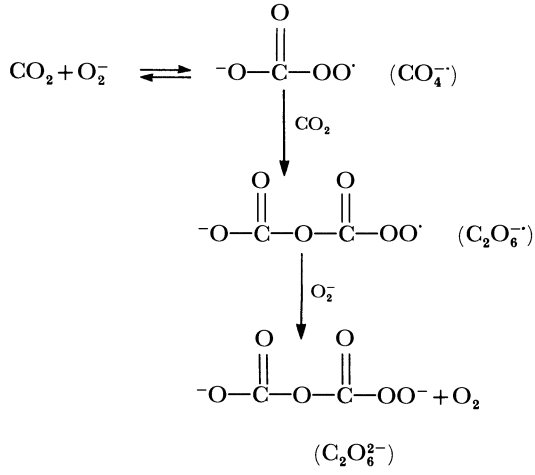
SCHEME 8

pathway in which the initial step is electron transfer from O_2^- to the carbonyl, followed by coupling of the benzil radical with dioxygen to give the cyclic dioxetane-like intermediate.

Carbon dioxide reacts rapidly with O_2^- in aprotic solvents. The net stoichiometry of the reaction in acetonitrile is given by

$$2CO_2 + 2O_2^- \rightarrow C_2O_6^{2-} + O_2 \qquad (10)$$

and the proposed mechanism is outlined in scheme 9. This reaction is significant because it provides a route to an activated form of carbon dioxide that may be involved in the vitamin K-dependent carboxylation of glutamic acid residues (Esnouf *et al.* 1978). The results indicate that likely candidates for active intermediates are the anion radical, $CO_4^{-\cdot}$, or a hydrolysis product of $C_2O_6^{2-}$ such as peroxybicarbonate, $HOC(O)OO^-$.



SCHEME 9

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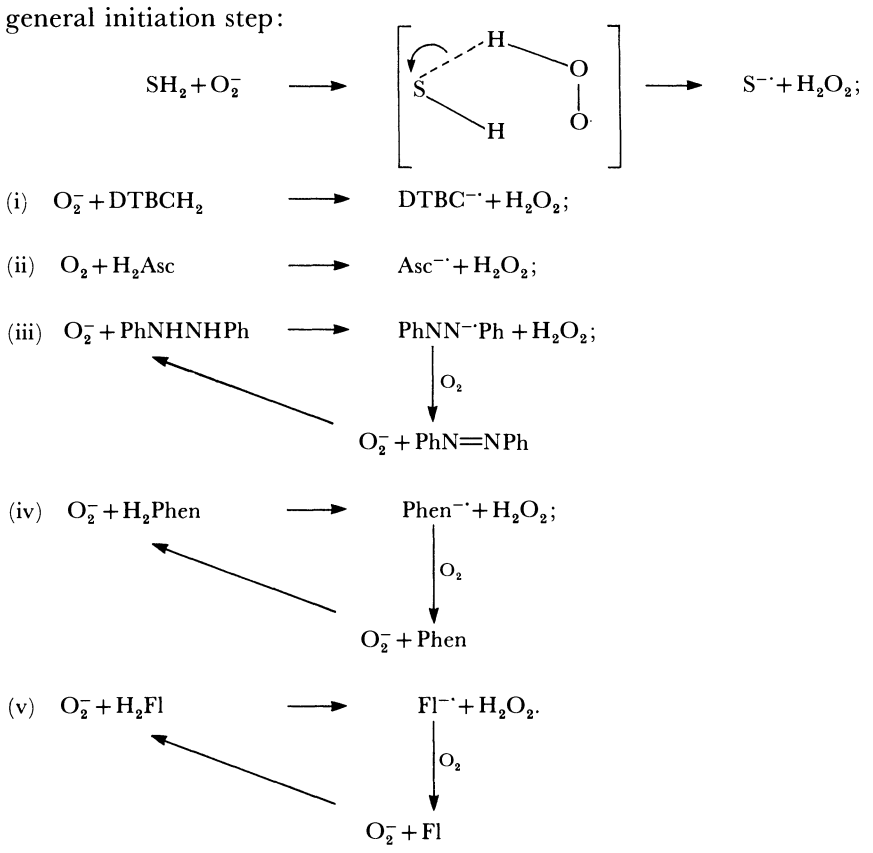
(iii) *Oxidation of compounds with hydrogen atoms on vicinal nitrogen or oxygen atoms*

Recent studies have demonstrated that 3,5-di-t-butylcatechol ($DTBCH_2$), ascorbic acid (H_2Asc), 1,2-disubstituted hydrazines, dihydrophenazine (H_2Phen), and dihydrolumiflavin (H_2Fl), are oxidized by O_2^- in aprotic media via a general mechanism (scheme 10) that involves the rapid sequential transfer to O_2^- of a proton and a hydrogen atom to form H_2O_2 and the anion radical of the dehydrogenated substrate (Sawyer *et al.* 1984; Calderwood *et al.* 1984; Sawyer *et al.* 1982*b*). Table 3 summarizes the stoichiometric and kinetic data for oxidation of these compounds by O_2^-.



SCHEME 10

The azobenzene, phenazine and lumiflavin anion radicals are rapidly oxidized by dioxygen; hence O_2^- acts as an initiator for the autoxidation of these compounds (see scheme 10). For 1,2-diphenylhydrazine, turnover numbers in excess of 200 substrate molecules per O_2^- species have been observed. The 1,2-diphenylhydrazine autoxidation cycle can be initiated by OH^-, which indicates that O_2^- is formed in the OH^--initiated process. Superoxide ion also initiates the autoxidation of dihydrophenazine, which is a model for dihydroflavin. For example, the addition of 1 mM $(Me_4N)O_2$ in DMF to 10 mM H_2Phen in an O_2-saturated DMF solution results in the complete oxidation of the substrate (about 80% recovered as phenazine) and the production of 9–10 mM H_2O_2.

TABLE 3. PRODUCTS AND KINETICS FOR THE ONE-TO-ONE COMBINATION OF 2 mM $(Me_4N)O_2$ AND 2 mM SUBSTRATE IN DIMETHYLFORMAMIDE (0.1 M TETRAETHYLAMMONIUM PERCHLORATE) AT 25 °C

substrate, S[a]	anion radical/S[b]	H_2O_2/S	k_1/[S] (mol^{-1} s^{-1})[a]
$DTBCH_2$	0.8	0.9	10^4
H_2Asc	0.8	0.9	1.8×10^4
H_2Phen	0.9	1.0	> 560
H_2Fl	0.8	0.9	> 340
PhNHNHPh	1.0	1.0	> 100

[a] $DTBCH_2$, 3,5-di-t-butylcatechol; H_2Asc, ascorbic acid; H_2Phen, dihydrophenazine; H_2Fl, dihydrolumiflavin.
[b] The u.v.–visible absorption spectra for the anion radical products were compared with those for the products from controlled potential electrolytic reduction of 3,5-di-t-butyl-*o*-benzoquinone, dehydroascorbic acid, phenazine, lumiflavin and azobenzene.

Support for the general mechanism outlined in scheme 10 is provided by gas-phase Fourier-transform–mass spectrometric studies of the anionic reaction products of several substrates with O_2^- (produced by electron impact with O_2; OH^- can be produced by electron impact with H_2O). In these experiments neutral products are not detected. Both O_2^- and OH^- react rapidly with 1,2-diphenylhydrazine in the gas phase ($P \approx 10^{-7}$ Torr†) to give the anion radical of azobenzene ($PhNN^{-\cdot}Ph$; $m/z = 182$) and the anion from deprotonation (PhN^-NHPh; $m/z = 183$), respectively. When O_2^- is ejected from the experiment, the peak at $m/z = 182$ disappears. In contrast to the exponential decay that is observed for the OH^- peak with time, the ion current for O_2^- decays to a steady-state concentration. Apparently, the $PhNN^{-\cdot}Ph$ product reacts with residual O_2 (which cannot be ejected from the F.t.–m.s. cell) to give O_2^- and azobenzene in a process that is analogous to the O_2^--induced autoxidation in aprotic solvents.

Analogous F.t.–m.s. studies with 1,2-dihydroxybenzenes also provide support for the general mechanism. Superoxide ion reacts rapidly with 3,5-di-t-butylcatechol ($DTBCH_2$) in the gas phase to give the anion ($DTBCH^-$; $m/z = 221$) and the anion radical of 3,5-di-t-butyl-*o*-benzoquinone ($DTBSQ^{-\cdot}$; $m/z = 220$) in an approximate ratio of 3:1. With hydroquinone [*p*-$Ph(OH)_2$] the dominant product (*ca.* 70%) is the anion radical ($SQ^{-\cdot}$; $m/z = 108$). When OH^- is the gas-phase reagent, the only product for $DTBCH_2$ (and for *o*-$Ph(OH)_2$) is the anion from deprotonation.

Parenthetically, an earlier study (Nanni & Sawyer 1980) formulated the reaction between O_2^- and dihydrophenazine (or dihydrolumiflavin) as

$$H_2Phen + O_2^- \rightarrow Phen + OH^{\cdot} + OH^-. \qquad (11)$$

The experiments now appear to have been flawed by subsequent reaction of the anion radical of the dehydrogenated substrate with adventitious oxygen (or oxygen produced by base-catalysed decomposition of hydrogen peroxide). The more recent study (Calderwood *et al.* 1984) of these reactions confirms that they are not a source of hydroxyl radicals.

The fact that the anion radicals of the dehydrogenated substrates are produced in the gas phase as well as in aprotic solvents confirms that the reaction sequence deprotonation–

† 1 Torr = 101325/760 Pa.

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hydrogen-atom abstraction to form H_2O_2 must either be a rapid sequence or a nearly concerted process. Because O_2^- is expected to abstract hydrogen atoms much less easily than $HO_2^{\cdot}$, the initial step is deprotonation of the substrate by O_2^- to form $HO_2^{\cdot}$; the latter (contained within the solvent cage or weakly bonded to the substrate in a 'sticky collision' in the gas phase) then abstracts a hydrogen atom from the substrate anion to form H_2O_2 and the anion radical of the dehydrogenated substrate.

Thus, the O_2^-- (or OH^--) induced autoxidations of 1,2-disubstituted hydrazines, dihydrophenazines, and dihydroflavins in aprotic media provide a simple pathway for rapid conversion of dioxygen to H_2O_2, and one that does not involve catalysis by metal ions or metalloproteins. This is exemplified by the net reaction for dihydrolumiflavin,

$$H_2Fl + O_2 \xrightarrow{O_2^-,\, OH^-} Fl + H_2O_2. \qquad (12)$$

(*b*) *Formation and reactivity of* $HO_2^{\cdot}$

In aprotic media, proton sources induce the rapid disproportionation of O_2^- to H_2O_2 and O_2 via formation of the perhydroxyl radical, $HO_2^{\cdot}$ (Chin *et al.* 1982),

$$\begin{array}{l} O_2^- + HA \rightarrow A^- + HO_2^{\cdot} \\ \qquad\qquad \downarrow k \\ \qquad\qquad \rightarrow \tfrac{1}{2}H_2O_2 + \tfrac{1}{2}O_2. \end{array} \qquad (13)$$

In aqueous media $HO_2^{\cdot}$ has a pK_a value of 4.9 and a disproportionation rate constant k of 10^6 mol^{-1} s^{-1}, but in dimethylformamide is estimated pK_a is 12 and k is greater than 10^7 mol^{-1} s^{-1}. Hence, formation of superoxide ion in an aprotic medium (biological membrane), that is at neutral pH, will result in $HO_2^{\cdot}$ as the dominant species.

Some years ago Howard & Ingold (1967) used radical-initiated autoxidation experiments in acetonitrile and chlorobenzene to demonstrate that $HO_2^{\cdot}$ abstracts hydrogen atoms from allylic hydrocarbons (1,4-cyclohexadiene). Because linoleic acid and arachidonic acid esters contain allylic groups and are important components of lipids, there is reason to believe that *in situ* generation of $HO_2^{\cdot}$ (via O_2^- plus H^+) can initiate lipid peroxidation and autoxidation. This has prompted some preliminary experiments on the reactivity of O_2^- with 1,4-cyclohexadiene in acidified dimethyl sulphoxide; the results are summarized in table 4. A reasonable reaction

Table 4. Oxidation of 1,4-cyclohexadiene (1,4-CHD) by $HO_2^{\cdot}$ (O_2^- plus HA) in dimethyl sulphoxide[a]

proton source (HA)	[HA]/mM	[O_2^-]/mM	[1,4-CHD]/mM	reaction efficiency (percentage)[b]	product distribution (percentage) 1,3-CHD	PhH
H_2O	100	5.3	10.6	25	74	26
H_2O	1000	3.3	6.6	90	92	8
$HClO_4$	1.6	3.2	6.4	100	79	21
$HClO_4$	3.2	3.2	6.4	92	69	31
$HClO_4$	6.4	3.2	6.4	31	54	46
$HClO_4$	8.4	8.4	17.0	40	75	25

[a] A 10 mM O_2^- solution (Me_2SO) was slowly added to an Me_2SO solution that contained 1,4-CHD and the proton source (HA). The indicated concentrations represent the initial values after mixing.
[b] 100% represents the reaction of one 1,4-CHD molecule per O_2^- added.

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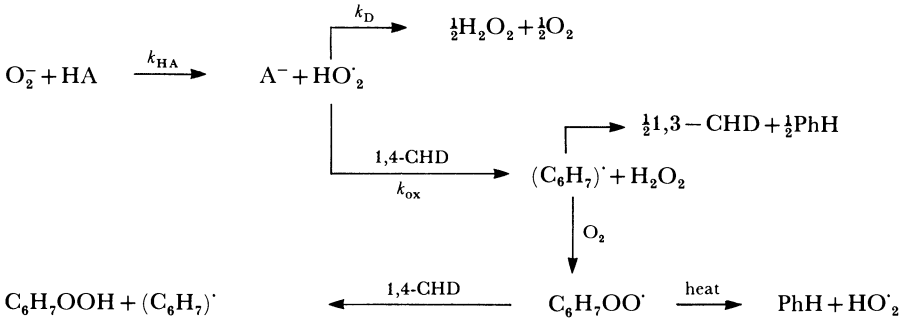
scheme involves the initial formation of $HO_2^{\cdot}$ with its subsequent disproportionation (second order in $HO_2^{\cdot}$) and attack of 1,4-cyclohexadiene (first order in $HO_2^{\cdot}$) (see scheme 11).

Other organic molecules with weak heteroatom—H bonds include Ph(Me)N—H, Ph(H)N—H and thiols (RS—H); these should be susceptible to $HO_2^{\cdot}$-initiated oxidations and autoxidations. Studies of the reactivity for $HO_2^{\cdot}$ with these substrates and with the esters of linoleic acid, arachidonic acid and other molecules with allylic groups are in progress.



SCHEME 11

(*c*) *Iron(II)-induced activation of* H_2O_2

Recent work (Sugimoto & Sawyer 1984) establishes that $Fe^{II}(MeCN)_4(ClO_4)_2$ in dry acetonitrile (MeCN) catalyses the rapid disproportionation of added 98% H_2O_2 to O_2 and H_2O, but all of the catalyst remains in the Fe^{II} oxidation state. Table 5 summarizes the results from the addition of dry H_2O_2 (98%, dissolved in MeCN) to solutions of various organic substrates in the presence of the Fe^{II} catalyst. Three classes of reaction occur on the basis of the substrate: (*a*) monoxygenations, (*b*) dehydrogenations and oxidations and (*c*) dioxygenations.

The products are in marked contrast to those observed for aqueous Fenton chemistry. However, the presence of 1% H_2O in the MeCN reaction system results in the oxidation of Fe^{II} and substrate products that are characteristic of the Fenton process. Fenton chemistry is generally believed to be induced by the $OH^{\cdot}$ radical that is produced from the reduction of H_2O_2 by Fe^{II} (Walling 1975):

$$Fe^{II} + H_2O_2 \rightarrow Fe^{III}(OH) + OH^{\cdot}. \qquad (14)$$

The unique feature of the anhydrous system for the activation of H_2O_2 is that the acetonitrile matrix for the Fe^{II} catalyst causes the Fe^{III}/Fe^{II} redox potential to be greater than +1.8 V against n.H.e., compared to about +0.4 V against n.H.e. in water at pH 7. This large shift of redox potential precludes the reduction of H_2O_2 by Fe^{II}. As a result, the Fe^{II} catalyst remains in its reduced state for all of the reactions in dry acetonitrile.

At present, little is known about the structure of the activated Fe^{II}–H_2O_2 complexes. The disproportionation reaction for H_2O_2 and the three types of substrate reactions indicate that more than one kind of complex may be present, perhaps in dynamic equilibrium. Scheme 12 presents possible models for the oxidase–monoxygenase function and the disproportionase (catalase)–dioxygenase function.

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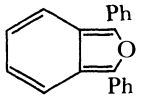
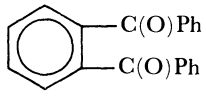
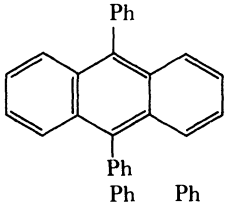
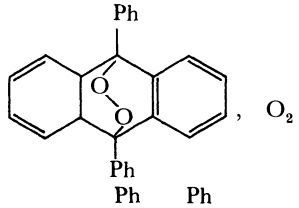
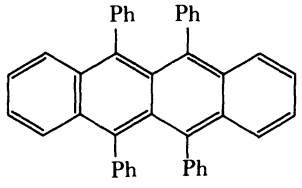
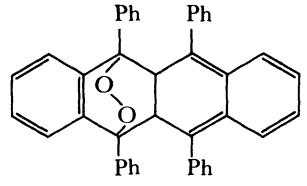
TABLE 5. PRODUCTS FROM THE IRON(II)-INDUCED MONOXYGENATION, DEHYDROGENATION, AND DIOXYGENATION OF ORGANIC SUBSTRATES (RH) BY H_2O_2 IN DRY ACETONITRILE[a]

substrate	reaction efficiency (percentage)	products
monoxygenation		
blank (H_2O_2)	100	O_2, H_2O, Fe(II)
Ph_3P	100	Ph_3PO
Me_2SO	100	Me_2SO_2
Ph_2SO	100	Ph_2SO_2
EtOH	70	MeCH(O) (90%), MeC(O)OH (10%), O_2
$PhCH_2OH$	100	PhCH(O)
c-$C_6H_{11}OH$	47	$C_6H_{10}(O)$, O_2
MeCH(O)	20	MeC(O)OH, O_2
$Me_2C(O)$	NR	O_2
PhCH(O)	28	PhC(O)OH, O_2
dehydrogenation and oxidation		
cyclohexane	NR	O_2
1,4-*c*-C_6H_8	59	PhH, O_2
PhNHNHPh	100	PhN=NPh
H_2S	100	H_2SO_4
H_2O (56 mM)	100	Fe(III)
dioxygenation		
	100	
	69	 , O_2
	83	 , O_2
$Ph_2C=CPh_2$	22	$Ph_2C(O)$, O_2
PhC≡CPh	42	PhC(O)C(O)Ph, O_2
PhC≡CMe	26	PhC(O)C(O)Me, O_2
PhC≡CH	11	PhC(O)CH(O), O_2
c-PhCH=CHPh	52	PhCH(O) (98%), PhC≡CPh (2%), O_2
t-PhCH=CHPh	28	PhCH(O), O_2
PhCH=CHMe	32	PhCH(O) + MeCH(O) (85%), PhCHCHOMe (15%), O_2

[a] (Sugimoto & Sawyer 1984). Product solution [from the slow addition (*ca.* 5 min to give a final 2 mM concentration) of 1 M H_2O_2 (98% H_2O_2 in MeCN) to a solution of 1 mM [Fe^{II} $(MeCN)_4$] $(ClO_4)_2$ plus 2 mM substrate] analysed by gas chromatography and assayed for residual Fe^{II} by MnO_4^- titration and by colorimetry with 1,10-*o*-phenanthroline.

[b] 100% represents one substrate oxygenation or dehydrogenation per H_2O_2 added. For dioxygenations, 100% represents one substrate converted per two H_2O_2 added.

[c] 100% represents one H_2S converted to H_2SO_4 per four H_2O_2 added.

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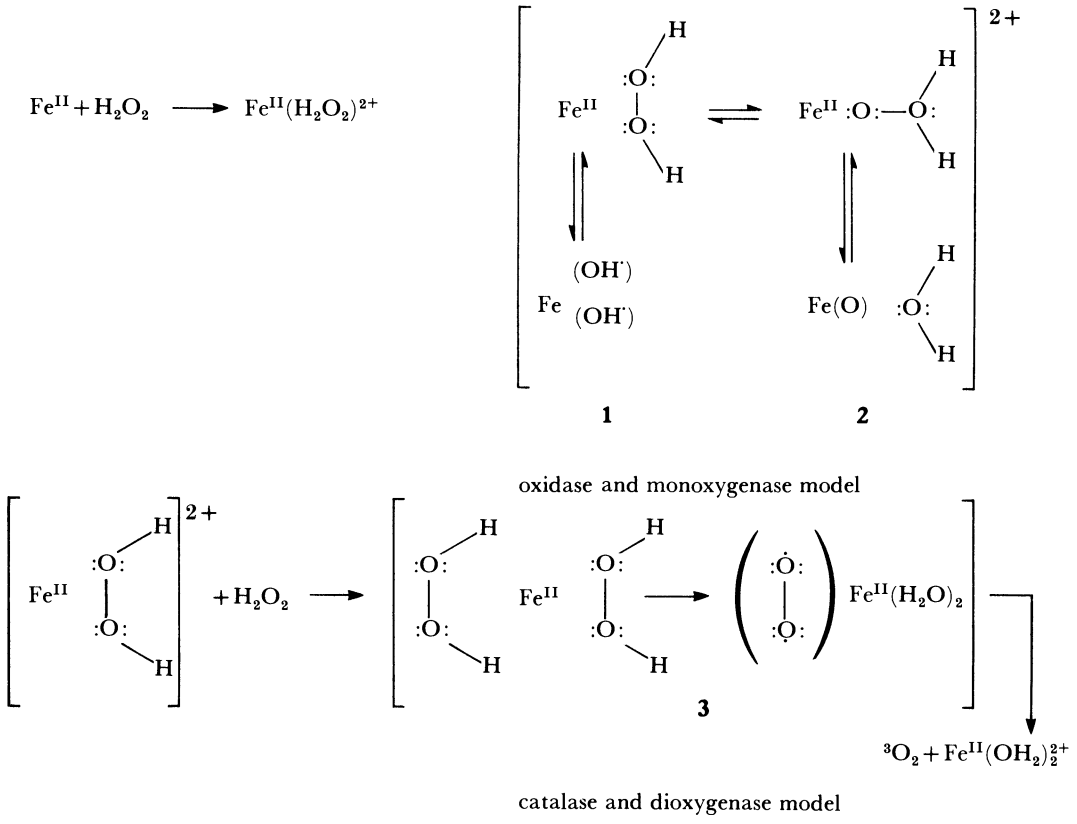

SCHEME 12

The elements of this chemistry can be coupled with the observed base-induced autoxygenation described in §2*c* to form a model chemical system for cytochrome P_{450} monoxygenase (Sawyer *et al.* 1984*b*). This is characterized by the net overall reaction

$$RH + DH_2 + O_2 \rightarrow ROH + D + H_2O, \qquad (15)$$

where RH represents the substrate and DH_2 a two-electron reductant (donor), such as reduced flavin or ascorbic acid.

The model consists of (i) an O_2-activation segment that produces H_2O_2 from the base-initiated autoxidation of 1,2-diphenylhydrazine (a model for reduced flavin),

$$PhNHNHPh + O_2 \xrightarrow{O_2^-,\, OH^-} PhN{=}NPh + H_2O_2 \qquad (16)$$

and (ii) a H_2O_2-activation component via the Fe^{II}–H_2O_2 complex in dry acetonitrile, shown as complex **2** in scheme 12. The combination of (i) and (ii) provides a catalytic system for the autoxygenation of organic substrates with reaction cycles that are similar to those for cytochrome P_{450} monoxygenases. Thus, when catalytic quantities of base (O_2^- or OH^-) are introduced into a dry acetonitrile solution that contains excess substrate (RH), ambient air (O_2), 1,2-diphenylhydrazine (PhNHNHPh) and Fe^{II}, the substrate is rapidly and efficiently monoxygenated (e.g. triphenylphosphine → triphenylphosphine oxide; benzyl alcohol → benzaldehyde; diphenylsulphoxide → diphenylsulphone) or dehydrogenated-(1,4-cyclohexadiene → benzene).

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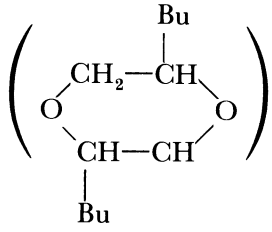
(*d*) $Fe^{III}Cl_3$-*induced activation of* H_2O_2

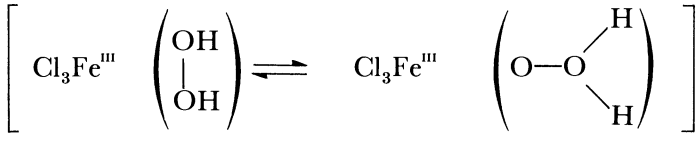
The observation (Sugimoto & Sawyer 1984) that iron(II) in ligand-free acetonitrile activates hydrogen peroxide to act as a monoxygenase and dehydrogenase (but not as an initiator of radical reactions via Fenton chemistry) has prompted the considerations of other iron salts. Thus, anhydrous ferric chloride ($Fe^{III}Cl_3$) in dry acetonitrile (MeCN) activates hydrogen peroxide to epoxidize alkenes, and to monoxygenate or dehydrogenate other organic substrates (Sugimoto & Sawyer 1985).

Table 6*a* summarizes the conversion efficiencies and product distributions for a series of alkene substrates subjected to the $Fe^{III}Cl_3$–H_2O_2/MeCN system. The extent of the $Fe^{III}Cl_3$-induced monoxygenations is enhanced by higher reaction temperatures and increased concentrations of the reactants (substrate, $Fe^{III}Cl_3$ and H_2O_2). For 1-hexene (representative of all of the alkenes) a substantial fraction of the product is the dimer of 1-hexene oxide, a disubstituted dioxane



With other organic substrates (RH), $Fe^{III}Cl_3$ activates H_2O_2 for their monoxygenation and the reaction efficiencies and product distributions are summarized in table 6*b*. In the case of alcohols, ethers and cyclohexane, a substantial fraction of the product is the alkyl chloride, and with aldehydes [PhCH(O)] the acid chloride represents one-half of the product. In the absence of substrate the $Fe^{III}Cl_3$/MeCN system catalyses the rapid disproportionation of H_2O_2 to O_2 and H_2O.

Because $Fe^{III}Cl_3$ is an exceptionally strong Lewis acid and electrophilic centre, it activates H_2O_2 (which acts as a nucleophile) for the dehydrogenation of a second H_2O_2. On the basis of this disproportionation process, as well as the monoxygenation and dehydrogenation reactions of table 6, the activation of H_2O_2 by $Fe^{III}Cl_3$ probably involves the initial formation of at least two reactive forms of an $Fe^{III}Cl_3$ (HOOH) adduct that are in dynamic equilibrium,



The disproportionation of H_2O_2 occurs via a concerted transfer of the two hydrogen atoms from a second H_2O_2 to the $Fe^{III}Cl_3(H_2O_2)$ adduct. This dehydrogenation of H_2O_2 is a competitive process with the $Fe^{III}Cl_3$–substrate–H_2O_2 reactions. The controlled introduction of dilute H_2O_2 into the $Fe^{III}Cl_3$–substrate solution limits the concentration of H_2O_2 and ensures that the substrate–H_2O_2 reaction can be competitive with the second-order disproportionation process. The substrate reaction efficiencies in table 6 are proportional to the relative rates of reaction ($k_{RH}/k_{H_2O_2}$). The mode of activation of H_2O_2 by $Fe^{III}Cl_3$ is analogous to that of

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TABLE 6. PRODUCTS AND CONVERSION EFFICIENCIES FOR THE FERRIC CHLORIDE ($Fe^{III}Cl_3$)-INDUCED OXYGENATION–DEHYDROGENATION OF OLEFINS AND ORGANIC SUBSTRATES (RH) BY H_2O_2 IN ACETONITRILE

substrate, RH	reaction efficiency (percentage)[a, b]	products[c]
	(*a*) *olefins* (−5 °C; 10 *min reaction times*)	
blank (H_2O_2)	100	O_2, H_2O
1-hexene	10	epoxide (1-hexene oxide) (71%), dimer (dioxane) (10%), others (19%)
1-hexene (+5°)	23	epoxide (55%), dimer (15%), others (30%)
1-octene	60	epoxide (53%), dimer (10%)
cyclohexene	25	epoxide (45%), dimer (30%)
$Me_2C{=}CMe_2$	40	$Me_2C(O)CMe_2$ (50%), dimers and others (50%)
	(*b*) *other substrates* (+5 °C, 20 *min reaction times*)	
cyclohexanol	52	cyclohexanone (88%)
$PhCH_2OH$	63	PhCH(O) (51%), $PhCH_2Cl$ (21%), PhC(O)OH (14%), PhC(O)Cl (14%)
$PhCH_2OCMe_3$	56	PhCH(O) (72%), $PhCH_2Cl$ (11%), PhC(O)OH (3%), PhC(O)Cl (14%)
PhCH(O)	75	PhC(O)OH (55%), PhC(O)Cl (45%)
$PhCH_3$ (25 °C)	2	$PhCH_2OH$, PhCH(O), PhC(O)Cl, PhC(O)OH, cresols
cyclohexane	22	cyclohexylchloride (45%), cyclohexanol (40%), cyclohexanone (15%)
Ph_2S	58	Ph_2SO (100%)
Ph_2SO	60	Ph_2SO_2 (100%)
Ph_3P	80	Ph_3PO (100%)

[a] RH and $Fe^{III}Cl_3$ (1.0 mmol of each) combined in 10–20 ml dry MeCN, followed by the slow addition of 1 mmol H_2O_2 [1 M H_2O_2 (98%) in MeCN].
[b] Percentage of substrate converted to products.
[c] After the indicated reaction time, the product solution was quenched with water, extracted with diethylether and analysed by capillary gas chromatography and g.c.–m.s.

$Fe^{II}(MeCN)_4^{2+}$; both are strong electrophiles in ligand-free dry MeCN and induce H_2O_2 to monoxygenate organic substrates.

The epoxidation of alkenes (table 6*a*) appears to involve an O-atom transfer from the end-on configuration of the $Fe^{III}Cl_3(HOOH)$ adduct. The electrophilicity of $Fe^{III}Cl_3$ promotes the initial activation of the alkene bond before the binding of H_2O_2. The resulting epoxides are rapidly dimerized to dioxanes. Hence, the complete conversion of an alkene to its epoxide is precluded; the more complete the conversion the higher the fraction of dioxane in the product mixture.

The results in table 6*b* indicate that the $Fe^{III}Cl_3(HOOH)$ adduct monoxygenates alkanes, alcohols and aldehydes. A mechanism that is consistent with this involves the homolytic scission of the HO–OH bond in the side-on configuration, induced by the bound substrate, and the subsequent abstraction by one OH· of an H-atom from the α-carbon and addition of the second HO· to the resulting carbon radical (equation (17)).

$$PhCH_2OH + Fe^{III}Cl_3(H_2O_2) \rightarrow [PhCH(OH)_2]Fe^{III}Cl_3(OH_2) \rightarrow PhCH(O) + Fe^{III}Cl_3(OH_2)_2. \quad (17)$$

An analogous process appears to occur for the oxygenation of benzaldehyde by the $Fe^{III}Cl_3$ (HOOH) adduct, but 50% of the product is the acid chloride.

This result indicates that the activated side-on complex has some hypochlorous acid (HOCl) character and can add a chlorine atom to the carbon radical that results from the H-atom abstraction by the $OH^{\cdot}$ group. This also occurs with alkanes, alcohols, and ethers (table 6*b*). Such chemistry is similar to the activation of chloride ion and H_2O_2 to HOCl by a haem protein, myeloperoxidase (Rosen & Klebanoff 1977; Held & Hurst 1978).

Phosphines, dialkylsulphides and sulphoxides are monoxygenated by the $Fe^{III}Cl_3$(HOOH) adduct in a manner that appears to be analogous to that for the epoxidation of alkenes.

3. Model systems for biological O_2 and H_2O_2 activation

The base-catalysed autoxidations and the Lewis-acid-catalysed (iron(II) and $FeCl_3$) reactions of H_2O_2 with various substrates exhibit parallels to reactions that are catalysed by metalloenzymes. They may, therefore, be useful models for various enzyme-catalysed reactions. For example, xanthine oxidase (XO) normally acts *in vivo* to catalyse the oxidation of reduced flavin,

$$H_2Fl + O_2 \xrightarrow{XO} H_2O_2 + Fl, \tag{18}$$

but is known to produce a flux of superoxide ions in the presence of xanthine and O_2 (Fridovich 1983). Thus there is a superficial resemblance between the xanthine oxidase-catalysed reaction and the superoxide ion-catalysed autoxidation of reduced flavin that is described in §2*b*(iii).

The Fe^{II}- and $FeCl_3$-catalysed disproportionation of H_2O_2 in dry acetonitrile (see §§2*c* and 2*d*) is analogous to that of catalase,

$$2H_2O_2 \xrightarrow{Fe^{II}} O_2 + 2H_2O \tag{19}$$

and in the presence of appropriate substrates these Lewis acids display a peroxidase-like activity,

$$H_2O_2 + RH \xrightarrow{Fe^{II}} H_2O + ROH \tag{20}$$

that is illustrated by the oxidation of benzaldehyde to benzoic acid.

Finally, the modelling of the cytochrome P_{450} monoxygenation reaction cycles, which is discussed in §2*c*, involves (i) an O_2-activation component whereby a donor molecule (DH_2; PhNHNHPh) is autoxidized to produce H_2O_2 and (ii) a peroxidase-like component whereby Fe^{II} activates H_2O_2 for the monoxygenation

$$DH_2 + O_2 \xrightarrow{O_2^-} H_2O_2 + D, \tag{21}$$

$$H_2O_2 + RH \xrightarrow{Fe^{II}} ROH + H_2O. \tag{22}$$

Although the reactions shown in table 5 parallel the 'oxene' monoxygenase chemistry that is catalysed by cytochrome P_{450}, the lack of significant reactivity with cyclohexene and norbornene indicates that $Fe^{II}(H_2O_2)^{2+}$ is an inadequate model for the reactive iron-oxygen centre of the enzyme (Sawyer *et al.* 1984). The $Fe^{III}Cl_3$–H_2O_2 system that is discussed in §2*d* is a much more effective monoxygenase and epoxidizing agent.

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These models demonstrate that the chemistry of $Fe^{II}(MeCN)_4^{2+}$ and $Fe^{III}Cl_3$ is dramatically altered when water is removed to provide an aprotic and ligand-free environment and support the thesis that oxygen-activation processes in aprotic solvents are useful models for the chemistry of dioxygen in biological membranes and in the hydrophobic regions of metalloproteins.

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Discussion

P. Wardman (*Gray Laboratory, Mount Vernon Hospital, Northwood, Middlesex HA6 2RN*). Professor Sawyer has stressed the increased oxidizing power of the superoxide radical compared with oxygen: this is indeed a most important point. However, this behaviour is not 'unique', at least in respect of physiological conditions. It seems the norm that oxidants capable of adding two electrons sequentially generally become more powerful oxidants when the first electron is added. Common examples include quinones, flavins, nicotinamides and dehydroascorbic acid as well as oxygen. (An alternative statement of this generalization is that the semiquinone formation constant, $[Q^{-\cdot}]^2/([Q]\,[QH_2])$, is less than unity in water at pH 7.) The only exceptions I can recall are those of the 2,2′- and 4,4′- bipyridinium dications (viologens; paraquat, diquat, etc.), where the reduction potential for adding the second electron is more negative than that for the first. This characteristic is responsible for some of their exceptional properties, of course.

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