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IMPORTANCE OF SINGLE ELECTRON-TRANSFER IN SINGLET OXYGEN REACTION IN AQUEOUS SOLUTION

OXIDATION OF ELECTRON-RICH THIOANISOLES

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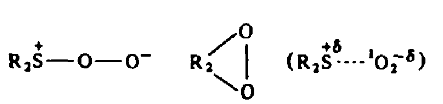
Abstract—Oxidation of substituted thioanisoles by chemically generated singlet oxygen was investigated in polar aqueous media. The formation of the superoxide ion was observed during sulphoxidation of 4-hydroxythioanisole (4) in phosphate buffer at pH 7.5. Control experiments indicated that the superoxide ion was formed by a direct reaction between singlet oxygen and 4. The kinetics of the trapping reaction by diphenylsulphoxide indicated the involvement of a single intermediate. The overall rate constants of the reaction of thioanisoles with singlet oxygen in methanol–water (1 : 1) are one order of magnitude larger than those in benzene. On the basis of these results, a mechanism involving a charge-transfer complex has been proposed for the reaction of electron-rich thioanisoles with singlet oxygen, whereby the charge-transfer complex would produce persulphoxide directly or dissociate to the cation radical and superoxide ion in polar aqueous media.

Single electron-transfer from substrate to singlet oxygen ($^1O_2$) giving rise to a substrate cation radical and the superoxide ion ($O_2^{-\cdot}$) has become more and more important in the interaction of $^1O_2$ with electron-rich substrates in aqueous solvents.[1,2] Since our first observation of $O_2^{-\cdot}$ formation in the reaction between $^1O_2$ and N,N-dimethyl-*p*-anisidine,[3] a number of electron-rich substrates have been shown to undergo single electron-transfer to $^1O_2$ to produce $O_2^{-\cdot}$. These examples include N,N,N′,N′-tetramethyl-*p*-phenylenediamine,[4,5] substituted N,N-dimethylanilines,[5] NAD(P)H,[6–8] and 5-hydroxytryptophan.[8] All of these substrates have oxidation potentials as low as *ca* 0.5 V vs SCE and can serve as a quencher of $^1O_2$. In our experiments, substrates with oxidation potentials more than 0.5 V vs SCE never produced $O_2^{-\cdot}$ even in polar aqueous solvents. In order to know the generality of the single electron-transfer process involving $^1O_2$, we have examined the reaction of electron-rich sulphides with $^1O_2$ in aqueous solvents.

The mechanism of $^1O_2$ oxidation of diethylsulphide has been extensively studied by Foote *et al.*[9–12] and is known to be highly dependent on solvent proticity and temperature. In protic solvent a single intermediate, persulphoxide (1), has been proposed, whereas the possibility of the involvement of two intermediates, persulphoxide (1) and cyclic sulphurane (2), has been suggested in aprotic solvent based on the kinetics of



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† In the present experiments we calculated the amount of $O_2^{-\cdot}$ by using the stoichiometry in which 1 mol of NBT is reduced by 4 mol of $O_2^{-\cdot}$ to form 1 mol of formazan.[15]

trapping experiments. However, single electron-transfer from sulphide to $^1O_2$ via a charge-transfer complex (3) would become important particularly in polar aqueous solvents, if the sulphide possessed a low enough oxidation potential to undergo electron-transfer to $^1O_2$. In fact, single electron-transfer from thiophenolate to $^1O_2$ yielding $O_2^{-\cdot}$ has previously been proposed in the photo-oxidation of the thiophenolate ion.[13]

We describe herein the observation of $O_2^{-\cdot}$ formation in the $^1O_2$ oxidation of electron-rich thioanisoles such as *p*-hydroxythioanisole ($E_{1/2} = 0.48$ V vs SCE) and N,N-dimethylamino-*p*-thioanisole ($E_{1/2} = 0.47$ V vs SCE) by employing the assay method recently developed in our laboratory.[5]

RESULTS AND DISCUSSION

Formation of superoxide ion

p-Hydroxythioanisole (4) was readily oxidized to sulphoxide (5) in the presence of 3-(1,4-epidioxy-4-methyl-1,4-dihydro-1-naphthyl)propionic acid (EP), a water soluble $^1O_2$ source,[5] at 35° at neutral pH in methanol–water (1 : 1). Attempts were then made to detect $O_2^{-\cdot}$ during oxidation of 4 by utilizing a combination of nitro blue tetrazolium (NBT) and superoxide dismutase (SOD) in phosphate buffer at pH 7.5. As shown in Fig. 1, the reduction of NBT to formazan (UV $\lambda_{max} = 560$ nm) was observed when 4 was added to the solution containing EP and NBT. The formation of formazan increased with increasing incubation time. The addition of SOD to the reaction system results in a decrease of the formazan formation. This indicates the involvement of $O_2^{-\cdot}$ in the reaction of $^1O_2$ with 4, since SOD is known to react specifically with $O_2^{-\cdot}$ with rate constant of $2 \times 10^9\ M^{-1}s^{-1}$.[14] The yield of $O_2^{-\cdot}$ was calculated to be 2.1% on the basis of the amount of 4 initially used.† The various control

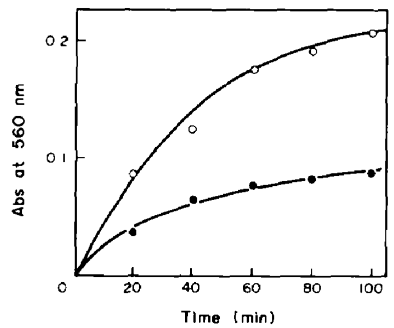


Fig. 1. Reduction of NBT during incubation of EP and 4 in the presence (●) and absence (○) of SOD (140 units) in phosphate buffer (pH 7.5) at 35° [4] = 1 mM; [EP] = 2.5 mM; [NBT] = 2 mM.

Table 1. Effect of various additives on the formation of $O_2^{-\cdot}$ in the reaction of 4 with $^1O_2$ in phosphate buffer (pH 7.5) at 35°

System	Additives	% yield of $O_2^{-\cdot a}$
EP, 4, NBT	—	2.1
	—	0^b
	NaN_3 (10 mM)	0.2
	Dabco (10 mM)	0.9
	2-PrOH (2 M)	2.0
	Mannitol (10 mM)	1.9
4, NBT	O_2 bubbling	0

[EP] = 2.5 mM; [4] = 1 mM; [NBT] = 2 mM.
^{a}Yield based on the amount of 4 initially used.
^{b}At 15°.

experiments indicate that $O_2^{-\cdot}$ is formed by a direct reaction between 4 and $^1O_2$ (Table 1). For example, addition of $^1O_2$ quenchers (NaN_3, Dabco) suppressed the formation of $O_2^{-\cdot}$. Furthermore, the possibility of the formation of radical ion intermediate by the reaction between EP and 4[16] may be ruled out since the decomposition rate of EP is not affected by addition of 4. All the results shown in Table 1 strongly suggest the direct involvement of $^1O_2$ in the formation of $O_2^{-\cdot}$.

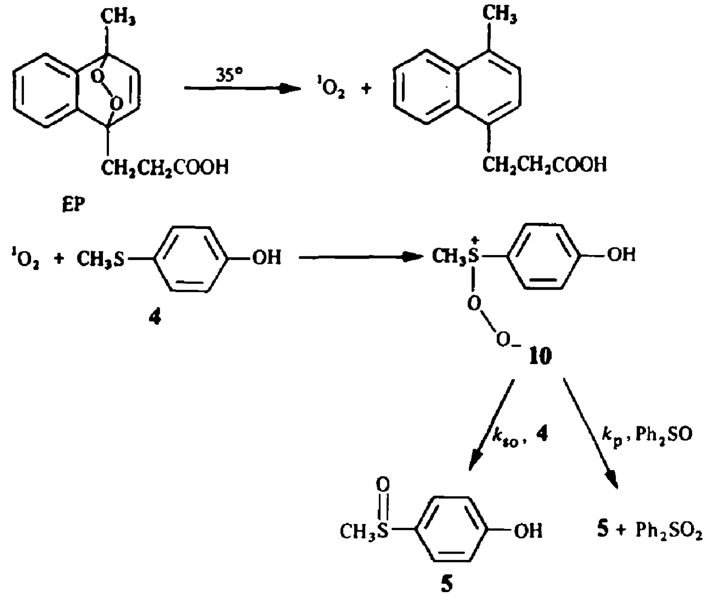
In the reaction of *p*-dimethylaminothioanisole (6, $E_{1/2} = 0.47$ V vs SCE) in phosphate buffer–ethanol (85:15), the formation of $O_2^{-\cdot}$ was also observable but in low yield (0.4%). In contrast, thioanisoles with higher oxidation potentials, such as *p*-methoxythioanisole (7, $E_{1/2} = 1.05$ V vs SCE), *p*-methylthioanisole (8, $E_{1/2} = 1.15$ V vs SCE) and thioanisole (9, $E_{1/2} = 1.20$ V vs SCE), did not produce $O_2^{-\cdot}$, while these thioanisoles were readily oxidized to the corresponding sulphoxides under such conditions. These results are consistent with the previous finding that the $O_2^{-\cdot}$ formation is strongly dependent on the oxidation potentials of substrates.[3,5,8]

Trapping by diphenylsulphoxide

Foote and co-workers[10–12] have proposed a persulphoxide intermediate, 1, in the $^1O_2$ oxidation of diethylsulphide in protic solvent on the basis of the trapping reaction in which the intermediate 1 reacts with diphenylsulphoxide as a nucleophile.[10–12] In order to get information on the intermediate formed in the reaction of 4 with $^1O_2$, trapping with diphenylsulphoxide was examined in methanol. If Scheme 1 holds, the steady-state equation 1 can be derived according to the kinetics as reported by Foote and co-workers[10–12]

$$\frac{[CH_3\overset{\overset{\displaystyle O}{\|}}{S}C_6H_4OH]}{[Ph_2SO_2]} = 1 + \frac{2k_{so}[CH_3SC_6H_4OH]}{k_p[Ph_2SO]} \quad (1)$$

A singlet oxygen reaction of two different concentrations of 4 in the presence of various amounts of diphenylsulphoxide was carried out. Figure 2 shows the results plotted according to equation 1. The result implies that 4 and diphenylsulphoxide are competing



Scheme 1.

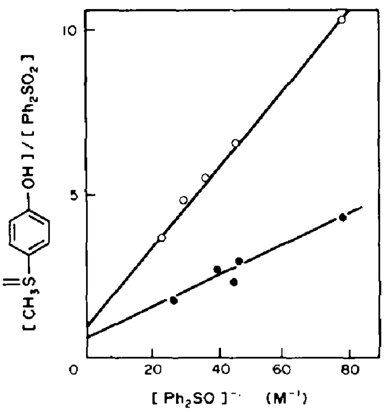


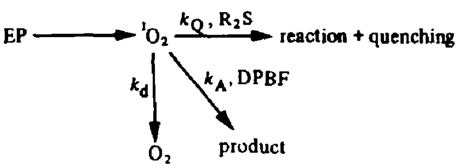
Fig. 2. Trapping by diphenylsulfoxide in the singlet oxygenation of 4 in methanol. [4] = 2 mM (●); 10 mM (○). [EP] = 6.5 mM.

for a single intermediate, presumably persulphoxide (10).

Determination of the rate constant

The overall rate constants ($k_Q = k_r + k_q$) for the reaction of $^1O_2$ with thioansioles were determined in methanol–water (1:1) and in benzene by equation 2 derived from the steady-state treatment of Scheme 2.[17]

$$\frac{S_O}{S_s} = 1 + \frac{k_r + k_q}{k_d}[R_2S] \qquad (2)$$



Scheme 2.

where S_O and S_s are slopes of the first-order plots of disappearance of $^1O_2$ acceptor, DPBF, in the absence and presence of sulphide, respectively. The overall rate constants (k_Q) are calculated from an average k_d of $^1O_2$ in methanol–water ($k_d = 2.8 \times 10^5$ s^{-1})[18] and in benzene ($k_d = 5.4 \times 10^4$ s^{-1})[18a,19] and summarized in Table 2 together with the reported values.[20,21] The rate constants (k_Q) in benzene are similar to the reported values within experimental errors. However, k_Q values in methanol–water are larger by one order of magnitude than those in benzene. Thus, the presence of water shows a remarkable effect on the overall rate constants, suggesting the involvement of a quite polar transition state in the $^1O_2$ reaction of 4. The rate constant of reaction (k_r) between 4 and $^1O_2$ was determined by competitive reaction with DPBF as 1.3 $\times 10^8$ $M^{-1}s^{-1}$ in aqueous methanol. This indicates that reaction is more favourable than quenching in $^1O_2$ oxidation of 4, consistent with the result obtained by Foote and co-workers[9–12] in the reaction of diethylsulphide in methanol.

Mechanism of oxidation

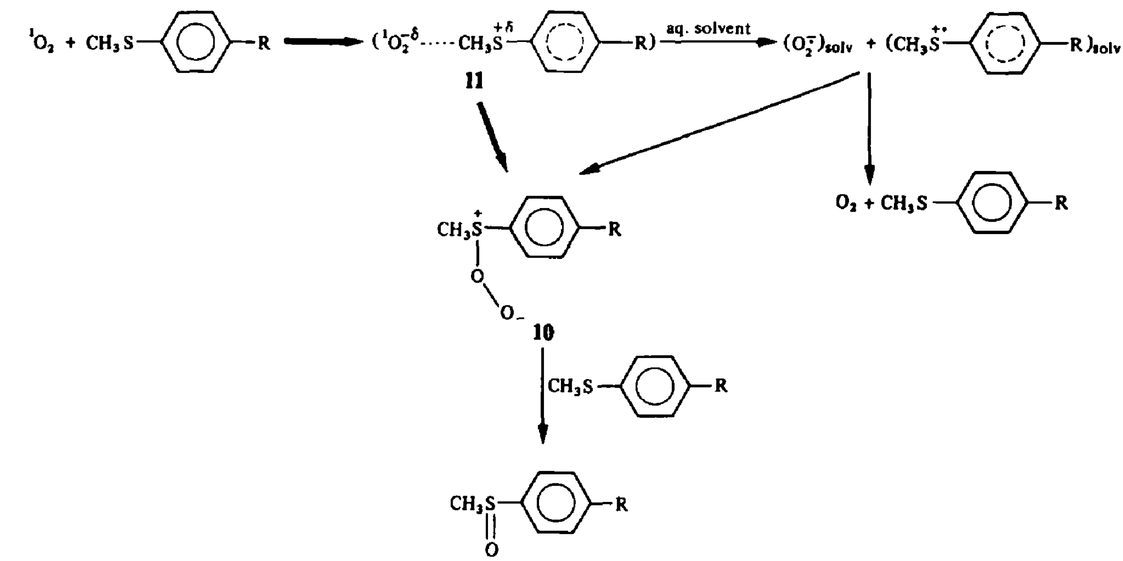
4-Hydroxythioanisole (4) produced sulphoxide in high yield upon oxidation with $^1O_2$ generated from EP in aqueous methanol. The kinetics of the oxidation in the presence of diphenylsulphoxide showed that competitive trapping of a single intermediate species occurs in the protic solvent. This seems to indicate the participation of persulphoxide (10) as precursor of sulphoxide (5). The overall rate constant (k_Q) of the oxidation of 4 is one order of magnitude larger than that in benzene. It was also confirmed that the rate constant for reaction ($k_r = 1.3 \times 10^8$ $M^{-1}s^{-1}$) is much larger than the quenching rate constant ($k_q = 0.32 \times 10^8$ $M^{-1}s^{-1}$).

All of these results strongly suggest that a charge-transfer-like complex (11) is initially formed between 4 and $^1O_2$ as shown in Scheme 3. This complex would then transform to persulphoxide (10) which gives sulphoxide on reaction with another molecule of thioanisole. In a highly polar solvent such as aqueous methanol, the CT complex (11) would dissociate to some extent to the cation radical and $O_2^{-\cdot}$. In fact, the formation of $O_2^{-\cdot}$ was confirmed in the $^1O_2$ oxidation of 4 in phosphate buffer. However, the yield of $O_2^{-\cdot}$ (2.1%) is extremely low compared with the sulphoxide yield (*ca* 85%), even though a small rate constant of $O_2^{-\cdot}$ with

Table 2. Rate constant (k_Q) of the reaction of $^1O_2$ with thioanisoles and the yield of $O_2^{-\cdot}$

Thioanisole	k_Q[a] ($\times 10^{-7}$ $M^{-1}s^{-1}$)	k_Q[b] ($\times 10^{-7}$ $M^{-1}s^{-1}$)	$E_{1/2}$[c] (V vs SCE)	$O_2^{-\cdot}$ yield[d] (%)
p-OH (4)	16.2	1.1	0.48	2.1
p-$N(CH_3)_2$ (6)	36.0	3.0	0.47	0.4
p-OCH_3 (7)	4.6	0.2 (0.53,[e] 0.76[f])	1.05	0
p-CH_3 (8)	3.2	0.15 (0.31,[e] 0.46[f])	1.15	0
p-H (9)	2.3	0.08 (0.20,[e] 0.23[f])	1.20	0

[a] Determined in $MeOH–H_2O$ (1:1). Errors ± 5%.
[b] Determined in benzene.
[c] Measured in Britten–Robinson buffer (pH 7.5).
[d] Obtained from the reaction of EP (2.5 mM), substrate (1 mM) and NBT (2 mM).
[e] In MeOH, Ref. 20.
[f] In $CHCl_3$, Ref. 21.



Scheme 3.

NBT ($k_{NBT} = 6 \times 10^4$ $M^{-1}s^{-1}$)[22] is considered. It is, therefore, reasonable to assume that a large portion of the CT complex would produce persulphoxide directly. Dissociation to the cation radical and $O_2^{-\cdot}$ is, indeed, a feasible process in a polar aqueous solvent but this process is a minor path for **4** even in aqueous solution.

The radical cation and $O_2^{-\cdot}$ pair thus formed would undergo back electron-transfer resulting in quenching of $^1O_2$ or would combine to give persulphoxide (**10**). In fact, independent reaction of the cation radical of **4**, generated by the action of tris-(*p*-bromophenyl) ammoniumyl hexachloroantimonate with KO_2 in dry acetonitrile produced sulphoxide (**5**) in 47% yield. A similar reaction between the cation radical and $O_2^{-\cdot}$ has been reported.[23] Thioanisole (**8**, **9**), with higher oxidation potentials, also produced the corresponding sulphoxides in high yields under these conditions. However, in none of these cases was $O_2^{-\cdot}$ formation observed, indicating that single electron-transfer is not involved in these cases. As observed in other cases,[3,5,8] single electron-transfer is only possible for thioanisoles with oxidation potentials less than 0.5 V vs SCE.

EXPERIMENTAL

UV spectra were recorded with a Shimazu UV-200 spectrophotometer. High performance liquid chromatography (HPLC) was performed on a Shimazu LC-3A equipped with a Radial Pak A (Waters). The cyclic voltamagram was recorded with a Yanagimoto P-1000 in 0.04 M Britten–Robinson buffer as described previously.[5] All potentials in volts are referred to the saturated calomel reference electrode (SCE).

Superoxide dismutase (SOD, type I, 3400 units) was purchased from Sigma. Nitro blue tetrazolium (NBT), *p*-hydroxythioanisole, and thioanisole were purchased from Wako Chemicals. Other thioanisoles were prepared by standard methods. The $^1O_2$ (EP) was prepared by the reported procedure.[5] Other chemicals were commercially available and used without further purification. Doubly distilled water was used in all cases.

Detection of $O_2^{-\cdot}$. Solns containing EP (2.5 mM), **4** (1 mM) and NBT (2 mM) with and without SOD (140 units) in phosphate buffer (pH 7.5) were incubated at 35° under rigorous shaking. After a fixed time of incubation, a constant volume of DMF was added to dissolve formazan. The amount of $O_2^{-\cdot}$ was calculated from the difference between the absorbance in the absence and that in the presence of SOD. A mixture of phosphate buffer and EtOH (85 : 15) was also used when the substrate is insoluble in phosphate buffer. Control experiments were carred out as follows. Solns containing EP (2.5 mM), **4** (1 mM) and NBT (2 mM) with and without SOD in 0.26 M phosphate buffer (pH 7.5) were prepared, and appropriate amounts of additives were added. The soln was incubated as described above.

Trapping by diphenylsulphoxide. Solns containing EP (6.5 mM), **4** (2 or 10 mM) and diphenylsulphoxide (1.2–4.2 mM) in MeOH were incubated at 35° for 2 hr. The appearance of sulphoxide (**5**) and diphenylsulphone was monitored by HPLC (eluent, $MeOH$–H_2O, 7 : 3) with naphthalene as internal standard. The peak areas were integrated with a Shimazu C-RIA autointegrator.

Reaction of $^1O_2$ *with* **4**. A soln containing EP (2.5 mM) and **4** (1 mM) in $MeOH$–H_2O (1 : 1) was shaken at 35° for 2 hr. The yield of sulphoxide (**5**) was determined by HPLC as described above.

Determination of rate constant. The total rate constant (k_Q) for the reaction of $^1O_2$ with thioanisoles was determined by the technique of Young *et al.*[17] Solns containing EP (4.5×10^{-4} M), DPBF (5×10^{-5} M) and various amounts of **4** (0–18 mM) in $MeOH$–H_2O (1 : 1) were shaken at 35°. The disappearance of DPBF was monitored at 411 nm with a UV spectrophotometer. The rate constants of reaction (k_r) were obtained from a competition reaction. To a $MeOH$–H_2O soln of EP (3 mM) and **4** (3.3, 6.7, 10.0 and 14.0 mM) was added a MeOH soln of DPBF (0.25, 0.64, 1.28 and 1.90 mM), respectively, and the solns were incubated at 35°. Changes in the concentrations of **4** and DPBF were periodically measured by the decrease of the peak area in HPLC and of the absorbance at 411 nm respectively.

Reaction of KO_2 *in the presence of tris-(*p*-bromophenyl) ammoniumyl hexachloroantimonate.* Solns of **4** (0.5 mM), KO_2 (5 mM) and tris-(*p*-bromophenyl) ammoniumyl hexachloroantimonate $[(p\text{-}BrC_6H_5)_3N^+ \cdot SbCl_6^-]$ in dry CH_3CN were prepared separately and each soln was evacuated by the freeze–pump–thaw technique. A soln of **4** was transferred into the soln of $(p\text{-}BrC_6H_5)N^+ \cdot SbCl_6^-$ followed by addition of a suspension of KO_2 in CH_3CN. The mixture was poured into

MeOH and filtered off. After evaporation of the solvent, the residue was subjected to preparative TLC (silica gel; ether–MeOH, 9:1) to give **5** (47%).

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