Kinetics and Mechanism of the Reactions of Superoxide Ion with Hydrogen Peroxide and Hydroperoxides in an Aprotic Medium

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The interaction of electrochemically generated superoxide ion with hydrogen peroxide and t-butyl and cumyl hydroperoxide has been studied in acetonitrile. It was shown that the first step of the reaction was the splitting off of a proton. HOO^- which was forming in subsequent steps reacted further with H_2O_2 *via* a chain mechanism again forming $O_2^{\cdot-}$ and possibly HO·. The interaction of excess of $O_2^{\cdot-}$ with alkyl hydroperoxides resulted in an autocatalytic process in which hydroperoxides played the role of catalysts of protonation of superoxide ion. The mechanism and kinetics of this process were studied.

Recently, much attention has been given to the mechanism of the interaction of superoxide ion with hydrogen peroxide and hydroperoxides. This attention was stimulated by biochemical investigations which showed that the process leads to the formation of hydroxyl radicals and can therefore result in lipid peroxidation.[1] However, it was shown later (see ref. 2 and references therein) that the Haber–Weiss reaction (1),[3] which

$$O_2^{\cdot-} + H_2O_2 \longrightarrow O_2 + HO\cdot + HO^- \quad (1)$$

was regarded earlier as the main source of hydroxyl radicals, has a negligible rate in aqueous solutions or does not proceed at all. Therefore a catalytic mechanism for the Haber–Weiss reaction with participation of Fe^{3+} ions became generally accepted.[1]

$$O_2^{\cdot-} + Fe^{3+} \longrightarrow O_2 + Fe^{2+} \quad (2)$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO\cdot + HO^- \quad (3)$$

The results obtained in aprotic media are less definite. Peter and Foote concluded[4] that $(NMe_4)^+O_2^{\cdot-}$ reacts with t-butyl hydroperoxide in acetonitrile *via* reaction (4). However, Gibian

$$(NMe_4)^+O_2^{\cdot-} + Bu^tO_2H \longrightarrow O_2 + Bu^tO\cdot + (NMe_4)^+OH^- \quad (4)$$

and Ungermann[5] showed that $O_2^{\cdot-}$ is only able to deprotonate t-butyl hydroperoxide in aprotic media and that t-butyl alcohol and acetone so obtained can be formed in the reaction of $Bu^tO_2^-$ with acetonitrile. Recently, the Haber–Weiss mechanism was once more suggested for the reaction of superoxide ion with hydrogen peroxide in acetonitrile[6] and linoleate hydroperoxide in micelles.[7]

In this work we have studied the kinetics and mechanism of reactions of superoxide ion with hydrogen peroxide and t-butyl and cumyl hydroperoxide in acetonitrile. Preliminary results obtained for the interaction of $O_2^{\cdot-}$ with hydrogen peroxide have been published.[8]

Results

Experiments with Excess of Hydroperoxides.—A fast reaction was observed between $O_2^{\cdot-}$ and excess of hydroperoxides to form HO_2^- ($\lambda_{max.}$ 212—215 nm), $Bu^tO_2^-$ ($\lambda_{max.}$ 208 nm), and $PhCMe_2O_2^-$ ($\lambda_{max.}$ 240 nm) anions. (Analogous spectra were also obtained in the reaction of hydroperoxides with NBu_4OH.) In the reaction of superoxide ion with hydrogen peroxide the absorption spectrum of $O_2^{\cdot-}$ disappeared and that of HO_2^- appeared immediately on mixing the reactants. Then HO_2^- slowly decomposed to form superoxide ion.[8] Titration of the final solution with $KMnO_4$ showed that the hydrogen peroxide content had decreased more than two times, that indicating a chain mechanism for the reaction.

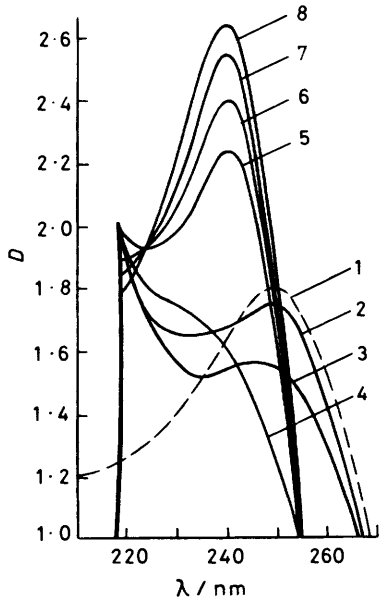

Figure 1. Interaction of $O_2^{\cdot-}$ with cumyl hydroperoxide in acetonitrile, $[O_2^{\cdot-}]_0$ 5.76 × 10^{-3}M, $[PhMe_2O_2H]_0$ 6.96 × 10^{-3}M. 1, Absorption spectrum of $O_2^{\cdot-}$. 2—8, Absorption spectra of reaction mixture in 20, 60, 120, 180, 250, 390, and 570 s after mixing the reactants

A typical example of the reaction of $O_2^{\cdot-}$ with cumyl hydroperoxide is shown in Figure 1. The disappearance of the maximum for $O_2^{\cdot-}$ at 249 nm was accompanied by an increase in the maximum of $PhCMe_2O_2^-$ at 240 nm. The reaction of $O_2^{\cdot-}$ with Bu^tO_2H proceeded similarly. Contrary to HO_2^-, RO_2^- formed in the reaction of $O_2^{\cdot-}$ with t-butyl and cumyl hydroperoxide was not decomposed.

Experiments with Excess of Superoxide Ion.—Under these conditions, $O_2^{\cdot-}$ slowly reacted with H_2O_2 to form HO_2^-. However, the reaction of $O_2^{\cdot-}$ with t-butyl and cumyl hydroperoxide accelerated after the first slow stage and then proceeded further at a constant rate; the reaction kinetics corresponded to an autocatalytic process (Figure 2). During the reaction a coloured intermediate with maxima at 355 and 512 nm was formed (Figure 3) which rapidly decomposed when

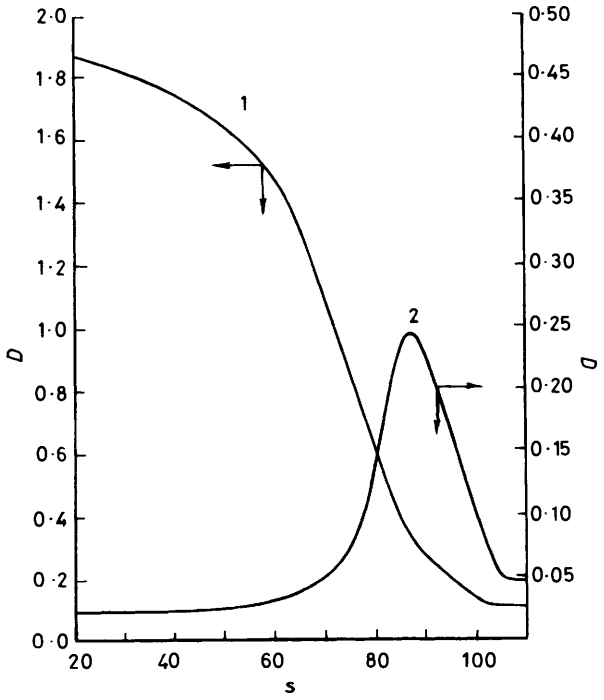

Figure 2. Kinetics of the reaction of $O_2^{\cdot-}$ with Bu^tO_2H, $[O_2^{\cdot-}]_0$ 6.99×10^{-3}M, $[Bu^tO_2H]_0$ 3.32×10^{-3}M. 1, Change in optical density at 249 nm (an absorption maximum of $O_2^{\cdot-}$). 2, Change in optical density at 355 nm (an absorption maximum of the free radical formed)

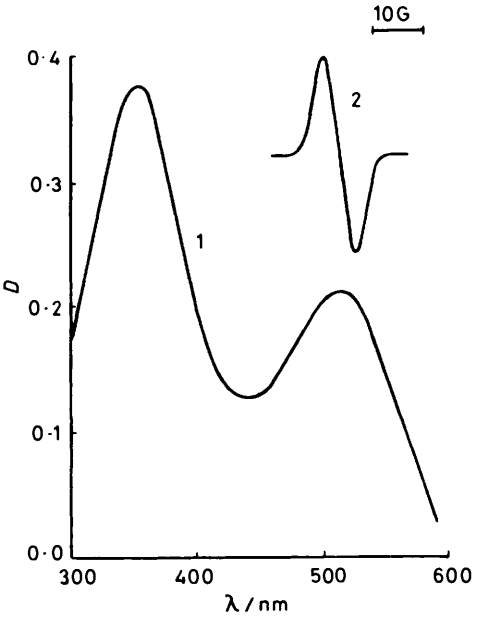

Figure 3. Absorption spectrum (1) and e.s.r. spectrum (2) of the free radical formed, $[O_2^{\cdot-}]_0$ 3.94×10^{-3}M, $[PhCMe_2O_2H]_0$ 3.46×10^{-3}M

the stationary stage was complete (Figure 2, curve 2). The intermediate was a free radical, because it yielded an e.s.r. spectrum with g 2.010 (Figure 3). The spectral characteristics of the free radical were independent of the hydroperoxide structure.

Discussion

Our results show that $O_2^{\cdot-}$ does react with hydroperoxides in aprotic media, but the reaction proceeds *via* the deprotonation mechanism (5) and (6) and not *via* the Haber–Weiss mechanism (1). It is possible that the origin of the repeated formation of

$$O_2^{\cdot-} + RO_2H \longrightarrow HO_2\cdot + RO_2^- \quad (5)$$

$$HO_2\cdot + O_2^{\cdot-} \longrightarrow HO_2^- + O_2 \quad (6)$$

$O_2^{\cdot-}$ and the cause, consequently, of a chain mechanism of the interaction of superoxide ion with excess of hydrogen peroxide is reaction (7).[9]

$$HO_2^- + H_2O_2 \longrightarrow O_2^{\cdot-} + HO\cdot + H_2O \quad (7)$$

It is known[1] that $O_2^{\cdot-}$ reacts with H_2O_2 in the presence of Fe^{3+} ions *via* the Fenton mechanism [reactions (2) and (3)]. Under our conditions, *i.e.* in acetonitrile solution, Fe^{3+} accelerated the whole process by increasing the rate of HO_2^- decay possibly *via* reactions (8) and (9). However, the main

$$Fe^{3+} + HO_2^- \longrightarrow (FeO_2H)^{2+} \quad (8)$$

$$(FeO_2H)^{2+} + H_2O_2 \longrightarrow O_2^{\cdot-} + Fe^{3+} + HO\cdot + H_2O \quad (9)$$

direction of the interaction of $O_2^{\cdot-}$ with H_2O_2 [*via* reaction (5)] did not change. Fe^{3+} ions did not affect the rates of the interaction of superoxide ion with t-butyl and cumyl hydroperoxide.

RO_2^- seems not to be able to react with alkyl hydroperoxides *via* reaction (7), and therefore a chain process analogous to that of $O_2^{\cdot-}$ with H_2O_2 was not observed in excess of hydroperoxides. However, as already mentioned, an autocatalytic process was observed when an excess of superoxide ion was used. This process is characterized by the following: (a) acetonitrile takes part in the reaction as the autocatalytic process was not observed in dimethylformamide; (b) the structure of a free radical intermediate is independent of the hydroperoxide used; (c) contrary to the experiments with excess of hydroperoxides, practically all $O_2^{\cdot-}$ disappeared before RO_2^- was formed in significant quantities. We proposed the mechanism (5), (10)—(12), (6) for the autocatalytic process.

$$O_2^{\cdot-} + RO_2H \xrightarrow{k_1} HO_2\cdot + RO_2^- \quad (5)$$

$$HO_2\cdot + MeCN \xrightarrow{k_2} \underset{\textbf{(1)}}{HO_2(MeCN)\cdot} \quad (10)$$

$$HO_2(MeCN)\cdot + O_2^{\cdot-} \xrightarrow{k_3} \underset{\textbf{(2)}}{^-O_2(MeCN)\cdot} + HO_1\cdot \quad (11)$$

$$^-O_2(MeCN)\cdot \longrightarrow \text{products} \quad (12)$$

$$O_2^{\cdot-} + HO_2\cdot \xrightarrow{k_4} HO_2^- + O_2 \quad (6)$$

In accordance with this mechanism, hydroperoxides play the role of catalysts of $O_2^{\cdot-}$ decomposition which proceeds with participation of a free radical formed from $HO_2\cdot$ and MeCN. It should be noted that Gibian and Ungermann[5] concluded that the protonation of $O_2^{\cdot-}$ by t-butyl hydroperoxide is accompanied by the interaction of $Bu^tO_2^-$ with acetonitrile. However, we found that $Bu^tO_2^-$ obtained from Bu^tO_2H and NBu_4OH does not react with acetonitrile in a time equal to that of the autocatalytic process. Moreover, a significant quantity of

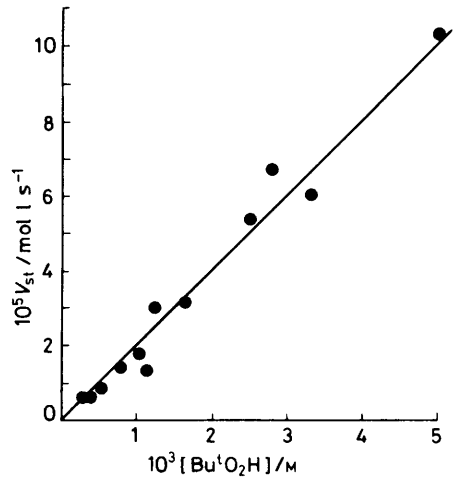

Figure 4. Dependence of stationary rate of the reaction of $O_2^{\cdot-}$ with Bu^tO_2H on the initial concentration of hydroperoxide, $[O_2^{\cdot-}]_0$ (1—7) × 10^{-3}M

$RO_2^{\cdot-}$ was formed in the autocatalytic process only after $O_2^{\cdot-}$ had disappeared completely. Therefore, the interaction of $RO_2^{\cdot-}$ with acetonitrile cannot be a stage of the autocatalytic process.

In accord with the mechanism proposed, the rate of the reaction of $O_2^{\cdot-}$ with hydroperoxides is given by equation (13).

$$-d[O_2^{\cdot-}]/dt = k_1[O_2^{\cdot-}][RO_2H] + k_3[O_2^{\cdot-}][(1)] + k_4[O_2^{\cdot-}][HO_2\cdot] \quad (13)$$

Under steady-state conditions for $HO_2\cdot$ and (1), we have (14). As

$$-d[O_2^{\cdot-}]/dt = k_1k_2\,[RO_2H][MeCN]_0/k_4 + 2k_1[O_2^{\cdot-}][RO_2H] \quad (14)$$

the main stage of the autocatalytic process has an invariable rate (see Figure 2), $d[O_2^{\cdot-}]/dt$ = constant. Furthermore, RO_2H is not consumed in the autocatalytic process, so $[RO_2H] = [RO_2H]_0$. As $-d[O_2^{\cdot-}]/dt$ = constant, the second term of equation (14) (which is not constant) should be omitted. Finally, we have equation (15).

$$-d[O_2^{\cdot-}]/dt = k_1k_2\,[RO_2H]_0[MeCN]_0/k_4 \quad (15)$$

It follows that the rate of the stationary stage must depend on the hydroperoxide concentration and be independent of the $O_2^{\cdot-}$ concentration. The dependence of this rate on the Bu^tO_2H concentration is shown in Figure 4. $[O_2^{\cdot-}]_0$ varied in the range (1—7) × 10^{-3}M and it obviously did not affect the linear relationship. We found that $(k_1k_2):k_4 = (0.99 \pm 0.19) \times 10^{-3}$ l mol^{-1} s^{-1}. At the beginning of the reaction, in the absence of the autocatalytic process, $-d[O_2^{\cdot-}]/dt = k_1[O_2^{\cdot-}]_0[RO_2H]_0$. From this equation we determined $k_1 = 0.64 \pm 0.23$ l mol^{-1} s^{-1}, giving $k_2:k_4 = 1.54 \times 10^{-3}$. Analogously, we determined $(k_1k_2):k_4 = (0.93 \pm 0.32) \times 10^{-3}$ l mol^{-1} s^{-1}, $k_1 = 0.78 \pm 0.23$ l mol^{-1} s^{-1}, and $k_2:k_4 = 1.19 \times 10^{-3}$ for the reaction of $O_2^{\cdot-}$ with cumyl hydroperoxide. Similar values of the $k_2:k_4$ ratio obtained for the reaction of superoxide ion with two different hydroperoxides confirm the correctness of the proposed mechanism of the autocatalytic process and the identical structure of a free radical intermediate. Similar values of k_1 are explained by the practically identical pK_a values for these compounds (12.8 and 12.6 in water[10]).

It was found that $k_4 = (1.02 \pm 0.49) \times 10^8$ l mol^{-1} s^{-1} in water.[11] Although for acetonitrile solution the k_4 value is unknown, on the basis of a comparison with the rates of other electron-transfer reactions in this solvent[12] one can suggest that in acetonitrile k_4 will be about one order of magnitude greater than in water. In such a case $k_2 = 10^5$—10^6 l mol^{-1} s^{-1}, and from the steady-state conditions for $HO_2\cdot$, $[HO_2\cdot]_{st} = 10^{-11}$—$10^{-12}$M. This value proves correct a suggestion that reaction (6) and not reaction (16) is the chain-termination step, as under the conditions studied {*i.e.* at $[O_2^{\cdot-}]_0 = (1—7) \times 10^{-3}$M}, the rate of reaction (6) is 10^8 times greater than that of (16).

$$2\,HO_2\cdot \longrightarrow H_2O_2 + O_2 \quad (16)$$

Now we may consider the structure of the free radical intermediate. Even taking into account some uncertainty in the k_2 value, one can assume that this value is too high for a rate constant for the addition of $HO_2\cdot$ to acetonitrile to form a covalent bond. Indeed, the largest value of the addition rate constant known to us for this radical is 6.6×10^3 l mol^{-1} s^{-1} for the reaction of $HO_2\cdot$ with 5,5-dimethyl-1-pyrroline *N*-oxide.[13] Bielski *et al.*[14] found that the rate constants for the reaction of $HO_2\cdot$ with unsaturated fatty acids are (1—3) × 10^3 l mol^{-1} s^{-1}. We therefore concluded that the product of reaction (10) is not an adduct but a complex of hydroperoxyl radical with acetonitrile.

The formation and decay kinetics of the free radical (Figure 2, curve 2) indicate that it is radical (**2**) and not (**1**) as the latter must have a very short lifetime. The absence of hyperfine structure in the e.s.r. spectrum and the magnitude of the *g* factor (2.010) appear to point to a peroxyl radical, although the value of the *g* factor is 0.004 smaller than that for $HO_2\cdot$.[15] One could suggest that (**2**) has the structure $NCMe{\cdot}O_2^{\cdot-}$, especially if one takes into account the fact that complexation of $HO_2\cdot$ with metal cations usually decreases its *g* factor.[15] However, our experiments show that a similar complex is not formed, as expected, in acetonitrile solutions of superoxide ion. In addition, radical (**2**) did not decompose into $O_2^{\cdot-}$ and MeCN but disappeared to form unknown products. Therefore it is possible that radical (**2**) was converted into another radical, for example $^-N{=}CMeO_2\cdot$.

In conclusion, it should be noted that the interaction of superoxide ion with hydroperoxides appears to be the first example of catalytic protonation of $O_2^{\cdot-}$. This process has two peculiarities which might be of importance especially for biological systems where superoxide ion is formed: a chain mechanism of $O_2^{\cdot-}$ decay in the presence of substrates capable of reacting with $HO_2^{\cdot-}$ (this reaction can proceed in lipid membranes) and the ability of $HO_2\cdot$ to react with active substrates and thus escape the decay reaction (6).

However, the kinetic conditions which are needed for the realization of the autocatalytic process seems to be restricted. We found that radical (**2**) was not formed in the reactions of $O_2^{\cdot-}$ with either strong or weak proton donors (phenols and water or ethanol, respectively). For strong proton donors, it may possibly be accounted for by the fact that the rate of reaction (5) is considerably greater than that of (11). In the case of weak proton donors, the reason seems to be the low rate of reaction (5) [the k_1 values for the reaction of $O_2^{\cdot-}$ with H_2O and EtOH in acetonitrile are $(3.45 \pm 0.30) \times 10^{-3}$ and $(2.68 \pm 0.53) \times 10^{-3}$ l mol^{-1} s^{-1} [16]], which leads to an insignificant concentration of radical (**2**). We also found that the addition of water considerably diminishes the concentration of radical (**2**). It seems to explain the fact that radical (**2**) was not formed in the reaction of $O_2^{\cdot-}$ with hydrogen peroxide which was used as a 30% aqueous solution.

Experimental

Materials.—Hydrogen peroxide ('chemically pure') was used as a 27—30% aqueous solution. The concentration of H_2O_2 was

determined by titration with $KMnO_4$. t-Butyl hydroperoxide was purified by vacuum distillation. For experiments the b.p. 50—51 °C at 75 mmHg fraction of 99.0% purity was used (g.l.c. analysis). Cumyl hydroperoxide was purified by conversion into the sodium salt. A product of 99.8% purity was used (iodine titration). Acetonitrile was dried by refluxing over P_2O_5 and was distilled over anhydrous potassium carbonate.

Reaction of Superoxide Ion with Hydroperoxides.—As previously,[17] solutions of $O_2^{\cdot-}$ in acetonitrile were prepared by the electrochemical reduction of O_2 in the presence of tetrabutylammonium perchlorate. The half-life of superoxide ion was 30—35 h.[18] The reaction of $O_2^{\cdot-}$ with hydroperoxides was carried out in 1 and 2 mm cells of a Cary 218 spectrophotometer at room temperature. The decay of superoxide ion was followed spectrophotometrically at 249 nm. E.s.r. spectra were recorded with a Varian E-12A e.s.r. spectrometer.

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