Spin-Trapping of Alkyl Radicals by a Water-Soluble Nitroso Aromatic Spin-Trap, 3,5-Dibromo-4-nitrosobenzenesulfonate

Toshihiko OZAWA* and Akira HANAKI
National Institute of Radiological Sciences, 9-1 Anagawa-4-chome, Chiba 260
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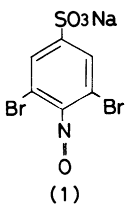
Synopsis. Some short-lived alkyl radicals generated by the oxidation with either Ti^{3+}-H_2O_2 or Fe^{2+}-H_2O_2 system were trapped by a water-soluble nitroso aromatic spin-trap, 3,5-dibromo-4-nitrosobenzenesulfonate (DBNBS, **1**), in aqueous solutions to yield the stable spin-adducts which could be easily detected and identified by electron spin resonance (ESR) spectroscopy.

The spin-trapping compounds react with the short-lived free radicals to form the more stable radical products, spin adducts, which can be detected and identified by electron spin resonance (ESR) technique.[1-6] Recently, a new water-soluble spin-trap, sodium 3,5-dibromo-4-nitrosobenzenesulfonate (DBNBS, **1**), was synthesized and was found to be an efficient radical trap in aqueous, or partially aqueous, solutions.[7] This new spin-trap does not yield observable spin adduct with hydroxyl radical (OH·) which is formed by the photolysis of hydrogen peroxide (H_2O_2), but dimethyl sulfoxide (DMSO) included in the aqueous H_2O_2 system gives the adduct with methyl radical which arises via hydroxyl radical attack on DMSO.[8]

In a previous paper,[9] we have reported that superoxide ion, O_2^-, can be easily trapped by DBNBS in aqueous solutions and that besides the intense signal due to O_2^- adduct, weak ESR signals assignable to methyl radical adduct are observed. Then, using DBNBS, we have further investigated the oxidation of some organic substrates by Fenton-type systems such as Ti^{3+}-H_2O_2 and Fe^{2+}-H_2O_2, both of which are known to be potent oxidizing agents,[10] and found that these Fenton-type systems give radical adducts similar to those obtained by the photolysis of the aqueous H_2O_2 system containing some organic substrates.

Experimental

Reagents. $TiCl_3$ (20% v/v), $FeCl_2$, and sulfuric acid (H_2SO_4) were purchased from Wako Pure Chemical Co. Ltd. and were used without further purification. H_2O_2 (30%) was obtained from Mitsubishi Gas Chemical Co. Ltd. Organic substrates were commercially available and were distilled prior to use. Deionized and triply distilled water was used throughout. Sodium 3,5-dibromo-4-nitrosobenzenesulfonate (DBNBS, **1**) was synthesized from 3,5-dibromosulfanilic acid by oxidation with H_2O_2 in glacial acetic acid.[7]



(1)

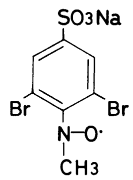
Procedure. Both 0.01 mol dm^{-3} metal ion (Ti^{3+} or Fe^{2+}) and 0.1 mol dm^{-3} H_2O_2 solutions were acidified by H_2SO_4 to pH 2.0. DBNBS (0.05 mol dm^{-3}) and organic substrates (0.1 mol dm^{-3}) were included in the aqueous H_2O_2 solutions. Reactions were started by adding the metal ion solutions to H_2O_2 solutions. The reaction solutions were immediately transferred to a JEOL quartz flat cell to measure ESR spectra. All the procedures were done in the air.

ESR Measurements. ESR spectra were obtained at room temperature on a JEOL-PE-1X (X-band) ESR spectrometer with 100 kHz field modulation. ESR parameters were calibrated by comparison with a standard Mn^{2+}/MgO marker and 2,2-diphenyl-1-picrylhydrazyl (DPPH, g=2.0036).

Results and Discussion

No ESR spectrum was observed from the following aqueous solutions: DBNBS, metal ion (Ti^{3+} or Fe^{2+}) with DBNBS, H_2O_2 with DBNBS.

When aqueous solutions of Ti^{3+} ion were mixed with aqueous solutions of H_2O_2 containing DBNBS, no ESR signal was observed. This result is consistent with the fact that DBNBS does not yield observable spin adduct with hydroxyl radical.[7,9,11] However, when DMSO was included in Ti^{3+}-H_2O_2-DBNBS system, an intensive ESR spectrum was observed as shown in Fig. 1. ESR parameters can be determined as follows: a^{N} (1)=13.9 G,[12] $a^{H}_{CH_3}$ (3)=12.8 G, a^{H}_{phenyl} (2)=0.7 G and g=2.0062 (1G=10^{-4} T). The ESR spectrum shown in Fig. 1 is almost identical with that of methyl radical adduct of DBNBS generated by the photolysis of aqueous solutions of H_2O_2 containing DMSO in the presence of DBNBS.[7] Therefore, the radical species observed in Fig. 1 can be assigned to the methyl radical adduct (**2**). In the Ti^{3+}-H_2O_2 system



(2)

containing DMSO, methyl radical may be formed as follows:

$$Ti^{3+} + H_2O_2 \rightarrow Ti^{4+} + \cdot OH + OH^- \quad (1)$$

$$\cdot OH + (CH_3)_2SO \rightarrow CH_3SO_2H + CH_3\cdot \quad (2)$$

$$CH_3\cdot + \text{DBNBS} \rightarrow \text{methyl radical adduct } (\mathbf{2}) \quad (3)$$

It is known that methyl radical is formed by the OH radical attack on DMSO (Eq. 2).[8] In Fig. 1, ESR spectrum is somewhat asymmetrical. Such a spectral pattern is often observed in even relatively small nitroxide molecules in aprotic media.[13] This phenomenon may be caused by anisotropic motion in isotropic media.[13]

When methyl alcohol was included in Ti^{3+}-H_2O_2-DBNBS system, ESR spectrum [a^{N} (1)=13.4 G, $a^{H}_{CH_2}$ (2)=7.4 G, a^{H}_{phenyl} (2)=0.8 G, and g=2.0064] was observed. Since these ESR parameters are almost identical with those

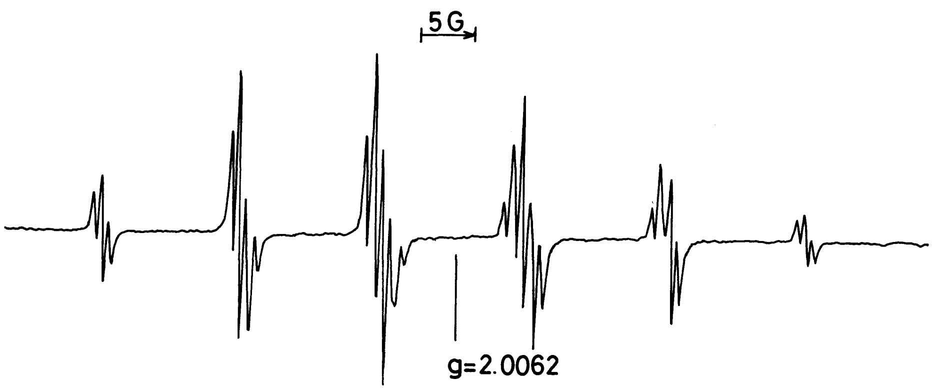


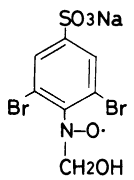
Fig. 1. ESR spectrum observed by the oxidation of DMSO with Ti^{3+}–H_2O_2 system in the presence of DBNBS in aqueous solutions.
Reaction conditions: 0.01 mol dm^{-3} Ti^{3+}, 0.1 mol dm^{-3} H_2O_2, 0.05 mol dm^{-3} DBNBS, 0.1 mol dm^{-3} DMSO. Instrument settings: microwave power, 10 mW; modulation amplitude, 0.5 G; amplitude, 10×100; time constant, 0.3 s; scan time, 8 min.

Table 1. ESR Parameters for Selected Spin Adducts of DBNBS

Substrate	Trapped radical	Method of generation of radical	Hyperfine splitting const (G)		
			a^N	a^H_{phenyl}	a^H_{other}
DMSO	$CH_3\cdot$	Ti^{3+}–H_2O_2	13.9	0.7	12.8 (CH_3)
	$CH_3\cdot$	Fe^{2+}–H_2O_2	14.1	0.7	13.3 (CH_3)
	$CH_3\cdot$	$h\nu$–H_2O_2[a]	14.50		13.50(CH_3)
CH_3OH	$\dot{C}H_2OH$	Ti^{3+}–H_2O_2	13.4	0.8	7.4 (CH_2)
	$\dot{C}H_2OH$	Fe^{2+}–H_2O_2	13.6	—[b]	9.1 (CH_2)
	$\dot{C}H_2OH$	$h\nu$–H_2O_2[a]	13.70		9.20(CH_2)
C_2H_5OH	$\dot{C}H_2CH_2OH$	Ti^{3+}–H_2O_2	14.2	—[b]	12.2 (CH_2) —[b] (CH_2)
	$\dot{C}H_2CH_2OH$[c]	Fe^{2+}–H_2O_2	14.3	—[b]	13.6 (CH_2) 9.2 (CH_2)
	$\dot{C}H_2CH_2OH$	$h\nu$–H_2O_2[a]	14.00		11.30(CH_2)
	$CH_3\dot{C}HOH$	$h\nu$–H_2O_2[a]	14.00		9.20(CH)

a) Deuteriated DBNBS was used as a spin-trap.[7] b) Not measured. c) A weak, unanalyzable signal was also observed.

of hydroxymethyl radical ($\cdot CH_2OH$) adduct of DBNBS formed by the photolysis of aqueous solutions of H_2O_2 containing methyl alcohol in the presence of DBNBS,[2] the radical species observed can be assigned to the radical (**3**). In this reaction, hydroxymethyl radical is formed as follows:

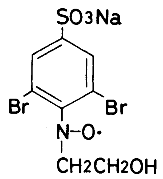


(3)

$$\cdot OH + CH_3OH \rightarrow \cdot CH_2OH + H_2O \quad (4)$$

$$\cdot CH_2OH + DBNBS \rightarrow \text{hydroxymethyl radical adduct } (\mathbf{3}) \quad (5)$$

When ethyl alcohol was included in Ti^{3+}–H_2O_2–DBNBS system, a weak ESR spectrum [a^N (1)=14.2 G, $a^H_{CH_2}$ (2)=12.2 G, and g=2.0063] which could be assigned to hydroxyethyl radical ($\cdot CH_2$-CH_2OH) adduct of DBNBS (**4**) was observed.



(4)

In this reaction system, another weak signal, which could not be analyzed, was also observed.

The same radical species were obtained from the Fe^{2+}–H_2O_2 system. These results are summarized in Table 1, along with the radical species observed by photolysis.

It is apparent from Table 1 that the same radical species were generated from both photolysis of H_2O_2 and Fenton-type reaction systems. However, the hyperfine coupling constants are somewhat different from each other, depending on the different reducing agents for H_2O_2.

The present experimental results indicate that a new water-soluble spin-trap, DBNBS, is an effective trapping agent for some alkyl radicals generated by the oxidation with Fenton-type systems such as Ti^{3+}-H_2O_2 and Fe^{2+}-H_2O_2 in aqueous solutions. Recently, nitrone spin-traps such as 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) and *N-t*-butyl-*α*-phenylnitrone (PBN) are often used in biological systems. DBNBS may be compared with DMPO and PBN. DBNBS has a high solubility in aqueous solutions compared with PBN and, while DMPO is susceptible to decomposition by light,[6] DBNBS in aqueous solutions is stable under light. Further, DBNBS does not yield the observable spin-adduct with OH radical. From these facts, it is indicated that DBNBS is a useful spin-trap in aqueous reaction systems in which OH radical is formed as a first intermediate.

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References

1) M. J. Perkins, *Adv. Phys. Org. Chem.*, **17**, 1 (1980).
2) E. G. Janzen, "Free Radicals in Biology," ed by W. A. Pryor, Academic Press, New York (1980), Vol. IV, pp. 116—154.
3) P. B. McCay, T. Noguchi, K.-L. Fong, E. K. Lai, and J. L. Poyer, "Free Radicals in Biology," ed by W. A. Pryor, Academic Press, New York (1980), Vol. IV, pp. 155—186.
4) E. Finkelstein, G. M. Rosen, and E. J. Rauckman, *Arch. Biochem. Biophys.*, **200**, 1 (1980).
5) E. G. Janzen, "Methods in Enzymology," ed by L. Packer, Academic Press, Orland, Florida (1984), Vol. 105, pp. 188—198.
6) G. M. Rosen and E. J. Rauckman, "Methods in Enzymology," ed by L. Packer, Academic Press, Orland, Florida (1984), Vol. 105, pp. 198—209.
7) H. Kaur, K. H. W. Leung, and M. J. Perkins, *J. Chem. Soc., Chem. Commun.*, **1981**, 142.
8) B. D. Flockhart, K. J. Ivin, R. C. Pink, and B. D. Sharma, *Chem. Commun.*, **1971**, 339.
9) T. Ozawa and A. Hanaki, *Biochem. Biophys. Res. Commun.*, **136**, 657 (1986).
10) M. J. Green and H. A. O. Hill, "Methods in Enzymology," ed by L. Packer, Academic Press, Orland, Florida (1984), Vol. 105, pp. 3—22.
11) T. Ozawa and A. Hanaki, *Biochem. Biophys. Res. Commun.*, **142**, 410 (1987).
12) In this paper, number of atom which interacts with unpaired electron is designated in parentheses.
13) S. Schreier, C. F. Polnaszenk and I. C. P. Smith, *Biochim. Biophys. Acta*, **515**, 375 (1978).