UNUSUAL SPIN-TRAP CHEMISTRY FOR THE REACTION OF HYDROXYL RADICAL WITH THE CARCINOGEN N-NITROSODIMETHYLAMINE

DAVID A. WINK[1] and MARC F. DESROSIERS[2]†

[1]Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD 21702, U.S.A. and [2]Ionizing Radiation Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, U.S.A.

Abstract—The reaction of the potent carcinogen N-nitrosodimethylamine (NDMA) with hydroxyl radical generated via radiolysis was studied using EPR techniques. Attempts to spin trap NDMA radical intermediates with 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS) produced only unusual DBNBS radicals. One of these radicals was shown to be generated by both reaction of DBNBS with nitric oxide, and direct oxidation of DBNBS with an inorganic oxidant (Br_2^-). Another DBNBS radical was identified as a sulfite spin adduct resulting from the degradation of DBNBS by a NDMA reactive intermediate. In the absence of DBNBS, hydroxyl radical reaction with NDMA gave the dimethylnitroxide radical. Unexpectedly, addition of DBNBS to a solution containing dimethylnitroxide produced an EPR spectrum nearly identical to that of NDMA solutions with DBNBS added before radiolysis. A proposed mechanism accounting for these observations is presented.

INTRODUCTION

The activation and detoxication of the potent carcinogen, N-nitrosodimethylamine (NDMA), has attracted much attention in the past two decades, yet its metabolic pathways have not been fully elucidated (Kroeger-Koepke *et al.*, 1981; Appel and Graf, 1982; Haussman and Werringloer, 1985). Only cytochrome P-450 oxidation has been clearly demonstrated *in vivo* (Preussman and Steward, 1982). Nonenzymatic oxidation of NDMA, mediated by hydroxyl radicals generated via xanthine oxidase-induced Fenton and Haber-Weiss chemistry, has been suggested as a potential metabolic pathway. Recently, Heur *et al.* (1989) have postulated that Fenton degradation of NDMA is initiated by hydroxyl radical abstraction of a hydrogen to form an alkyl radical,

$$\cdot OH + (CH_3)_2NNO \rightarrow H_2O + \cdot CH_2(CH_3)NNO \quad (1)$$

which then denitrosates to give methylamine and formaldehyde.

$$\cdot CH_2(CH_3)NNO \rightarrow CH_3NH_2 + CH_2O + \cdot NO \quad (2)$$

To investigate the validity of this proposal, we chose first to study the reaction of NDMA with hydroxyl radical in the absence of transition metal

mediated Fenton chemistry. It was hoped that by understanding this chemistry, correlations could be drawn to the metal- and/or enzyme-mediated metabolic pathways, and may provide insight for a mechanism to possible alternative metabolic pathways *in vivo*. We have examined the reaction of ·OH with NDMA using radiolytic techniques, and although NDMA reactive intermediates proved untrappable, unusual spin-trap chemistry was observed that is of importance to future uses of DBNBS as a spin trap.

MATERIALS AND METHODS‡

The following chemicals were used without further purification: N-nitrosodimethylamine, NDMA, 3,5-dibromo-4-nitrosobenzene sulfonate sodium salt, DBNBS (Sigma); 2-methyl-2-nitrosopropane, MNP, 5,5-dimethyl-1-pyrroline-N-oxide, DMPO, dimethylsulfoxide, DMSO, thallium sulfate (Aldrich); potassium bromide, sodium persulfate (Fisher); and ethanol (Warner–Graham). Aqueous solutions were purged with ultrahigh purity grade N_2O (Matheson) or nitric oxide (AGA) through a syringe needle into a septum-sealed sample tube. Gas mixtures were prepared with a gas mixer (Cole–Parmer) followed by purging of the solution. Sealed aqueous solutions were irradiated in a Gamma cell 220 [60]Co γ source (absorbed dose rate in water: 7.5 kGy/h). Samples were then transferred to a quartz EPR flat cell (Wilmad) via syringe. EPR spectra were measured with a Bruker ESP300 EPR spectrometer at ambient temperature. Spin concentrations were assessed by comparing the double integrals of measured first
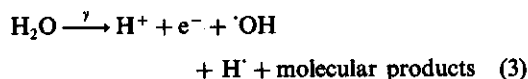
---

†Author to whom correspondence should be addressed.
‡The mention of commercial products does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the products identified are necessarily the best available for the purpose.

derivative spectra with those of freshly made solutions of 2,2,6,6-tetramethylpiperidine-N-oxyl, TEMPO (Sigma). Pulse radiolysis experiments were done using a 2 MeV Febetron electron accelerator (Simic and Hunter, 1983; Neta et al., 1986). For these studies, N_2O saturated thallium sulfate solutions, pH 2.7, or nitrogen-saturated persulfate solutions containing 2 mol·dm^{-3} tert-butanol (Fisher) were anaerobically transferred to a cell with an optical pathlength of 2 cm. Transient absorptions of Tl^{2+} and SO_4^- were monitored at 340 and 450 nm, respectively.

RESULTS

Radiolysis of a N_2O-saturated aqueous solution will produce ˙OH in high yield:

$$H_2O \xrightarrow{\gamma} H^+ + e^- + \text{˙OH}$$
$$+ \text{H˙} + \text{molecular products} \quad (3)$$
$$e^- + N_2O + H^+ \rightarrow \text{˙OH} + N_2 \quad (4)$$

As mentioned previously, it was postulated that ˙OH may abstract a hydrogen from NDMA to give an alkyl radical [reaction (2)]. Radiolysis of N_2O-saturated aqueous solutions of NDMA (20–150 mmol·dm^{-3}) in the presence of the spin trap DBNBS (5–50 mmol·dm^{-3}) did not produce detectable levels of NDMA/DBNBS adducts. However, DBNBS paramagnetic species were observed [Fig. 1(A)]. Figure 1(A) is a composite of two spectra: a broad triplet (radical 1) due to an unpaired spin coupled to a single nitrogen ($a_N = 9.7$ G) and a sharp triplet of triplets (radical 2) resulting from an unpaired spin coupled to one nitrogen atom ($a_N = 12.7$ G) and two hydrogen atoms ($a_H = 0.64$ G). Control experiments corroborated previous reports that radiolytically-produced ˙OH does not react with DBNBS to give detectable paramagnetic products (Kaur et al., 1981). Thus, the DBNBS radicals in Fig. 1(A) are formed by reaction of DBNBS with hydroxyl radical generated NDMA intermediates. The NDMA intermediates are apparently highly reactive, untrappable species since for similar conditions, in separate experiments, two other spin traps, DMPO and MNP, also failed to produce NDMA spin adducts.

Since denitrosation of NDMA generates nitric oxide, we investigated the possibility that nitric oxide may react with DBNBS to generate radicals 1 or 2. When an anaerobic solution of DBNBS (10 mmol·dm^{-3}) was exposed to nitric oxide, an EPR spectrum identical to that assigned to radical 1 was observed [Fig. 1(B)]. Thus, nitric oxide, produced by hydroxyl radical-induced denitrosation of NDMA, can be considered as potential reactant for DBNBS to produce radical 1. However, radical 1 has been detected (but not identified) by others as a secondary product in chemistry not involving nitric oxide (Kalyanaraman, 1990; Flitter and Mason, 1991).
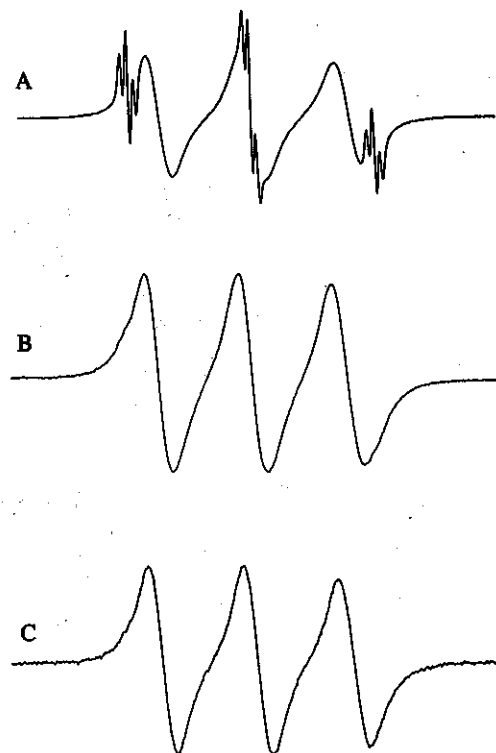

Fig. 1. First derivative EPR spectra of: (A) γ-irradiated aqueous solution (N_2O saturated) containing 25 mmol × dm^{-3} DBNBS and 70 mmol·dm^{-3} NDMA; (B) N_2-saturated, 25 mmol·dm^{-3} DBNBS solution bubbled with nitric oxide for 1 min; (C) γ-irradiated aqueous solution (N_2O saturated) containing 0.1 mol·dm^{-3} KBr and 25 mmol·dm^{-3} DBNBS. The field sweep for these spectra is 50 G.

Therefore, an alternative experimental design was invoked to prove the formation of radical 1. When the inorganic oxidant ˙Br$_2^-$, produced via reactions (3)–(6),

$$\text{˙OH} + Br^- \rightarrow OH^- + \text{˙Br} \quad (5)$$
$$\text{˙Br} + Br^- \rightarrow \text{˙Br}_2^- \quad (6)$$
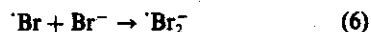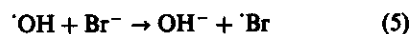
was reacted with DBNBS, a radical with a broad three line spectrum ($a_N = 9.7$ G) identical to radical 1 was produced [Fig. 1(C)]. The similarity of the coupling constants for radical 1 and a reported DBNBS-phenyl adduct (Kaur et al., 1981) lend support to the conclusion that radical 1 is a substituted phenyl adduct of DBNBS formed by oxidation (and subsequent decomposition) of DBNBS. Though the structure of radical 1 is unknown, it is important to note that oxidation of DBNBS is the first step in its production, and in this study, is produced either by nitric oxide or by an unidentified ˙OH-generated NDMA intermediate.

The second component of spectrum 1A (radical 2) is nearly identical to an EPR spectrum assigned (Stolze and Mason, 1987) to a sulfite spin adduct of DBNBS ($a_N = 12.7$ G; $a_H = 0.6$ G). The similarity of the two spectra suggests that the radical 2 may result

from decomposition of the spin trap in the presence of ·OH-generated NDMA intermediates, such that a DBNBS-derived sulfite radical is liberated and trapped by another DBNBS molecule to yield the observed spin adduct (radical 2).

Varying the concentration of DBNBS in the presence of NDMA changed the relative yields of radicals 1 and 2. When the NDMA concentration was maintained at 70 mmol·dm^{-3}, radical 2 became the more prevalent component of the EPR spectrum as the concentration of DBNBS was lowered to 5 mmol·dm^{-3} [Fig. 2(A)]; as the DBNBS concentration was incrementally increased, the EPR intensity of radical 1 increased. Conversely, when the DBNBS concentration was maintained at 25 mmol·dm^{-3} and the NDMA concentration varied, only radical 1 was detected at NDMA concentrations less then 40 mmol·dm^{-3}, whereas at higher concentrations of NDMA radical 2 appears, such that at 150 mmol·dm^{-3} NDMA

both radical 1 and 2 are present. This suggests that ·OH reacts with NDMA to produce a chemical species which can either react with DBNBS to yield radical 1, or reacts with another NDMA molecule to give a second chemical species which can react with the DBNBS to ultimately yield radical 2 (Scheme 1).

In the absence of spin trap, EPR analysis of irradiated N$_2$O-saturated aqueous solutions of NDMA (0.01–0.5 mmol·dm^{-3}) yielded a spectrum shown in Fig. 3(A). This spectrum is a triplet of septets derived from an unpaired electron coupled to one nitrogen (a$_N$ = 17.0 G) further split by six equivalent hydrogen atoms (a$_H$ = 14.8 G). This spectrum was assigned to dimethylnitroxide (below).
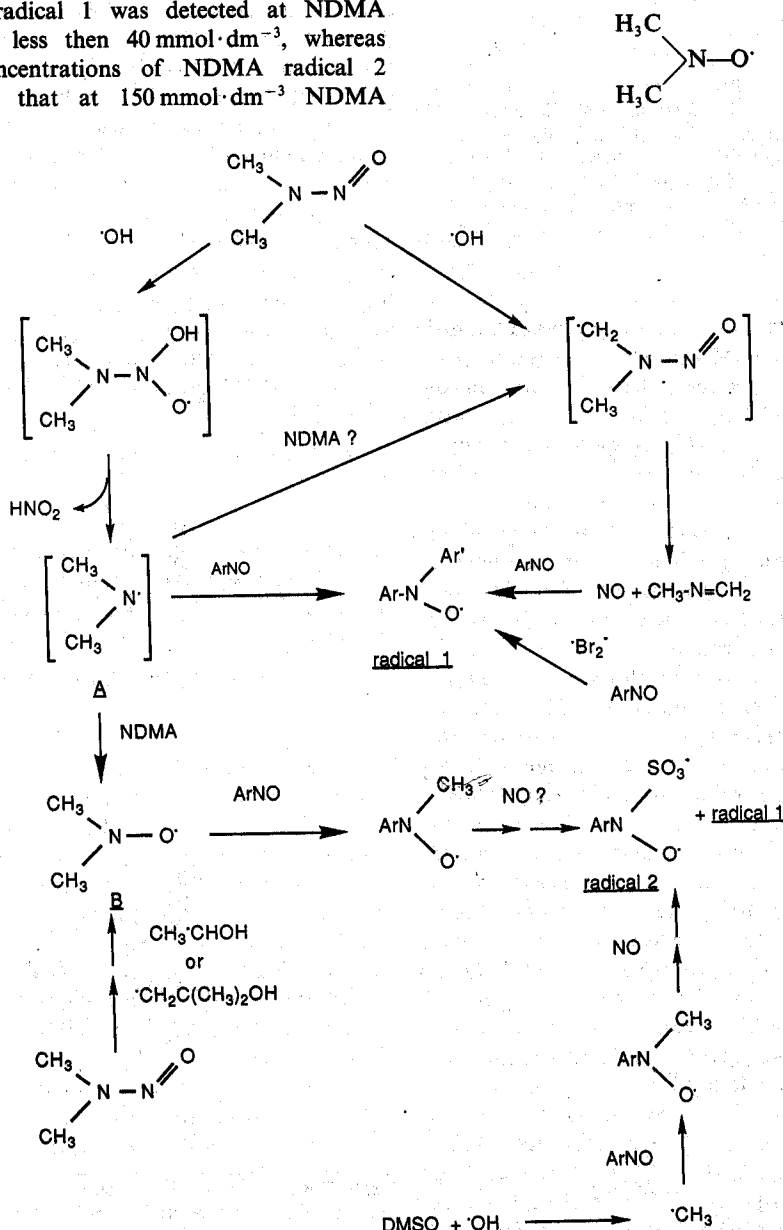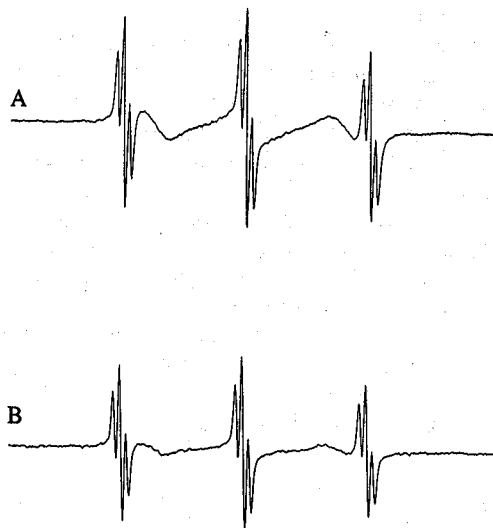

Scheme 1

Fig. 2. First derivative EPR spectra of γ-irradiated aqueous solutions (N$_2$O saturated) containing: (A) 70 mmol·dm^{-3} NDMA and 5 mmol·dm^{-3} DBNBS; (B) 70 mmol·dm^{-3} NDMA added, postirradiation, to DBNBS (final concentration: 5 mmol·dm^{-3}). The total spin concentrations estimated by double integration and comparison with TEMPO were: (A) $8 \pm 1 \ \mu$mol·dm^{-3}; (B) $7 \pm 1 \ \mu$mol·dm^{-3}. The field sweep for these spectra is 50 G.

Further confirmation of this structure was obtained by radiolysis of NDMA-d_6 under identical conditions [Fig. 3(B)]. Spectrum 3B results from a nitrogen triplet ($a_N = 17.0$ G) coupled to six equivalent deuterium atoms ($a_D = 2.3$ G). However, quantitative analysis of the spectra recorded above find that the maximum dimethylnitroxide spin concentration is less than 1% of the reaction. Though dimethylnitroxide is a minor product, a dose-dependence study demonstrates it is produced even at ·OH concentrations calculated to react with <1% of the initial NDMA concentration.

Increasing the NDMA concentration increased the intensity of the dimethylnitroxide EPR spectrum [Fig. 3(A)]. This implies that the reaction of ·OH with NDMA results in the formation of reactive chemical species which further react with additional NDMA, to produce dimethylnitroxide. Dimethylnitroxide was still measurable 24 h after radiolysis when protected from air. Upon exposure to oxygen its EPR spectrum rapidly (within minutes) decreased in intensity (this effect was found not to be due to EPR line broadening by O$_2$). Radiolysis of aqueous solutions of NDMA saturated with N$_2$O/O$_2$ mixtures (5–100% O$_2$) produced no detectable EPR signals of any kind, indicating that molecular oxygen does not participate in the formation of dimethylnitroxide.

Formation of dimethylnitroxide was found to be initiated by reaction of NDMA with radiolytially-generated alkyl radicals. When N$_2$O-saturated aqueous solutions of NDMA (70 mmol·dm^{-3}) containing ethanol (2 mol·dm^{-3}) or t-butanol (2 mol × dm^{-3}) were irradiated, EPR spectra identical to

Fig. 3(A) were observed. Under these conditions >99% of radiolytically-generated ·OH reacts with the alcohol to produce the corresponding alkyl radicals (Scheme 1). Therefore, the reaction pathway for NDMA activation to form dimethylnitroxide must be similar for both ·OH and alkyl radicals (see "Discussion").

An experiment was devised to determine the role, if any, of dimethylnitroxide in the radiolytic degradation of NDMA in the presence of DBNBS. A 70 mmol·dm^{-3} NDMA (N$_2$O-saturated) solution was irradiated and anaerobically transferred to a septum-sealed vial containing solid DBNBS, such that the final concentration of DBNBS was 5 mmol·dm^{-3}. The EPR spectrum of this mixture was predominately due to radical 2, with a small amount of radical 1. When compared with a 70 mmol·dm^{-3} NDMA solution irradiated with 5 mmol·dm^{-3} DBNBS, the resultant EPR spectra are essentially identical [Figs 2(A) and 2(B)]. This was taken as strong evidence that B in Scheme 1 is dimethylnitroxide. For spectrum 2B the small amount of radical 1 is likely due to residual nitric oxide present in the irradiated NDMA solution added to DBNBS. The remarkable result that identical spectra can be obtained regardless of whether spin trap is added before or after radiolysis is, to the authors' knowledge, unprecedented, and illustrates the unusual chemistry of DBNBS in this system. Furthermore, this reaction is apparently exclusive to
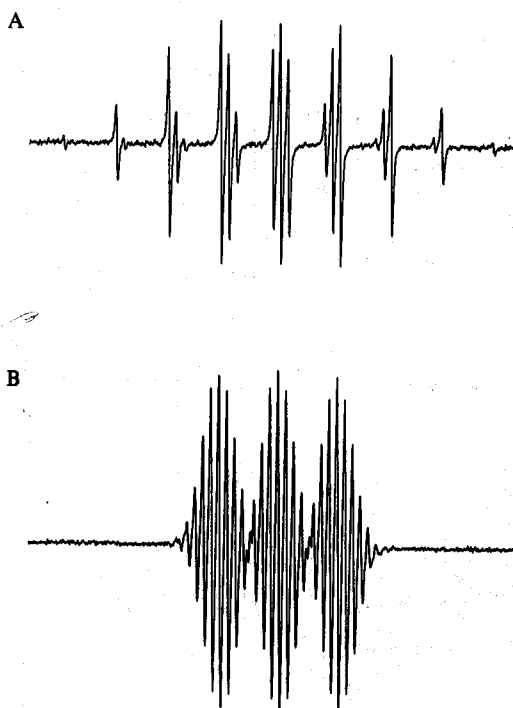

Fig. 3. First derivative EPR spectra of γ-irradiated aqueous solutions (N$_2$O saturated) containing: (A) 70 mmol·dm^{-3} NDMA; (B) 70 mmol·dm^{-3} NDMA-d_6. The field sweep for these spectra is 140 G.

DBNBS; addition of DMPO to dimethylnitroxide solutions produced no reaction.

The existence of any paramagnetic intermediates in the conversion of dimethylnitroxide to radical 2 was probed with rapid scan (4 s) EPR techniques. After the addition of DBNBS to dimethylnitroxide, a series of consecutive rapid scans detected production of a paramagnetic species ($a_N = 14.4$ G; $a_H = 13.5$ G; $a_{\gamma H} = 0.7$ G) assigned to a methyl adduct of DBNBS (Krishna et al., 1988) with concomitant decay of dimethylnitroxide EPR spectral features [Figs 4(A) and 4(B)]. Continued monitoring of this solution revealed that the DBNBS-methyl adduct decayed as EPR signals due to radical 2 increased in intensity [Figs 4(C) and 4(D)]. A clue as to what species could induce rapid decomposition of an otherwise stable DBNBS-methyl adduct was provided in a separate experiment. Radiolysis of N_2O-saturated solutions containing DMSO (0.3 mol·dm^{-3}) and DBNBS

Fig. 4. First derivative EPR spectra of: (A) γ-irradiated aqueous solutions (N_2O saturated) containing 70 mmol × dm^{-3} NDMA. The full EPR spectrum of dimethylnitroxide [Fig. 3(A)] is not represented here. Due to rapid scan EPR experimental limitations, the field sweep was reduced to 100 G, thus expanding the horizontal scale by approximately 29%. Spectrum (B) was recorded 1 min after mixing an aliquot of dimethylnitroxide solution with DBNBS (5 mmol·dm^{-3}); (C) same solution recorded 6 min after DBNBS addition; (D) same solution recorded 30 min after DBNBS addition. For spectra B, C the asterisks mark groups of resonances assigned to a DBNBS-methyl adduct.

(25 mol·dm^{-3}) produced a DBNBS-methyl adduct (long-term stability of this adduct was confirmed by continued monitoring of the EPR spectra, >5 h). Introduction of NO (via syringe) degraded the DBNBS-methyl adduct, and produced an EPR spectrum which contained both radical 1 and 2. Radical 2 is presumably derived from the reaction of DBNBS with dimethylnitroxide, via the DBNBS-methyl adduct (Scheme 1); radical 1 is produced by the reaction of DBNBS with NO (vide supra).

In order to determine whether any of the radicals above are the result of simple one electron oxidation of NDMA by OH, two strong oxidants were reacted with NDMA. Aqueous solutions of NDMA in the presence of radiolytically-generated oxidants, Tl^{2+} [reaction (7), $E^0 = +2.22$ V] and SO_4^- [reaction (8), $E^0 = +2.43$ V] (Stanbury, 1989), gave no detectable ESR signals.
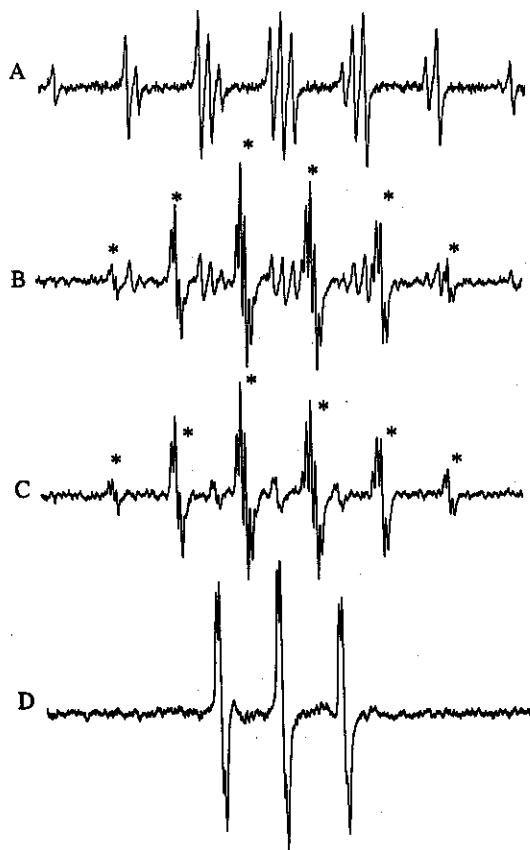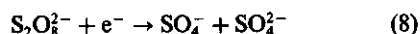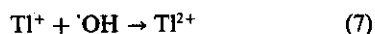
$$Tl^+ + \cdot OH \rightarrow Tl^{2+} \tag{7}$$

$$S_2O_8^{2-} + e^- \rightarrow SO_4^- + SO_4^{2-} \tag{8}$$

Furthermore, pulse radiolysis experiments have shown that Tl^{2+} and SO_4^- radical transients both react with NDMA, with rate constants of $(1.4 \pm 0.2) \times 10^7$ dm^3·mol^{-1} s^{-1} and $(1.5 \pm 0.3) \times 10^{10}$ dm^3 × mol^{-1} s^{-1}, respectively. Thus, both oxidizing species (Tl^{2+} and SO_4^-) react with NDMA, yet do not yield stable paramagnetic species. Therefore, OH ($E^0 = +1.9$ V) does not simply oxidize NDMA to give dimethylnitroxide, but must either add to NDMA or abstract a hydrogen to initiate the process.

DISCUSSION

There are several possible mechanisms by which hydroxyl radical may react with NDMA, including: (1) oxidation via electron transfer, (2) hydrogen abstraction, or (3) addition to the nitroso functional group. If oxidation of NDMA by hydroxyl radical is the primary mode of reaction, one would expect that either Tl^{2+} or SO_4^- would yield dimethylnitroxide. Therefore, OH oxidation via electron transfer is not likely. For hydrogen abstraction, the radiolysis studies using spin traps suggest that if the NDMA alkyl radical is formed by the reaction of OH with NDMA, if must either rapidly rearrange unimolecularly to form an untrappable species or, for DBNBS, react directly by electron transfer with the spin trap. The final possible reaction pathway is addition of OH at the nitroso group of NDMA. Stable hydroxyl adducts of α-carbon nitroso groups have not been reported. Furthermore, spin adducts of any kind for α-nitrogen nitroso groups are extremely rare (Perkins, 1970). Therefore, addition of OH, or an alkyl radical, to the nitroso group of NDMA should lead to an inherently unstable product, and is a possible precursor responsible for the observed free radical chemistry. The detailed mechanism for the reaction of NDMA with OH is difficult to elucidate

and may require study by pulse radiolysis–EPR detection techniques where both the structure and kinetics of the primary intermediates can be measured.

After OH reacts with NDMA, a chemical species (A in Scheme 1) is generated, which in the presence of DBNBS, yields radical 1. This species oxidizes DBNBS similar to NO and Br_2^-. It might be attractive to assign A as NO; however, this would not explain the NDMA concentration dependence on the observed radical 1 and 2 ratios. This assignment would require NO to react with NDMA to yield B. However, NO does not react with NDMA, thereby eliminating NO as species A, though NO may contribute to the radical 1 yield (Scheme 1). Since DBNBS would be expected to trap the alkyl radical of NDMA [equation (1)], if formed, the alkyl radical must denitrosate faster than it can be scavenged by DBNBS. Despite the absence of a DBNBS-NDMA alkyl adduct, the possibility of fast denitrosation along with observed chemistry attributable to NO, do not permit elimination of the NDMA alkyl radical from the mechanistic scheme. A likely candidate for A is the dimethylamino radical, produced by OH (or R) addition to the nitroso nitrogen, followed by N–N bond cleavage. Supporting evidence for this conclusion is derived from UV-photolysis of NDMA, which induces denitrosation to form the dimethylamino radical, and results in dimethylnitroxide formation (Joshi and Yang, 1981).

Species A reacts further, involving one or more NDMA molecules, to form dimethylnitroxide. In the presence of DBNBS, radical 2 is generated in a similar NDMA concentration dependence behavior. Also, the observation that DBNBS can be added *after radiolysis* to a solution containing dimethylnitroxide to give radical 2 [Fig. 2(B)], supports the assignment of species B in Scheme 1 as dimethylnitroxide. In this case, the small amount of radical 1 formed likely results from residual NO reacting with DBNBS, and is presumed not from species A, since A is likely to be short-lived. The mechanism by which dimethylnitroxide reacts with DBNBS to form a DBNBS-methyl adduct, which in turn decays rapidly to radical 2, cannot be determined here. It was determined that the conversion of DBNBS-methyl adduct to radical 2 may be initiated by NO, though the involvement of EPR-silent diamagnetic species cannot be discounted. Overall, very unusual chemistry is observed for DBNBS reactions with NDMA intermediates, which unfortunately did not permit us to fully elucidate the mechanism by which OH degrades NDMA, but provided examples of other modes of reaction for DBNBS other than simple spin-trap chemistry.

A number of observations important to spin-trap chemistry are summarized. First, that DBNBS can react by electron transfer with chemical intermediates from a substrate, without the accumulation of radical adducts of that substrate. Direct reaction instead results in DBNBS radicals that are derived from decomposition of the spin trap itself. Second, the observation that DBNBS added after radiolysis of NDMA produces paramagnetic products in amounts nearly identical to those produced in NDMA solutions irradiated with DBNBS is astonishing, and unprecedented (to our knowledge) in any spin-trap chemistry. Lastly, nitric oxide induced formation of radical 1 would suggest the use of DBNBS as a potential biological marker of NO. However, radical 1 is not specific to NO, and can be formed by other oxidative processes. These and other observations reported here illustrate the difficulties involved in spin-trap chemistry, and suggest caution should be exercised when using DBNBS as a spin trap.

REFERENCES

Appel K. E. and Graf H. (1982) Metabolic nitrite formation from *N*-nitrosoamines: evidence for a cytochrome P-450 dependent reaction. *Carcinogenesis* **3**, 293–296.

Flitter W. D. and Mason R. P. (1990) The horseradish peroxidase catalysed oxidation of deoxyribose sugars. *Free Rad. Res. Commun.*

Haussman H.-J. and Werringloer J. (1985) Oxidative denitrosation and activation of *N*-nitrosodimethylamine by rat liver microsomes. *Biochem. Pharm.* **34**, 411–412.

Heur Y. H., Streeter A. J., Nims R. W. and Keefer L. K. (1989) The Fenton degradation as a non-enzymatic model for microsomal denitrosation of *N*-nitrosodimethylamine. *Chem. Res. Toxicol.* **2**, 247–253.

Joshi A. and Yang G. C. (1981) ESR studies of nitroxide radicals formed in the low temperature photolysis of *N*-nitrosoamines. *Org. Magn. Resonance* **17**, 138–143.

Kalyanaraman B. (1990) Characteristics of radical adducts found during photochemical spin trapping in liposomes. *Photochem. Photobiol.* **52**, 323–331.

Kaur H., Leung K. H. W. and Perkins M. J. (1981) A water-soluble, nitroso-aromatic spin-trap. *J.C.S. Chem. Commun.* 142–146.

Krishna C. M., Kondo T. and Reisz P. (1988) Sonochemistry of aqueous solutions of amino acids and peptides. A spin trapping study. *Radiat. Phys. Chem.* **32**, 121–128.

Kroeger-Koepke M. B., Koepke S. R., McClusky G. A., Magee P. N. and Michejda C. J. (1981) α-Hydroxylation pathway in the *in vitro* metabolism of carcinogenic nitrosoamines: *N*-nitrosodiethlyamine *N*-nitroso, *N*-methylanaline. *Proc. Nat. Acad. Sci. U.S.A.* **78**, 6489.

Neta P., Silverman J., Markovic V. and Rabani J. (1986) Radiation-induced reactions of polymer radicals with ruthenium tris(bipyridl)-OH adducts in aqueous solutions. *J. Phys. Chem.* **90**, 703–707.

Perkins M. J. (1970) The trapping of free radicals by diamagnetic scavengers. In *Essays on Free-Radical Chemistry*, p. 99. Special Publication 24, Alden Press, Oxford.

Preussman R. and Stewart B. W. (1982) *N*-Nitroso carcinogens. In *Chemical Carcinogenesis* (Edited by Searle C. E.), 2nd edn, pp. 643–823. American Chemical Society Monograph. ACS, Washington, DC.

Simic M. G. and Hunter E. P. (1983) Interactions of free radicals and antioxidants. In *Radioprotectors and Anticarcinogens*, p. 449. Academic Press, New York.

Stanbury D. M. (1989) Reduction potentials involving inorganic free radicals in aqueous solution. *Adv. Inorg. Chem.* **33**, 69–138.

Stolze K. and Mason R. P. (1987) Spin trapping artifacts in DMSO. *Biochem. Biophys. Res. Commun.* **143**, 941–946.