Oxidation of the Spin Trap 5,5-Dimethyl-1-pyrroline *N*-Oxide by Singlet Oxygen in Aqueous Solution

P. Bilski,* K. Reszka, M. Bilska,† and C. F. Chignell

Contribution from the Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

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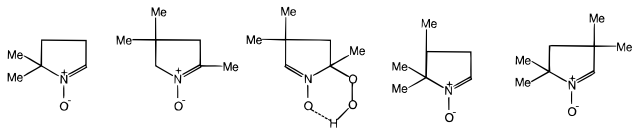
Abstract: The spin trap 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) is frequently used to identify free radicals that are generated photochemically using dyes as photosensitizers. When oxygen is present in such systems, singlet oxygen ($^1O_2$) may be produced and can react with DMPO. We have studied the reaction of DMPO with $^1O_2$ in aqueous solutions over a wide range of pH, using micellar Rose Bengal (pH 2−13) and anthrapyrazole (pH < 2) as photosensitizers. We found that DMPO quenches $^1O_2$ phosphorescence ($k_q = 1.2 \times 10^6$ M^{-1} s^{-1}), thereby initiating oxygen consumption that is slow at pH 10 but increases about 10-fold at pH < 6. This oxygen consumption is a composite process that includes efficient oxidation of both DMPO and its degradation products. The oxidation products include both products in which the DMPO pyrroline ring remains intact (DMPO/•OH and 5,5-dimethyl-2-oxo-pyrroline-1-oxyl (DMPOX) radicals) and those in which it becomes opened (nitro and nitroso products). The nitroso product itself strongly quenched $^1O_2$ phosphorescence, while (photo)decomposition of the nitroso group, presumably to nitric oxide (NO•), produced nitrite as a minor product. We propose that $^1O_2$ adds to the >C═N(O) bond in DMPO, producing a biradical, >C(OO•)—N•(O). This biradical may follow one of two pathways: (i) It may be protonated and rearrange to a strongly oxidizing nitronium-like moiety, which could be reduced to the DMPO hydroperoxide radical DMPO/•O_2H while oxidizing another DMPO moiety to ultimately form DMPOX. The DMPO/•O_2H could undergo further redox decomposition, e.g. *via* the known Fenton-like reaction, to produce both free •OH radical and the DMPO/•OH radical. (ii) The biradical >C(OO•)—N•(O) may cyclize to a 1,2,3-trioxide (ozonide), which could open the pyrroline ring to form 4-methyl-4-nitropentan-1-al and 4-methyl-4-nitrosopentanoic acid. Because the oxidation of DMPO by $^1O_2$ leads to both rapid O_2 depletion and the formation of transients and products that might interfere with trapping and identification of free radicals, DMPO should be used with caution in systems where $^1O_2$ is produced.

Introduction

Photoxidative processes often produce free radicals as transient species that can be identified by using radical trapping agents[1] ("spin traps"). Spin traps react with transient free radicals in solution to yield stable products, "spin adducts", which can be observed directly by EPR spectroscopy. The ideal spin trap should be reactive enough to scavenge the free radical-(s) of interest while at the same time being relatively inert toward reactive but nonradical species.

The nitrone spin trap 5,5-dimethyl-1-pyrroline *N*-oxide (**1**, DMPO) is widely used to provide evidence for the involvement of free radicals in many chemical and biological reactions.[2] DMPO is particularly useful for identifying oxygen-centered radicals, e.g. superoxide radical anion, peroxyl, alkoxyl, and hydroxyl radicals, because the resultant spin adducts have characteristic EPR parameters[1,2] and can be readily distinguished from other adducts. These oxygen-centered radicals are frequently generated in photochemical systems that use dyes as photosensitizers. However, such dyes frequently also generate singlet oxygen, $^1O_2$. Thus, when DMPO or other nitrones are used to identify radicals generated in photochemical systems, it is important to understand the possible reactions that may occur between the spin trap and $^1O_2$.

Singlet oxygen generated via methylene blue photosensitization is known to oxidize nitrones in organic and aqueous solutions. For example, Ching and Foote[3] have shown that irradiation of methylene blue in the presence of 2,4,4-trimethyl-1-pyrroline *N*-oxide (**2**) in $CDCl_3$ solution at −55 °C results in the *ene* addition of $^1O_2$ to yield peroxide **3**. In contrast, nitrone **4** was unchanged by similar treatment.[3] Harbour and co-workers



have studied the reaction of $^1O_2$ generated from methylene blue with several nitrones, including DMPO, in aqueous solution.[4a] On the basis of oxygen consumption measurements, the rate constant for $^1O_2$ quenching by DMPO was estimated by these workers to be 1.8×10^7 M^{-1} s^{-1} in D_2O, although the pD was not specified.[4a]

During photooxidation of DMPO by methylene blue,[4a] Harbour and co-workers observed the EPR spectrum of the DMPO/•OH adduct. DMPO/•OH was also detected by Feix and Kalyanaraman when an aqueous solution of Merocyanine-540 was irradiated in the presence of DMPO.[4b] These workers demonstrated that only about one-half of the DMPO/•OH resulted from the trapping of freely diffusible •OH by DMPO. They postulated that $^1O_2$ reacted with DMPO to yield an

†Guest Researcher.

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(1) Janzen, E. G. *Acc. Chem. Res.* **1971**, *4*, 31.

(2) Janzen, E. G. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: New York, **1980**; Vol. IV and references therein.

(3) Ching, T.; Foote, C. S. *Tetrahedron Lett.* **1975**, 3771.

(4) (a) Harbour, J. R.; Issler, S. L.; Hair, M. L. *J. Am. Chem. Soc.* **1980**, *102*, 7778. (b) Feix, J. B.; Kalyanaraman, B. *Arch. Biochem. Biophys.* **1991**, *291*, 43.

unidentified intermediate, [DMPO−$^1O_2$], which decomposed to DMPO/•OH and free hydroxyl radical.[4b]

In strongly oxidative environments, DMPO may be oxidized to 5,5-dimethyl-2-oxopyrroline-1-oxyl (DMPOX), which is readily identifiable by its characteristic EPR spectrum.[5] Sometimes other unexpected EPR signal(s) that are typical of nitroso spin adducts are also observed from chemically or enzymatically oxidized DMPO.[6] However, to our knowledge, the production of DMPOX, nitroso, and nitro compounds from $^1O_2$-oxidized DMPO has not been reported previously.

The purpose of the present study was to investigate the oxidation of DMPO by $^1O_2$ in aqueous solutions. We have found that $^1O_2$ initiates DMPO degradation, a process whose efficiency depends on pH. We have identified products that are EPR active or that may affect the spin-trapping process and propose a comprehensive DMPO photooxidation mechanism.

Materials and Methods

DMPO (Aldrich, Chemical Co. Milwaukee, WI) was vacuum distilled and stored at −20 °C. 2-Methyl-2-nitrosopropane dimer (MNP), 3,3,5,5-tetramethyl-1-pyrroline *N*-oxide (M_4PO), Rose Bengal (RB) phenalenone, and NADH were purchased from Aldrich Chemical Co. (Milwaukee, WI). $^{17}O_2$ containing 64.2% of ^{17}O atoms was purchased from Isotec Inc. (Miamisburg, OH). The anthrapyrazole PD 110095 was a gift from Prof. J. W. Lown of the University of Alberta, Canada. Chemicals used to prepare micellar solutions of RB and the properties of micellar RB have been described previously.[7b] CHELEX 100 (Bio-Rad, Richmond, CA) was washed four times with warm water, filtered, and dried in a desiccator. The buffers were prepared from components of reagent grade or better, and their pH was measured using a glass electrode. Unless indicated, phosphate D_2O and H_2O buffers were treated with CHELEX 100 ion exchange resin in the dark ("chelexed") to remove heavy metal impurities. 4-Methyl-4-nitropentan-1-al (**11**) and 4-methyl-4-nitrosopentanoic acid (**9**), putative photoproducts from DMPO photooxidation, were synthesized by the methods of Bonnett *et al.*[8] and Clark *et.al.*,[9] respectively.

The measurements of oxygen photoconsumption were performed using an assembly previously described.[10] Photosensitizers were irradiated using a combination of glass cutoff filters, 450 nm for RB and 300 nm for the anthrapyrazole sensitizer (*vide infra*). In experiments where MNP was added to the samples, we also used a liquid filter ($CuSO_4$, 1 M, pathlength 1 cm) in order to avoid irradiating MNP, which has a long-wave absorption around 680 nm.

The system for monitoring steady-state singlet oxygen phosphorescence has been described previously.[11] Oxygen was bubbled through the sample during irradiation to compensate for loss of oxygen due to its photoconsumption. The signal from $^1O_2$ phosphorescence was extrapolated to zero irradiation time to eliminate any contribution of DMPO photoproducts to $^1O_2$ quenching.

Absorption spectra were recorded on a HP 8451A diode array spectrophotometer (Hewlett-Packard Instrument Co., Palo Alto, CA). Transient absorption was measured using a PRA flash photolysis system.[7a] EPR spectra were recorded on a Varian EPR spectrometer (Palo Alto, CA), E-Line Century Series, as previously described.[10]

Using RB, a very efficient $^1O_2$ generator, we established that DMPO in concentrations up to 0.2 M does not quench the RB triplet. The lifetime of the radical cation of RB, which is produced *via* self-quenching, was decreased somewhat by DMPO. This suggests that DMPO may be oxidized by this radical cation. However, when oxygen was present in solution, the RB triplet was mainly quenched by energy transfer to dioxygen with little or no formation of dye radicals *via* self-quenching. Therefore, we believe that in our samples DMPO was oxidized solely by the $^1O_2$ pathway.

Rose Bengal in appropriate micellar solutions[12] sensitizes $^1O_2$ generation over a wider pH range (pH 2−12) than free dye.[7b] Rose Bengal in acidic solutions (pH < 5) forms a colorless lactam. In contrast, RB sequestered in cationic micelles is less accessible to protons and, as a result, the dye not only retains its red color at pH 2 but also shows much less photobleaching than free dye.[7b] Finally, the triplet state of RB does not interact with ionic quenchers and is quenched only by oxygen when the dye is solubilized in micelles[13b] (*vide infra*).

To generate $^1O_2$ in HCl solutions (pH < 2), we used the anthrapyrazole derivative PD 110095. This compound has been previously shown to generate singlet oxygen.[14] In the present work, we also established that PD 110095 efficiently sensitized $^1O_2$ formation in strongly acidic HCl solutions; this was determined both by measuring $^1O_2$ phosphorescence and by measuring oxygen consumption using furfuryl alcohol as a $^1O_2$ trap. Perinaphthenone[15a] was used as a $^1O_2$ producer to examine $^1O_2$ quenching by MNP using a time-resolved technique.[15b]

Nitrite anion was assayed in the presence of RB as previously described.[15c] Acidification of nonbuffered samples during DMPO photooxidation was monitored by using a glass electrode and a cell for oxygen photoconsumption.

Alkyl nitro (RNO_2) and nitroso (RNO) photoproducts are formed simultaneously from $^1O_2$-oxidized DMPO; we monitored $^1O_2$ phosphorescence as an indicator of RNO accumulation because the nitroso product is a strong $^1O_2$ quencher. In these experiments, a solution containing RB (50 μM), DMPO (20 mM), and the appropriate buffer in D_2O was irradiated for about 10 min through a combination of glass (450 nm) and $CuSO_4$ liquid filter using a 200 W xenon−mercury lamp. Oxygen was bubbled through the sample during irradiation, and $^1O_2$ phosphorescence was monitored. Irradiation was continued until $^1O_2$ phosphorescence dropped to near baseline level. Products were identified by comparing the EPR spectra that were observed during a photoreduction procedure (see Results) with those produced when standards **9** and **11** were added to nonirradiated samples.

Results

Quenching of $^1O_2$ Phosphorescence. We first wished to measure the $^1O_2$ phosphorescence quenching rate constant for

(5) (a) Black, D. St. C.; Watson, K. G. *Aust. J. Chem*. **1973**, *26*, 2505. (b) Finkelstein, E.; Rosen, G. D. M.; Rauckman, E. J. *Arch. Biochem. Biophys*. **1980**, *200*, 1. (c) Rosen, G. M.; Rauckman, E. J. *Mol. Pharmacol*. **1980**, *17*, 233. (d) Nishi, M.; Hahi, A.; Ide, H.; Murakami, A.; Makino, K. *Biochem. Int.* **1992**, *27*, 651. (e) Hill, H. A. O.; Thornalley, P. J. *Inorg. Chim. Acta* **1982**, *67*, L35. (f) Bernofsky, C.; Bandara, B. M. R.; Hinojosa, O. *Free Rad. Biol. Med.* **1990**, *8*, 231. (g) Mao, G. D.; Thomas, P. D.: Poznansky, M. *Free Rad. Biol. Med.* **1994**, *16*, 493. (h) Floyd, R. A.; Soong, L. M. *Biochem. Biophys. Res. Commun*. **1977**, *74*, 79. (i) Thornalley, P. J.; Trotta, R. J.: Stern, A. *Biochim. Biophys. Acta* **1983**, *759*, 16.

(6) (a) Mottley, C.; Mason, R. P. In *Biological Magnetic Resonance;* Berliner, L. J.; Reuben, J., Eds.; Plenum Publishing Corp.: New York 1989; p 489. (b) Reszka, K.; Chignell, C. F. *Free Rad. Res. Commun*. **1991**, *14*, 97 and references therein.

(7) (a) Bilski, P.; Dabestani, R.; Chignell, C. F. *J. Phys. Chem*. **1991**, *95*, 5784−5791. (b) Bilski, P.; Chignell, C. F. *J. Photochem. Photobiol., A: Chem*. **1993**, *77*, 49.

(8) Bonnett R.; Clark, V. M.; Giddey, A.; Todd, A. *J. Chem. Soc*. **1959**, 2087.

(9) Clark, V. M.; Sklartz, B.; Todd, A. *J. Chem. Soc*. **1959**, 2123.

(10) Bilski, P.; Li, A. S. W.; Chignell, C. F. *Photochem. Photobiol*. **1991**, *54*, 345.

(11) Hall, R. D.; Chignell, C. F. *Photochem. Photobiol*. **1987**, *45*, 459.

(12) Chemical $^1O_2$ detection using furan derivatives, together with the direct observation of $^1O_2$ phosphorescence, confirmed that the quantum yield of $^1O_2$ production by RB in cationic or zwitterionic micelles is high and independent of pH.[7] Moreover, excited state RB is protected by the micellar envelope from interaction with quenchers in the aqueous phase.[7] The same photosensitization system was previously used to investigate the photooxidation of *N,N*-diethylhydroxylamine by both $^1O_2$ and the RB triplet.[13a]

(13) (a) Bilski, P.; Motten, A. G.; Bilska, M.; Chignell, C. F. *Photochem. Photobiol*. **1993**, *58*, 11. (b) We have also tried to use the thermal decomposition of the endoperoxide of 3,3′-(1,4-naphthylidene)dipropionate as an alternative source of $^1O_2$ to oxidize DMPO. However, under some conditions, particularly in the presence of redox metals, we observed additional radical adducts of DMPO besides DMPO/•OH. Furthemore, the thermal decomposition of endoperoxides is affected by pH, which makes them unsuitable for use over the wide pH range examined in our study. Thus, micellar RB proved to be the cleanest source of $^1O_2$.

(14) Reszka, K.; Tsoungas, P. G.; Lown, J. W. *Photochem. Photobiol*. **1986**, *43*, 499.

(15) (a) Schmidt, R., Tanielian, C., Dunsbach, R.; Wolff, C. J. *Photochem. Photobiol., A: Chem.* **1994**, *79*, 11. (b) Bilski, P.; Chignell, C. F. *J. Biochem. Biophys. Methods* **1995**, in press. (c) Bilski, P.; Reszka, K,; Chignell, C. F. *J. Am. Chem. Soc.* **1994**, *116*, 9883.

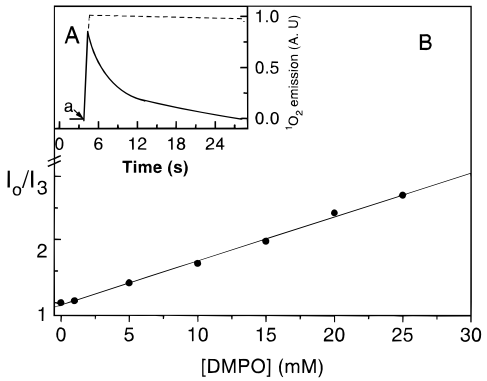


Figure 1. Quenching of $^1O_2$ phosphorescence by DMPO under steady-state conditions: (A) phosphorescence intensity of $^1O_2$ as a function of irradiation time for RB alone (dashed line) and in the presence of DMPO (30 mM) in alkaline H_2O solution (solid line); (B) quenching of $^1O_2$ phosphorescence by DMPO in D_2O at pD 9.5. RB, 25 μM. Deuterated buffers: 50 mM.

DMPO. We found that the addition of DMPO to an aqueous RB solution caused an immediate reduction in $^1O_2$ phosphorescence by about 20% during steady-state irradiation. However, as irradiation continued, the intensity of $^1O_2$ phosphorescence decreased further, reaching 50% of the initial value in approximately 6 min (Figure 1A). This gradual decrease can only be explained by the *in situ* production of a very strong $^1O_2$ quencher as the sensitizer was not bleached during irradiation and oxygen concentration was maintained by continuous O_2 purging of the samples. A similar pattern of steady-state $^1O_2$ phosphorescence quenching was observed for all pH values studied.

To differentiate quenching by DMPO itself from quenching caused by DMPO oxidation product(s), we extrapolated the intensity of $^1O_2$ phosphorescence to zero irradiation time (no products) for several different DMPO concentrations. The Stern−Volmer plot gave a quenching rate constant of $k_q = 1.2 \times 10^6$ M^{-1} s^{-1}, taking the $^1O_2$ lifetime as 54 μs in our D_2O buffer[16a] (Figure 1B). We also measured this k_q in pulse experiments in which the decay of $^1O_2$ phosphorescence was monitored after a single laser pulse at 532 nm (Figure 2B) to eliminate quenching byproducts. Pulse experiments yielded an identical k_q value ($k_q = (1.2 \pm 0.2) \times 10^6$ M^{-1} s^{-1}), which was virtually pH independent (Figure 2A). However, this k_q value

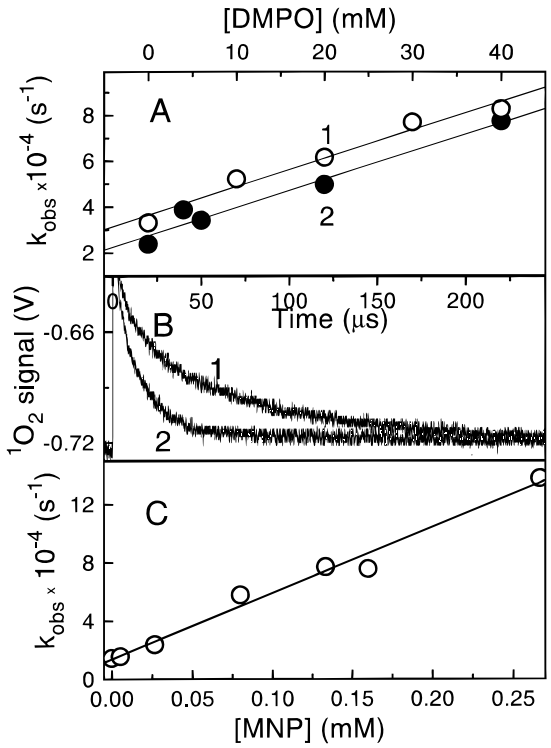


Figure 2. Quenching of $^1O_2$ phosphorescence by DMPO and MNP observed in pulse experiments: (A) observed rate constant of $^1O_2$ phosphorescence decay as a function of DMPO concentration at pD 4 (line 2) and pD 10 (line 1); (B) examples of $^1O_2$ decay in D_2O (plot 1) and in the presence of MNP, 3 mM (plot 2); (C) observed rate constant of $^1O_2$ phosphorescence decay as a function of the MNP concentration in D_2O containing 5% of acetonitrile (MNP dissolved in acetonitrile was a stock solution).

is significantly lower than the previously reported[4a] value of 1.8×10^7 M^{-1} s^{-1}.

Because our EPR experiments performed on preirradiated RB/DMPO samples showed the presence of nitro and nitroso photoproducts (*vide infra*), we examined the possibility that the very strong $^1O_2$ quencher observed above could be a nitroso compound. Nitroso compounds are known to be strong quenchers of $^1O_2$ in organic solvents.[16b] To determine what effect a DMPO-derived nitroso product might have on $^1O_2$ phosphorescence, we used 2-methyl-2-nitrosopropane (MNP) as a model $^1O_2$ quencher in time-resolved experiments. In Figure 2C, we plotted the observed rate constants as a function of the MNP concentration, which yields $k_q(\text{MNP}) = (4.5 \pm 0.3) \times 10^8$ M^{-1} s^{-1}; this value is more than 2 orders of magnitude higher than the k_q(DMPO) determined under similar conditions (*vide supra*). Thus, the character of the plot in Figure 1A may be caused by the *in situ* production of a similar nitroso quencher.

Oxygen Photoconsumption. The quenching of $^1O_2$ by DMPO is accompanied by oxygen consumption[16d] (Figure 3). In buffered solutions, the oxygen concentration decreased linearly until more than 50% of the O_2 was consumed but the overall consumption process was quasi-exponential over time (Figure 3A, plot 2). The initial consumption rates were practically linear and were used to investigate the influence of pH on the DMPO photooxidation process.

At all pH values examined, the initial consumption rate was a linear function of DMPO concentration (Figure 3A). These initial rates were higher in both acidic (pH < 6) and strongly alkaline solutions, with a minimum around pH 10 and a plateau in the pH range 2−6 (Figure 4). The pH dependence of oxygen photoconsumption was the same at two different DMPO concentrations[16f] (30 and 5 mM). Similar plots were also

(16) (a) We obtained this value from the exponential decay of $^1O_2$ phosphorescence using our time-resolved spectrometer.[15b] (b) There are few oxidation products that can be formed from DMPO that would quench $^1O_2$ efficiently. Nitro, nitroxyl, and hydroxylamine compounds are no better $^1O_2$ quenchers than DMPO itself;[16c] the only putative oxidation product that would be an efficient quencher is an aliphatic nitroso compound[16c] ($k_q \sim 10^{10}$ M^{-1} s^{-1} in organic solvents). (c) Wilkinson, F.; Brummer, J. G. *J. Phys. Chem. Ref. Data* **1981**, *10*, 809. (d) Oxygen photoconsumption could be affected by the *in situ* generation of a new $^1O_2$ nitroso quencher (*vide infra*). If a photoproduct is a purely physical quencher, then the rate of O_2 consumption should decrease. By contrast, a chemical quencher might accelerate the rate of oxygen consumption. In buffered solutions, the curves describing the rate of O_2 photoconsumption did not exhibit features expected for either purely autocatalytic or inhibitory processes (Figure 3A, plot 2). We used 2-methyl-2-nitrosopropane (MNP) to determine how a nitroso quencher might affect oxygen consumption. Irradiations were carried out through a $CuSO_4$ filter to prevent photodecomposition of MNP ($\lambda_{max} =$ 680 nm). The irradiation of RB in a solution containing MNP (2.5 mM) initiated oxygen consumption. However, this process was many times slower than that observed for the efficient chemical $^1O_2$ substrate furfuryl alcohol.[16e] Moreover, the presence of MNP (2.5 mM) during DMPO (30 mM) oxidation at pH 6 decreased the overall rate of O_2 consumption by about a factor of 2. This suggests that a nitroso product from DMPO decomposition may, like MNP, be more a physical than a chemical $^1O_2$ quencher. (e) Braun, A. M.; Oliveros, E. *Pure Appl. Chem.* **1990**, *62*, 1467. (f) Similar plots observed for different DMPO concentrations proved that quenching saturation did not occur in the DMPO concentration range employed in these experiments.

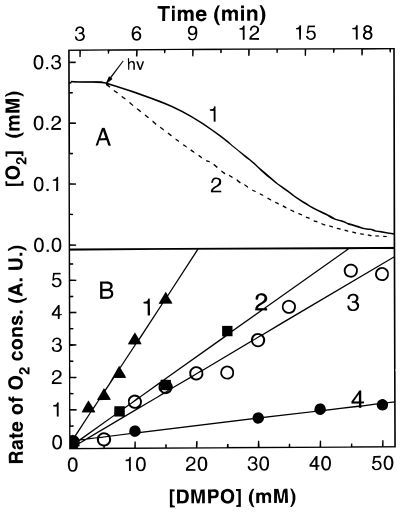


Figure 3. Oxygen photoconsumption by DMPO: (A) time profile of oxygen consumption during the oxidation of DMPO (50 mM) in deionized water (**1**) and in pH 10.8 phosphate buffer (**2**); (B) rate of oxygen photoconsumption by DMPO as a function of DMPO concentration at different pH values (1, pH 6; 2, pH 12.6; 3, pH 7; 4, pH 9.5) RB: 25 μM. Buffer: 60 mM.

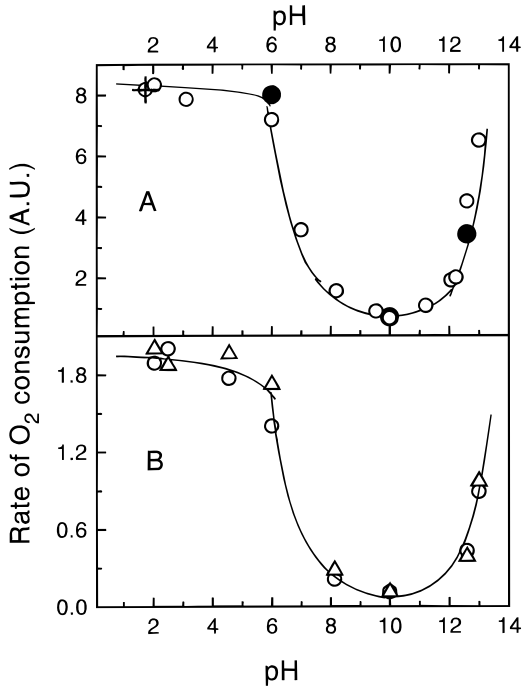


Figure 4. Rate of oxygen photoconsumption by DMPO or M_4PO sensitized by micellar or nonmicellar (-●-●-) RB (25 μM) as a function of pH. Appropriate buffers, 50 mM; surfactant, 10 mM: (A) DMPO, 30 mM; (B) DMPO (circles) and M_4PO (triangles), 5 mM.

observed when the DMPO analog 3,3,5,5-tetramethyl-1-pyrroline *N*-oxide (M_4PO)[17a] (**5**) was used (Figure 4B). In the pH range where either RB alone or micellar RB can be used as a photosensitizer, similar results were obtained (Figure 4A). Thus the micelles did not affect DMPO photooxidation but only extended the pH range for photosensitization.

In unbuffered samples the rate of O_2 consumption accelerated strongly during irradiation (Figure 3A, plot 1). This acceleration was caused by a decrease in the pH from 6.6 to 4.7 during irradiation, which is consistent with the pH effect shown in Figure 4. A contributor to this acidification may be carboxylic acids formed from DMPO (*vide infra*). Another possible mechanism is the spontaneous oxidation of nitric oxide to nitrous

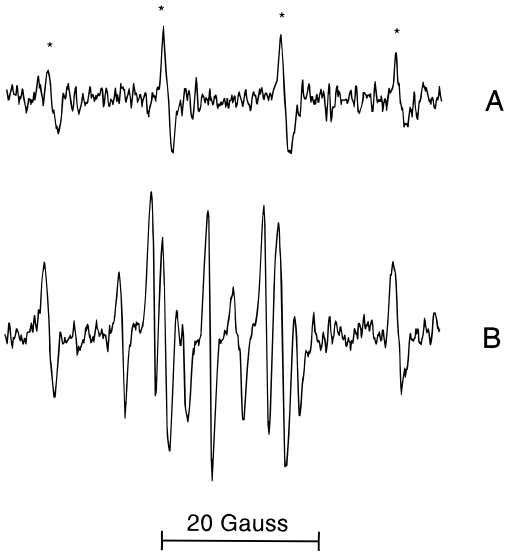


Figure 5. EPR spectrum observed before (A) and during (B) the irradiation of the micellar RB (50 μM) and DMPO (40 mM) in water at pH 2. DMPO/•OH adduct is marked with asterisks; the spectrum intensity did not increase upon longer irradiation. Unmarked signal belongs to the oxidation product of DMPO, 5,5-dimethyl-2-oxopyrroline-1-oxyl (DMPOX); $a_N = 7.2$, $a_H(2) = 4.2$. Instrumental settings: gain, 2×10^4; modulation amplitude, 0.33 G; microwave power, 20 mW; time constant, 0.25 s; scan rate, 4 min/100 G.

and nitric acids; nitric oxide is known to be the main product of nitroso compound (photo)degradation. In support of this hypothesis, we have detected nitrite as a minor product of DMPO photooxidation. However, nitrite production was small and accounted for only ca. 7% of the oxygen consumed at neutral pH. In control experiments, nitrite was also detected when MNP was photooxidized by RB under similar conditions.

Samples containing DMPO and RB became practically anaerobic when irradiated for a sufficiently long time. However, a portion of the photoconsumed oxygen was recovered in the dark. The O_2 recovery was slow (ca. 10 min to reach a constant level) but could be slightly accelerated by either catalase or $CuSO_4$. The recovery was 6%, 13%, and up to 50% at pH 7, 9, and 12, respectively. Upon the addition of catalase there was an immediate recovery of a small part of the O_2 consumed, suggesting that H_2O_2 was a minor product.[17b] However, the major part of the recovery was not specific for catalase and was also initiated by the addition of Cu^{2+}.

EPR Studies. Radical Photoproducts. In aqueous buffer (pH 2), the oxidation of DMPO by $^1O_2$ produced a weak EPR signal from 5,5-dimethyl-2-oxopyrroline-1-oxyl (DMPOX, **15**) (Figure 5, spectrum B). When the oxidation was carried out in a D_2O buffer at pD 2, a strong EPR spectrum of the DMPO/

(17) (a) We used M_4PO, which has a quaternary carbon at position 3 (γ), to see whether dissociation of DMPO (γ-protons) influences photooxidation in NaOH solution. (b) In control experiments, when H_2O_2 was added to the nonirradiated sample, O_2 release showed similar kinetics. Hydrogen peroxide was claimed to be observed previously during the photosensitized oxidation of a number of nitrones including DMPO in neutral solution; aliphatic nitrones showed higher yields of H_2O_2 formation than DMPO. While traces of H_2O_2 may be formed over a broad pH range in our system, its presence could not be conclusively demonstrated. It was difficult to accurately separate the formation of H_2O_2 from the formation of other peroxides by measuring oxygen recovered by catalase. (c) The spectra of DMPO/•OH and DMPO/•OD radicals are similar because the splitting by deuterium is small and is not resolved within the line width of the signal. (d) We did not observe the DMPO/•N_3 adduct, which is formed when azide is oxidized by •OH radical in the presence of DMPO. This shows that a route leading to free •OH is totally dependent on the initial reaction of DMPO with $^1O_2$. (e) Most of the buffers were "chelexed" using an ion exchange resin for the removal of heavy metal impurities (see Materials and Methods).

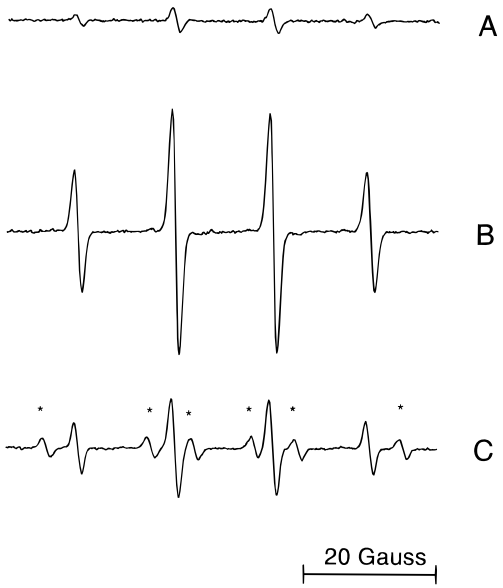


Figure 6. EPR spectrum of DMPO/•OD adduct observed before (A) and during (B, C) the irradiation (11 min) of the micellar RB (50 μM) and DMPO (40 mM) in D_2O at pD 2. (C) Same as B in the presence of MeOH. DMPO/•CH_2OH adduct is marked with asterisks. Instrumental settings are the same as in Figure 4 but gain was 5×10^3.

•OD[17c] (**14**) adduct was observed (Figure 6, spectrum B). The addition of EtOH to the sample resulted in the appearance of the DMPO/•$CH(OH)CH_3$ adduct, which suggests the presence of the hydroxyl radical (Figure 6, spectrum C). However, a portion of the DMPO/•OH adduct was not quenched by alcohol, which indicates that not all of this adduct was formed by the reaction of DMPO with •OH. In more acidic undeuterated solutions (0.25 M HCl in H_2O), we did not observe any EPR signal. In acidic D_2O solution (0.25 M DCl in D_2O), we detected the DMPO/•OH(D) signal, which was about 10 times less intense than that formed at pD 2. Control experiments showed that in strongly acidic solutions (1 M HCl) DMPO still functioned as a spin trap. Thus, the weaker DMPO/•OH signals observed in HCl solutions are probably caused by lower adduct stability. The stronger signal of DMPO/•OD in DCl solution can be explained by faster adduct production in D_2O due to the longer $^1O_2$ lifetime (roughly 10 times longer in D_2O), which may compensate for lower adduct stability.

In alkaline solutions (pH > 10), we did not detect any significant accumulation of the DMPO/•OH adduct. Most likely low photostability and a slower rate of adduct formation prevented its accumulation during steady-state irradiation in alkaline solutions.

The DMPO/•OH adduct was always formed during DMPO oxidation at neutral pH. The formation of DMPO/•OH was almost completely inhibited by N_3^- (Figure 7, spectrum D), confirming that essentially all DMPO/•OH and •OH[17d] are $^1O_2$ dependent. The signal intensity was much stronger when nonchelexed[17e] phosphate buffers were used (Figure 7, spectra C and E), which would seem to implicate metal-catalyzed decomposition of peroxides. Catalase had little effect on signal intensity, which excludes the involvement of H_2O_2. However, other organic hydroperoxide(s) may decompose in Fenton-like reactions (catalyzed by metal cations) to produce •OH and DMPO/•OH radicals.

Next we investigated the source of oxygen in the DMPO/•OH adduct. When a solution containing DMPO and RB was saturated with oxygen containing 64% $^{17}O_2$ isotope and then irradiated, the spectrum in Figure 8A was observed. This spectrum contains contributions from three adducts: DMPO/

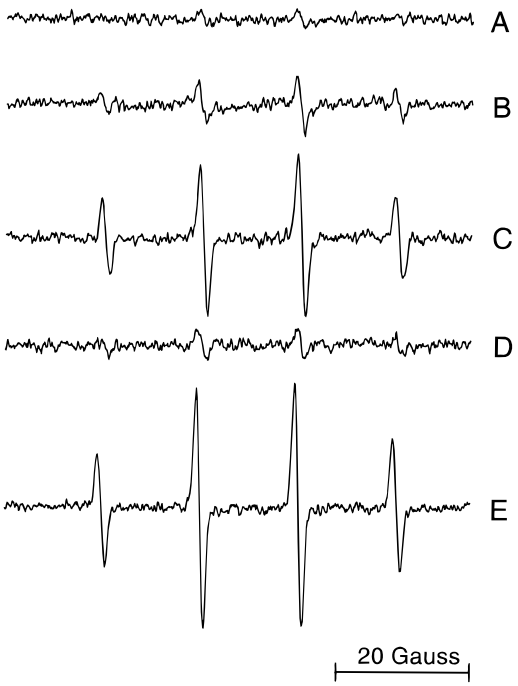


Figure 7. EPR spectrum of DMPO/•OH adduct observed during 4 min of irradiation (B−E) of the micellar RB (50 μM) and DMPO (40 mM) in H_2O at neutral pH: (B) in water; (C) in nonchelated phosphate buffer, 40 mM; (D) same as C in the presence of N_3^- (20 mM); (E) in nonchelated phosphate buffer, 400 mM. Control A shows the spectrum before irradiation. Instrumental settings are the same as in Figure 4.

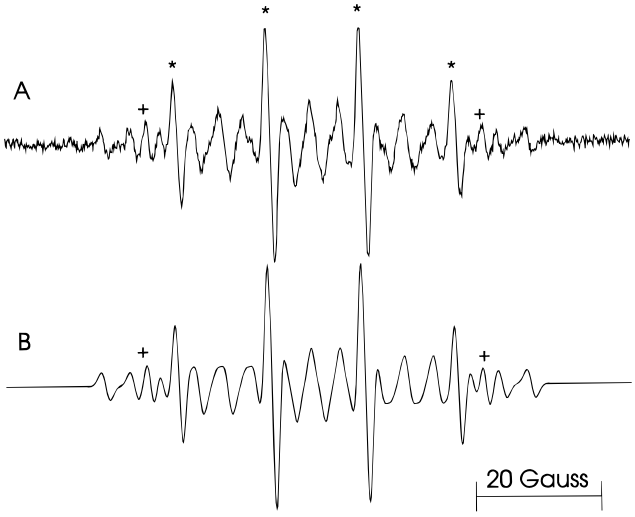


Figure 8. (A) EPR spectrum observed during the irradiation of RB (50 μM) and DMPO (30 mM) in phosphate buffer (pH 7) saturated with $^{17}O_2$ (64.2%). The spectrum contains contribution from DMPO/^{16}•OH (*, $a_N = 15.17$, $a_H^\beta = 14.62$ G) and DMPO/^{17}•OH ($a_N = 15.11$, $a_H^\beta = 14.96$; $a(^{17}O) = 4.65$ G). Better simulation (B) was obtained when a DMPO adduct of unknown carbon-centered radical was introduced (minor species, 6%), whose two outer lines from the six-line signal are marked +, $a_N = 17.33$, $a_H^\beta = 19.53$ G). Instrumental settings: gain, 2×10^4; modulation amplitude, 0.33 G; microwave power, 20 mW; time constant, 0.25 s; scan rate, 4 min/100 G.

^{17}•OH, DMPO/^{16}•OH, and DMPO/•C, an unknown adduct of a carbon-centered radical (ratio 59:35:6, respectively). A simulated spectrum and the values of the splitting constants are shown in Figure 8, spectrum B. These data show that practically all of the oxygen in the hydroxyl group of the DMPO/•OH radical adduct originates from dissolved molecular oxygen and not from DMPO or H_2O.[18]

Detection of Nitroso and Nitro Products. The presence of the nitro (RNO_2) and nitroso (RNO) products from $^1O_2$-induced DMPO decomposition was confirmed by the EPR detection of radicals $RNO_2^{\bullet -}$ and RN(D)O$^\bullet$, respectively. Both of these radicals were generated by photoreduction of RNO_2 and RNO products that were accumulated during DMPO photooxidation (Materials and Methods).

The photoreduction procedure requires irradiation of RB in the presence of NADH and the products of DMPO oxidation. The mechanism involves the RB triplet, $(^3RB^{2-})^*$, which is quenched by NADH in anaerobic solution,[20] forming the $RB^{\bullet 3-}$ anion (reduced RB) and oxidized NADH ($NAD^\bullet$ radical) (eqs 1 and 2). Both $RB^{\bullet 3-}$ and $NAD^\bullet$ are strongly reducing species.

$$RB^{2-} + h\nu \rightarrow {}^1(RB^{2-})^* \rightarrow {}^3(RB^{2-})^* \quad (1)$$

$$^3(RB^{2-})^* + NADH \rightarrow RB^{\bullet 3-} + NAD^\bullet + H^+ \quad (2)$$

We have found that such a photoreduction procedure gives clear EPR spectra of $RNO_2^{\bullet -}$ and RN(D)O$^\bullet$ radicals without any contribution from the DMPO/$^\bullet$H adduct, which had dominated when we tried to reduced RNO_2 and RNO chemically.[21]

The above photoreduction procedure was then used for RN(D)O$^\bullet$ detection. Even though RNO was formed at all pH values studied (indicated by the progressive quenching of $^1O_2$ phosphorescence), we used acidic solutions to facilitate RN(D)O$^\bullet$ detection without adjusting the pD. The solution containing RB (50 μM) and DMPO (20 mM) in D_2O buffer (pD 6) was irradiated as described in the Material and Methods. Then NADH (0.4 mM) was added, the sample was deoxygenated with N_2, and the solution was irradiated in the RB visible band at 550 nm in an EPR cell. The irradiation resulted in RNO reduction (eq 3). The EPR spectrum observed was that of a

$$RB^{\bullet 3-}/NAD^\bullet/D_2O + RNO \rightarrow RN(D)O^\bullet + RB^{2-}/NAD^+ \quad (3)$$

hydronitroxide, RN(D)O$^\bullet$ (Figure 9A). This spectrum has the same splitting constants as that produced by either chemical[22a] or photochemical[22b] reduction of MNP in D_2O solution. In control experiments, when DMPO was not photooxidized, we never observed EPR signals characteristic of the R−N(D)−O$^\bullet$ radical when the same photoreduction procedure was employed. Instead, a strong signal from the $RB^{\bullet 3-}$ radical anion was observed (Figure 9, spectrum B). When oxygen was present, we also observed the spectrum of the DMPO superoxide adduct (not shown). When 4-methyl-4-nitrosopentanoic acid, a known product of DMPO oxidation,[23] was added to nonirradiated samples, an EPR spectrum identical to that shown in Figure 9A was obtained during the photoreduction procedure.

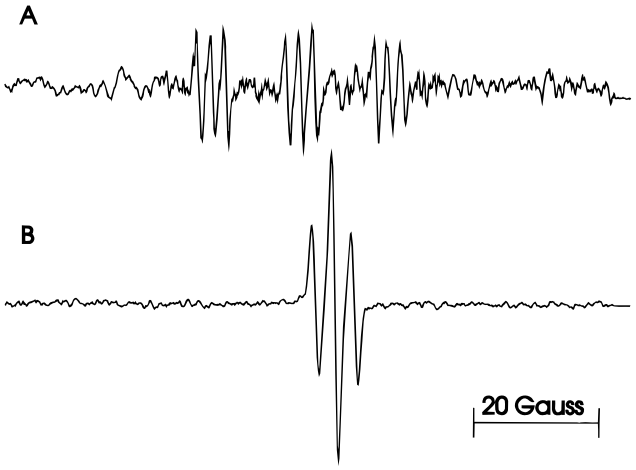


Figure 9. (A) EPR spectrum observed from a sample which contained the products of DMPO oxidation by $^1O_2$. Characteristic R−N(D)−O$^\bullet$ deuterium adduct (a_N = 14.11, a_{D^β} = 2.22 G) was developed as described in the Materials and Methods. Control spectrum B was obtained using nonirradiated sample; the three major line signal belongs to the reduced Rose Bengal ($RB^{\bullet 3-}$) with the splitting constants on two hydrogen atoms, a_H = 3.13 G. Instrumental settings: gain, 2.5 × 10^4; modulation amplitude, 0.66 G; microwave power, 10 mW; time constant, 0.25 s; scan rate, 4 min/100 G.

The nitro product was also detected by EPR after photochemical reduction using 1 mM NADH and RB in alkaline solutions because radical anions of nitro compounds are more stable than neutral radicals, R−$NO_2^\bullet$H. We usually used pD 10.5, although photoreduction in 0.1 M NaOH solution gave similar results. The irradiation of RB led to RNO_2 reduction (eq 4). The EPR signal observed was a triplet with a large

$$RB^{\bullet 3-}/NAD^\bullet/D_2O + RNO_2 \rightarrow RNO_2^{\bullet -} + RB^{2-}/NAD^+ \quad (4)$$

splitting by the nitrogen atom (Figure 10, spectrum A, asterisks). This spectrum is characteristic of a nitro anion radical, indicating the presence of a tertiary aliphatic nitro product **11** derived from DMPO. In a control nonirradiated sample, only the RB^{3-} radical anion was detected (Figure 10, spectrum B). The photoreduction of 4-methyl-4-nitropentan-1-al under the same conditions produced a spectrum identical to that generated by the reduced nitro product from DMPO photooxidation (Figure 10, spectrum C). These results confirm the generation of a nitro product during the reaction of DMPO with $^1O_2$.

Discussion

The oxidation of DMPO is initiated by the chemical quenching of singlet oxygen by DMPO. Singlet oxygen can oxidize C═N double bonds in many organic compounds, including oximes, nitronates, hydrazones, and nitrones.[24,25a] Generally, the reaction of $^1O_2$ with double bonds leads to either 1,2-addition or *ene* addition. However, the *ene* addition pathway is not available to DMPO because the carbon atom at position 5 is quaternary.

We propose that the major pathway of singlet oxygen quenching by DMPO occurs *via* $^1O_2$ addition to the carbon atom

(18) It has been suggested that the DMPO cation radical ($DMPO^{\bullet +}$) may hydrolyze to produce the hydroxyl radical adduct.[19] In a strongly oxidative environment, such as that created by irradiated RB, the formation of $DMPO^{\bullet +}$ and its hydrolysis is a possible nonradical source for the adduct. However, even the quenching of the oxidized RB by DMPO in an anaerobic solution of concentrated RB (0.25 mM), which is more likely to produce the $DMPO^{\bullet +}$ *via* electron transfer, did not lead to the appearance of DMPO/$^\bullet$OH (not shown).

(19) Chandra, H.; Symons, M. C. R. *J. Chem. Soc., Chem. Commun.* **1986**, 1301.

(20) Sarna, T.; Zajac, J.; Bowman, M. K.; Truscott, T. G. *J. Photochem. Photobiol., A: Chem.* **1991**, *60*, 295.

(21) When we used sodium borohydride instead of RB and NADH to reduce the nitro or nitroso products in "dark" reactions, a strong EPR signal from DMPO/$^\bullet$H was produced, which overshadowed the signals from other radicals present in the system.

(22) (a) Kalyanaraman, B.; Perez-Reyes, E.; Mason, R. P. *Tetrahedron Lett.* **1979**, *50*, 4809. (b) Reszka, K. J.; Chignell, C. F. *Photochem. Photobiol.* **1994**, *60*, 450.

(23) Breuer, E. In *Nitrones Nitronates and Nitroxides*; Patai, S., Rappoport, Z., Eds.; John Wiley: New York 1989; p 168 and references therein.

(24) Castro, C.; Dixon, M.; Erden, I.; Ergonenc, P.; Keeffe, J. R.; Sukhovitsky, A. *J. Org. Chem.* **1989**, *54*, 3732 and references therein.

(25) (a) Erden, I.; Griffin, A.; Keeffe, J. R.; Brinck-Kohn, V. *Tetrahedron Lett.* **1993**, *34*, 793. (b) Interaction of $^1O_2$ with the oxygen atom in $N^{\partial +}-O^{\partial -}$ nitrone group, which may also contribute to physical quenching cannot be totally excluded.

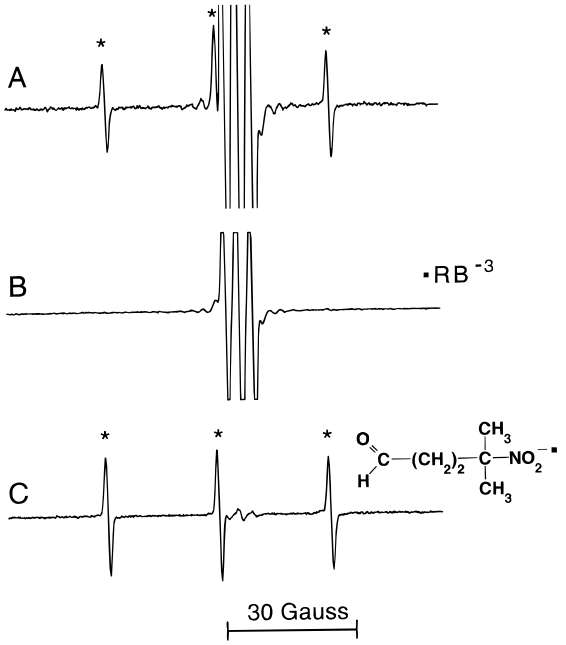
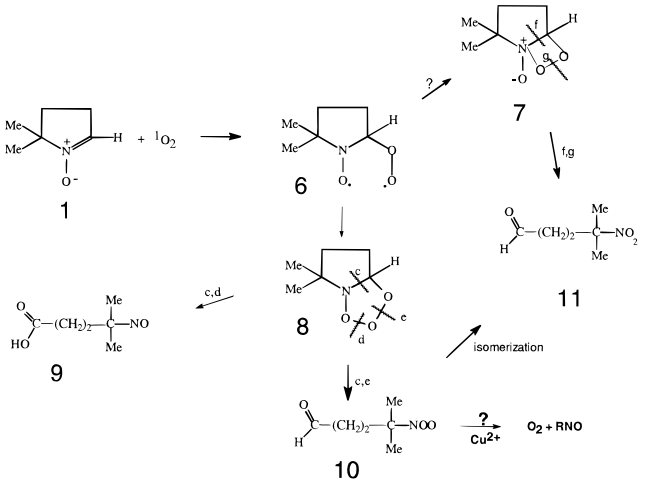


Figure 10. (A) EPR spectrum observed from a sample which contained the products of DMPO oxidation by $^1O_2$. EPR spectrum of the nitro radical anion (*, a_N = 26.14 G) was developed as described in the Materials and Methods. Control spectrum B was obtained using a nonirradiated sample. Spectrum C (gain, 3×10^3) was produced when 4-methyl-4-nitropentan-1-al was added to the nonirradiated sample before the developing procedure. Unmarked signal in the spectra belongs to $RB^{\bullet 3-}$ radical anion (see Figure 8). Instrumental settings: gain, 1.25×10^4; modulation amplitude, 0.66 G; microwave power, 10 mW; time constant, 0.25 s; scan rate, 4 min/100 G.

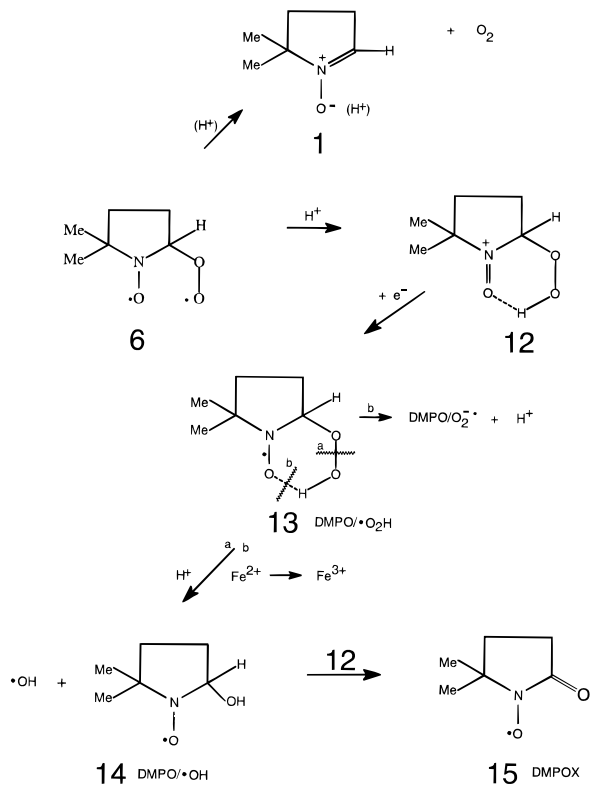
Scheme 1



in the C═N bond,[25b] initially forming a biradical (**6**) (Scheme 1) Singlet oxygen would be quenched physically by the dissociation of **6** to DMPO and O_2 (Scheme 2). Chemical quenching would proceed *via* the cyclization of biradical **6** either through the carbon atom (constrained 1,2-addition, **7**) or through the nitrone oxygen atom (1,3-addition), yielding a non-constrained 1,2,3-trioxide[26a] (ozonide, **8**; Scheme 1).

Decomposition of the ozonide (**8**) may generate 4-methyl-4-nitrosopentanoic acid (**9**) (Scheme 1). Nitroso and nitro products are known to be generated by oxidation of DMPO and related nitrones by strong oxidizers, such as periodate.[23] The same products are formed during the ozonation of Schiff bases,[26a] which is thought to proceed *via* a 1,2,3-trioxide (ozonide) intermediate. Thus it is plausible that **8** may decompose to a nitroso product; its decomposition to a strong

Scheme 2



singlet oxygen quencher such as **9**[26c] (Scheme 1) is strongly supported by the time-dependent decrease in $^1O_2$ phosphorescence (Figure 1A). (It is possible that a small part of the nitro product might be formed *via* the (photo)oxidation of nitrosopentanoic acid.)

The ozonide **8** may also decompose (*via* fission of bonds c and e) to give **10**, which is unstable; **10** may either rearrange to **11** or decompose and release oxygen[26d] (Scheme 1). This latter pathway is suggested by the Cu^{2+}-dependent oxygen release in alkaline solutions. 4-Methyl-4-nitropentanal (**11**) could also be produced by the decomposition of 1,2-adduct (**7**); however, this mechanism does not provide a route to **9**. While we cannot distinguish between these reactions, we believe that the ozonide **8** is the main source of **11**.

In acidic solution there is a second mechanism of chemical quenching *via* a redox pathway; it involves a putative "nitronium like" intermediate (**12**) formed by proton addition to biradical **6** accompanied by rearrangement (Scheme 2). This process seems to diminish physical quenching by opening a new reaction channel, which results in O_2 consumption in acidic solutions that is about 20 times faster than at pH 10.[26b] As we detected both products in which the pyrroline ring remains intact (DMPO/•OH, DMPOX) and products that require fission of the pyrroline

(26) (a) Bailey, P. S. In *Ozonation in Organic Chemistry*; Academic Press: New York, 1978; Vol. I, pp 225−453 and references therein. (b) The complexity of oxygen photoconsumption makes it difficult to determine the relative contributions of chemical and physical pathways to the total quenching of $^1O_2$ phosphorescence. Faster O_2 consumption in acidic solutions than at pH 10 cannot be explained by nitrite (ca. 7%), or even nitrate, production. Their contribution, together with a potential complete oxidation of carbon−center radicals formed when NO• splits, could only double O_2 consumption by a high estimate. (c) The −COOH group cannot be formed directly; it must result from a rearrangement of the unstable biradical HC•O−O•. (d) It is known that peroxynitrous acid undergoes such isomerization to nitrate[30] and that during this rearrangement peroxynitrite shows oxidative properties.[31] In addition, it is also well-known that O_2 can be released from peroxynitrite anion upon catalysis with Cu^{2+} in alkaline solution.[32] Thus, it is possible to explain both oxygen release and the isomerization of the DMPO photooxidation product in terms of peroxynitrite-like chemistry.

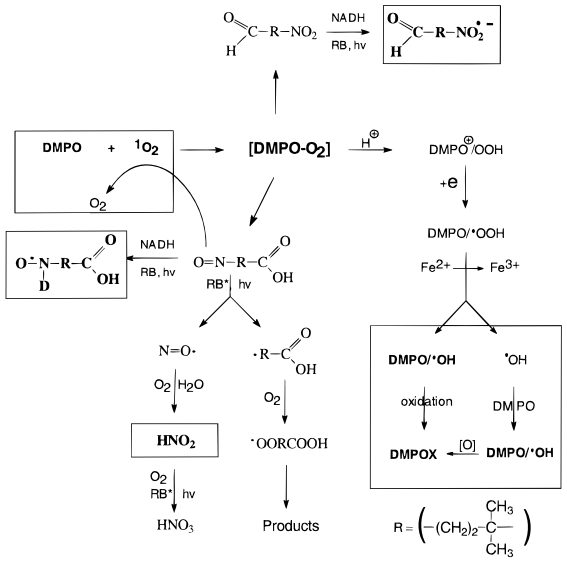
ring (nitroso and nitro products), our results are consistent with both of the above mechanisms.

Two EPR adducts are frequently detected during photochemical experiments: DMPO/•OH (**14**) and DMPOX (**15**). These products must be generated *via* redox reactions that spare the pyrroline ring (Scheme 2). The more persistent spectrum belongs to the DMPO/•OH adduct. We believe that this radical product is formed *via* the nitronium transient **12** (*vide supra*), which is known to be a strong oxidant. Not surprisingly, such an intermediate may be easily reduced to DMPO/•O_2H (**13**), most likely formed in the conformation stabilized by intramolecular hydrogen bonding.[27a] A structurally similar hydroperoxide **3** has been isolated during the photosensitized oxidation of 2,4,4-trimethyl-1-pyrroline *N*-oxide in methylene chloride at low temperature.[3] The DMPO hydroperoxide, DMPO/•O_2H, may ionize, and a weak signal from DMPO/$O_2^{\bullet-}$ was observed during the oxidation of DMPO by Merocyanine-540.[4b]

DMPO/•O_2H may be an intermediate leading to the DMPO/•OH adduct *via* both spin-trapping (reaction of •OH with DMPO) and non-spin-trapping reactions. The well-known Fenton-like reaction of such a hydroperoxide can produce the free •OH radical and DMPO/$O^{\bullet-}$, which protonates to yield the DMPO/•OH radical (Scheme 2). As expected for hydroperoxide reduction, adduct formation is sensitive to redox impurities present in phosphate buffers. Indeed, we observed that, in nonchelexed phosphate buffers (>50 mM), the signal intensity of the DMPO/•OH adduct was greater than in water alone or in chelexed buffers[17e] (Figure 7, spectra B and E). In the absence of redox-active impurities in distilled water, the reduction process can probably be sustained by reducing products from DMPO degradation like hydroxylamines because even very thoroughly chelexed solutions always contained a weak signal from the DMPO/•OH adduct.[28] Thus, dissolved oxygen, *via* its singlet state, is responsible for all of the DMPO/•OH adduct produced during DMPO photooxidation in the dye system, as confirmed by $^{17}O_2$ experiments (Figure 8). Our findings that the DMPO/•OH generated during $^1O_2$ oxidation is formed by reaction of DMPO with freely diffusible •OH and also by a nonspin-trapping route also fully agree with previously published results.[4b]

The second important oxidation product detected by EPR is DMPOX (**15**), which we observed at pH 2. DMPOX is formed only in the presence of strong oxidizers.[6] One possible source is oxidation of DMPO/•OH;[23] in one plausible hypothesis, the strong oxidizer would be the nitronium transient **12** (*vide supra*). While we cannot provide any direct experimental evidence to support this hypothesis, the presence of DMPO/•OH and DMPOX EPR signals in acidic solutions tends to confirm the redox mechanism in which the nitronium intermediate may be a strong oxidant.

Scheme 3



We believe that the increase in O_2 photoconsumption for pH >10 (Figure 4) is caused by reactions that are different from those operating in acidic solutions. In alkaline solution, DMPO is known to undergo nucleophilic addition of a water molecule to the nitrone to yield a hydroxylamine. The hydroxylamine moiety (R−NOH) is oxidized by singlet oxygen,[10,13] and the main product is hydrogen peroxide. Moreover, R−NOH dissociation produces a very efficient chemical $^1O_2$ quencher, the R−NO^- anion.[13] We also considered the possibility that in strong alkali the dissociation of a γ-hydrogen in the DMPO molecule might produce an efficient $^1O_2$ quencher. However, this possibility can be eliminated because M_4PO, in which both γ hydrogen atoms are blocked by methyl groups, behaved exactly the same as DMPO during photooxidation (Figure 4B). Thus, the hydrolysis of DMPO to a DMPO hydroxylamine derivative is probably responsible for the increased oxygen (photo)consumption in strongly alkaline solutions.

The oxidation of DMPO by $^1O_2$ is a complex process which is summarized in Scheme 3 (with the processes and products we observed directly in boxes). The simplest measure of DMPO photooxidation is oxygen consumption; however, such data should be interpreted cautiously. A calculation based solely on O_2 consumption resulted in previous overestimation of the $^1O_2$ quenching constant by DMPO (1.8×10^7 M^{-1} s^{-1} [4a] *vs* our value of 1.2×10^6 M^{-1} s^{-1}); it is likely that the k_q values for other nitrones[4a] have also been overestimated. This may not be surprising considering that 4-methyl-4-nitrosopentanioc acid, an efficient $^1O_2$ quencher, is an important photoproduct of DMPO. Other (photo)products are also likely to be involved (Scheme 3) in oxygen consumption, which depends strongly on pH (Figures 3 and 4), even though the total quenching of $^1O_2$ by the nitrone moiety[27b] is virtually pH independent.

Conclusions

We have shown that DMPO oxidation by $^1O_2$ depends strongly on pH; while pH does not influence the primary $^1O_2$ quenching, it strongly affects the subsequent oxidation reactions. We have documented for the first time that $^1O_2$ is able to open

(27) (a) Reszka, K.; Bilski, P.; Sik, R. H.; Chignell, C. F. *Free Rad. Res. Commun.* **1993**, *19S*, 33. (b) The efficiency of $^1O_2$ quenching by DMPO in HCl solution was the same as at pH 4, even though the protonation of the nitrone moiety seems to occur at very low pH. While the UV spectrum of DMPO (band at 228 nm) did not change from pH 2 to concentrated NaOH solution, in concentrated HCl solution (0.25 M), the absorbance of DMPO in the UV region decreased reversibly suggesting that DMPO protonation occurred at the nitrogen atom, p$K \sim 1.5$.

(28) When the DMPO concentration was high the intensity of the DMPO/•OH signal depended less on the concentration of phosphate in nonchelexed buffer (not shown). Probably, the rapid accumulation of reducing products from DMPO degradation, or perhaps DMPO itself, can substitute for the metal reductants.

(29) The exact mechanism of H_2O_2 formation is unknown. It is possible that a hydroxylamine derivative of DMPO contributes to the production of H_2O_2 during DMPO photooxidation. Stoichiometric formation of H_2O_2 from the photooxidation of *N,N*-diethylhydroxylamine by Rose Bengal was confirmed previously.[13] The hydroxylamine moiety may be formed in the redox reactions of DMPO and as a result of nucleophilic addition of H_2O/OH^- to the nitrone function in DMPO.[23] The formation and subsequent oxidation of the hydroxylamine should be easier in alkaline solutions. H_2O_2 produced *via* a DMPO hydroxylamine derivative would produce the ^{17}O-labeled hydroxyl adduct.

(30) Benton, D. J.; Moore, P. *J. Chem. Soc. A* **1970**, 3179.

(31) Koppenol, W. H.; Moreno, J. J.; Pryor, W. A.; Ischiropoulos, H.; Beckman, J. S. *Chem. Res. Toxicol.* **1992**, *5*, 834.

(32) Plumb, R. C.; Edwards, J. O. *Analyst* **1992**, *117*, 1639.

the DMPO pyrroline ring, leading to the formation of nitro and nitroso products. Thus, both DMPO itself and its nitroso photodecomposition product, a very strong $^1O_2$ quencher, may compete with any substrate(s) under investigation for $^1O_2$. The formation and (photo)decomposition of the nitroso product may contribute to acidification of nonbuffered solutions and result in nitrite accumulation.

Another potential complication is the formation of species that are not EPR silent. First, $^1O_2$ initiates the production of the DMPO/$^{\bullet}$OH adduct *via* both a spin-trapping and a non-spin-trapping route. Second, during the course of oxidation, a strong oxidizer such as the DMPO nitronium cation may be formed and lead to the DMPOX signal. Furthermore, the nitroso product (**9**) may itself act as a spin trap, which could produce nitroso-type radical adducts. Nitro products may also generate EPR signals in alkaline solution in the presence of electron donors.

The mechanisms, transients, and products that we found or postulated for the reaction between $^1O_2$ and DMPO are likely to occur during the thermal oxidation of DMPO by molecular oxygen in the dark. Usually, if a compound is easily oxidized by $^1O_2$, then a similar oxidation may proceed with ground state oxygen, but at a significantly slower rate. Thus, the products we found for the photochemical oxidation are likely to accumulate during long DMPO storage.

Finally, we should also mention that the light intensities and irradiation times that we used in our experiments were not extreme but were comparable to conditions normally used during photochemical spin-trapping experiments with DMPO. While DMPO is undoubtedly a valuable spin trap, data from systems in which $^1O_2$ can also be generated must be interpreted with caution, and appropriate control experiments should always be performed.

Acknowledgment. The authors thank Mr. R. H. Sik for conducting the experiments with the $^{17}O_2$ isotope.

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