Spin Trapping of the Nitrogen-centered Radicals. Characterization of the DMPO/DEPMPO Spin Adducts

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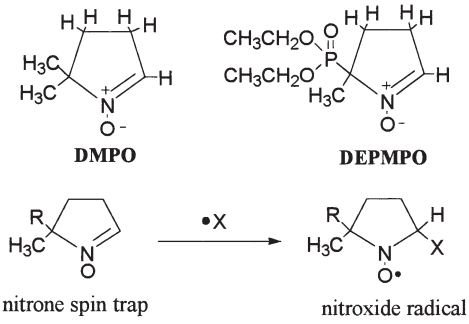
To develop the spin trapping utility for the detection of N-centered radicals generated in biological systems, 5,5′-dimethylpyrroline-1-oxide (DMPO) and 5-diethoxyphosphoryl-5-methyl-1-pyrroline-*N*-oxide (DEPMPO), have been applied for the detection of chemically generated N-centered radicals in aqueous solution. Both spin traps yielded N-radical adducts, which were characterized by the EPR parameters.

In the biochemical field, spin trapping technique has been widely used to detect short-lived radicals generated both in vivo and in vitro reactions. Active oxygen species such as hydroxyl and superoxide anion radicals are used to be detected as the DMPO[1–3] or DEPMPO[4] adducts (Scheme 1). Sulfur-centered radicals have also been trapped and characterized.[5–7] Other physiologically active species, NO, is detectable as the iron–dithiocarbamate nitrosyl complexes.[2,8–10] In contrast with these radicals, nitrogen-centered radicals (excluding NO) have rarely been detected by spin trapping,[11–14] in spite that possibility of the N-centered radical generation from peptide/protein or nucleotide/nucleic acid is potentially high in living systems. Therefore, to expand and develop the spin-trapping technique in detection and characterization of the N-centered radicals formed in various biological systems, we tried to assure the authentic N-radical trapping by use of popular O-radical spin traps of DMPO and DEPMPO.



Scheme 1. Nitron spin traps and their spin-adduct formation.

N-centered radicals were generated by the oxidation of alkyl amines using $K_2[IrCl]_6$ (Wako).[15] Normally, iridium chloride (15 mmol dm^{-3}) and DMPO (Sigma, 100 mmol dm^{-3}) or DEPMOP (Oxis, 100 mmol dm^{-3}) were added into 25 mmol dm^{-3} of amines in 0.1 mol dm^{-3} K-phosphate buffer (pH 7.0), in this order. The reaction mixture was transferred into a flat quartz cell (Jeol, LC11) soon after addition of the spin trap and measured X-band EPR (Bruker, E500) at room temperatures. EPR conditions were as follows: microwave power, 10 mW; field modulation frequency, 100 kHz; field modulation amplitude, 0.1 mT; field sweep width, 10 mT; receiver time constant, 0.64 ms; averaged scans, 5–50. Examined amines were methyl-, dimethyl-, trimethyl-, ethyl-, diethyl-, and triethylamines.

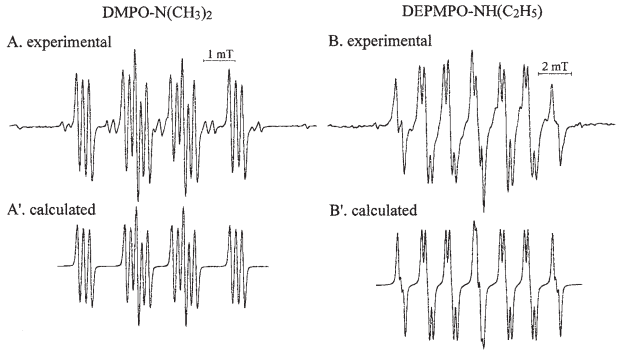


Figure 1. EPR spectra obtained from DMPO-NMe_2 and DEPMPO-NHEt and their calculated spectra.

Figure 1 exhibits the obtained spectra from the systems of $NH(CH_3)_2$-DMPO (A) and $NH_2C_2H_5$-DEPMPO (B) and their simulated spectra (A′ and B′), respectively.[16] The small triplet with equal intensity are indicative of N-centered radical adducts of DMPO and DEPMPO, which comes from the hyperfine interaction between electron spin and β-nitrogen nucleus. In trapping of the N-centered radical originated from dimethylamine, the EPR signal intensity of both DMPO and DEPMPO adducts maximized about 10-12 min after addition of the spin traps. After 16 min, the signal intensity reduced to 1/2 of the maximum value for the DMPO adduct and to 1/4 for the DEPMPO adduct. In N-radical trapping of the ethylamine system, the first acquisition of the spectrum, for 3 min after addition of spin traps, yielded the maximum intensity of the spectrum in both DMPO and DEPMPO systems. The spectral intensity of the DMPO adduct reduced to 1/3 after 12 min, while 19 min was needed to the same order of reduction for the DEPMPO adduct. Precise analysis for the stability of the N-radical adducts is now ongoing. Efficiency of the N-radical trapping by DMPO appears to be 2–4 times higher than that by DEPMPO. Among the amines examined, only dimethylamine and ethylamine yielded the N-centered radical adducts predominantly. By contrast, diethylamine yielded a mixture of N-centered radical and hydroxyl radical adducts. Methyl amine gave C-centered radical adducts mostly, while the tertiary amines did admixture of C-centered radical, N-centered radical, and hydroxyl radical adducts. The hyperfine coupling constants for the obtained radical adducts were summarized in Table 1.

The N-centered radical adducts are discriminated from the C- and O-centered radical adducts, first, by the hyperfine split-

Table 1. Hyperfine splitting constants (A, in mT)[a] of the DMPO-R and DEPMPO-R adducts

R[b]	A_α^N	A_β^H	A_β^N	A^P/others
DMPO-R				
• NHEt	1.55	1.77	1.85	—
• NMe_2	1.52	1.87	1.94	—
• OH	1.49	1.48	—	—
• OOH[c]	1.43	1.17	—	0.13(H_γ)
• CH_2OH	1.60	2.28	—	—
• CH_2NHMe	1.6	2.3	—	—
DEPMPO-R				
• NHEt	1.44	1.68	0.17	4.79
• NEt_2	1.5	1.5	~0.1	4.7
• NMe_2	1.42	1.73	0.16	4.80
• OH	1.40	1.37	—	4.73
• OOH[d]	1.30	1.17	—	5.06
• $CHMeNH_2$	1.49	2.21	—	4.94
• CHMeNHEt	1.49	2.20	—	4.76

[a]The hyperfine coupling constants are determined by the spectral simulation calculations.; [b]The radical adducts without references are all obtained in this work.; [c]taken from ref. 1; [d]taken from ref. 4, major component (63%).

ting due to the β-nitrogen. The coupling constant of A_β^N is 0.18–0.20 mT for the DMPO adducts and 0.15–0.17 mT for the DEPMPO adducts, where the smaller value in the latter is attributable to the larger spin delocalization onto the phosphoryl group. Second, the hyperfine splitting due to the β-hydrogen of C-, N-, and O-radical adducts (A_β^H) decrease in this order. This might be ascribed to the increased electronegativity of the atom attached to the β-carbon of the nitroxide.[10] Third, the hyperfine splitting due to the α-nitrogen (nitroxide nitrogen) of the N- and O-adducts (A_α^N) are slightly smaller than those of C-adduct. Interestingly, the hyperfine splitting due to the phosphorous nuclei in the DEPMPO adducts does not show distinct correlation with the sort of radical center atom trapped. Rather, that might depend on the planarity of the nitroxide ring system which mainly affects the spin delocalization to the peripheral phosphoryl group.

Structural optimization and spin density calculation were carried out with the Spartan ′02 Semi-Empirical Program: (PC/x86) Release 116. Geometry optimizations were performed at the UHF level using the AM1 method.[17] Calculated spin densities for the N-centered radical adducts of DMPO and DEPMPO have given consistent results with the experimentally obtained hyperfine coupling constants (Figure 2). The optimized structure has out-of-plane NO with *trans*-configuration to the N-substituent in both adducts, while the phosphoryl group of DEPMPO has *cis*-configuration with respect to the nitroxyl group. Such *cis*-configuration might contribute to the direct overlap of the spin orbitals on phosphorus and nitroxide-nitrogen, thereby enhancing the spin delocalization from nitroxide ring to the phosphoryl group. The optimized structure suggests that N-radical attack on the nitrone β-carbon is *trans*-addition to minimize steric repulsion.

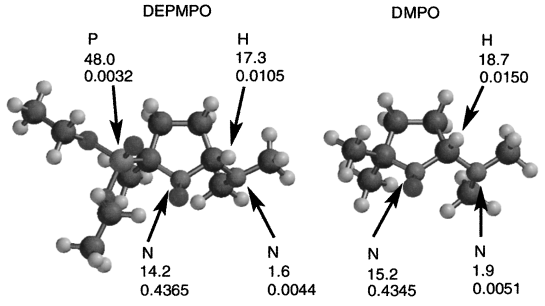


Figure 2. Optimized structures and spin densities on the atoms of DEPMPO- and DMPO-NMe_2 adducts. Numerals under each atom show hyperfine coupling constants (upper) and spin densities (lower).

Though amine oxidation generates alkylaminyl radicals primarily, most of the trapped N-centered radicals have origin of the secondary amines. Oxidation products of primary amines were predominantly trapped as the C-centered radicals, except for that of ethylamine. This implies that rearrangement of the β-hydrogen of the primarily generated radicals and/or hydrogen abstraction from the intact amines by the radicals generated might occur prior to the trapping reactions. The latter also could cause the multi-species detection in the tertiary amine systems. The product variation in spin trapping of the amine oxidation reactions has been under study by use of time-resolved EPR analysis.

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15 We chose iridium (IV) chloride as an one electron oxidant of amines because of its high E^0 value (0.86 V for $[IrCl_6]^{2-} + e = [IrCl_6]^{3-}$) compared with that of ferricyanide (0.36 V for $[Fe(CN)_6]^{3-} + e = [Fe(CN)_6]^{4-}$); see "Kagakubinran, Kisohen II," ed. by The Chemical Society of Japan, Maruzen, Tokyo (1986), p 475; This iridium (IV) complex has been occasionally applied for the oxidation of heme irons: e.g. in Y. Hayashi and I. Yamazaki, *J. Biol. Chem.*, **254**, 9101 (1979).
16 The small signals with wider spectral width than the main signals' observed in the experimentally obtained spectra (A and B) are considered to come from a ring-opening product of spin adducts.
17 AM1 calculation was executed for originally defined two structures, which have opposite configuration of N-substituent with respect to the nitroxide ring, each other.

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