Free Rad. Res. Comms., Vol. 14, No. 2, pp. 145-155
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DIFFICULTIES ENCOUNTERED IN THE DETECTION OF NITRIC OXIDE (NO) BY SPIN TRAPPING TECHNIQUES. A CAUTIONARY NOTE.

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(Received July 14, 1990; in final form August 24, 1990)

The spin trapping technique was used in an attempt to detect the free radical nitric oxide (NO) in solution. Five different spin traps were examined, α-phenyl-N-tert butyl nitrone (PBN), α-(4-pyridyl-N-oxide) N-tert-butylnitrone (POBN), 5,5-dimethyl-pyrroline-N-oxide (DMPO), 2-methyl-2-nitrosopropane (MNP) and 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS).

Our results suggest that the nitroso spin traps (MNP, DBNBS) are better suited for the identification of NO-related signals, than the nitrones, DMPO, PBN and POBN. In addition, it is shown that spin trapping of NO-related signals with nitroso and nitrone spin traps is subject to many artifacts.

KEY WORDS: Spin trapping, nitric oxide, endothelium-derived relaxing factor (EDRF), nitrone/nitroso spin traps, ESR.

INTRODUCTION

Nitric oxide (NO) gas in solution relaxes blood vessels with similar actions and pharmacodynamics as the endothelium derived relaxing factor (EDRF). This molecule has been proposed to be a component of the materials released from stimulated endothelial cells.[1] Palmer *et al.*[2] recorded a chemiluminescent signal indicative of NO following the reflux of endothelial cell perfusate in acidic conditions. In similar experiments using authentic NO producing a chemiluminescent signal of the same magnitude was shown to be sufficient to induce the relaxation of vascular smooth muscle. Similarly, NO-like material was demonstrated by Ignarro *et al.*[3] Because of the similar biological effects produced by NO and EDRF it has been proposed, based on the above observations in endothelial cells, that EDRF is identical to NO. Thus, it is likely that NO derived from endothelial cells may exist and be detected in an acidic medium. Although the possibility exists that nitric oxide gas, due to its paramagnetic nature, may be directly detected by ESR following acid treatment of endothelial cells, it must be noted that NO is chemically unstable in aqueous solution and is unlikely to exist in this form in a biological medium. A molecular grouping such as NO may be the active portion of a larger EDRF molecule, generated by free radical mechanisms and consequently a different pharmacological profile to NO may be rationalized. For this reason, the spin trapping technique, although having its limita-

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tions when applied to this type of study,[4-8] should play an important role in determining the structure of EDRF and its related mechanisms.

The technique of spin trapping consists of using a nitrone or nitroso compound to "trap" an initial unstable free radical forming a longer lived nitroxide which can be observed and identified at room temperature using conventional ESR spectrometry. In principle, the spin trapping of nitric oxide may appear to be a simple and straight forward system. However, it must be noted that the experiments, specifically in the case of nitroso spin traps deal with the addition of an NO radical to an -NO functional group on the spin trap. This gives rise, providing that the nitric oxide adds to the spin trap, to various questions regarding the nature of the potential electron spin density. Is the spin density located at the spin trap NO groups?, at the added nitric oxide NO group? or, is it shared by both in a resonating type structure? Extending these questions to a case where isotopically pure ^{15}NO gas is used, the system becomes more complex because in this case the experiments would deal with the addition of an ^{15}NO (^{15}N nuclear spin, $I = 1/2$) to the spin trap NO (^{14}N nuclear spin, $I = 1$) functional group. This study was undertaken to answer these questions and to establish criteria to determine whether ESR signals detected with different nitroso and nitrone-spin traps, may be a consequence of the reaction between NO and the spin traps and in addition, may be attributed to the formation of free NO radicals in cells and isolated tissues. The results suggest that the ESR spectra obtained from nitrone traps in the presence of NO results from the hydrolysis of these spin traps and not from the trapping of NO radicals. However, for nitroso traps nitroxides were formed in the reaction between NO and the traps and were observed at experimental conditions compatible with the biological requirements of cells. Thus, our data suggest that nitroso compounds may be the best spin traps for the detection of NO in a biological milieu.

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MATERIAL AND METHODS

α-Phenyl-N-tert-butylnitrone (PBN), α-(4-pyridyl-N-oxide) *N*-tert-butylnitrone (POBN), 2-methyl-2-nitrosopropane (MNP) were obtained from Aldrich Chemical Company (Milwaukee, WI). 3,5-Dibromo-nitrosobenzene sulfonate (DBNBS) was obtained from Sigma Chemical Co. (St. Louis, MO). 5,5-Dimethyl-1-pyrroline-1-oxide (DMPO) was synthesized according to the procedure of Bonnett, *et al.*[9] Aqueous solution of MNP were prepared by adding 20 mg of the spin-trap to 10 ml of H_2O and stirring in the dard at 45°C for 2 hours. All buffers were passed through a Chelex-100 (Bio-Rad, Richmond, CA) ion exchange resin to remove trace metal ion impurities prior to use. All solutions were prepared in doubly deionized and doubly distilled water. Air saturated and deaerated spin trap solutions were saturated directly with NO gas or NO produced chemically.

Air-free solutions were saturated by NO bubbling in a Pyrex glass tube (12 × 85 mm) containing a side arm with a ground glass joint connected to an ESR quartz flat cell (60 × 10 × 0.25 mm). The Pyrex tube was sealed with a serum bottle stopper containing a needle through which the nitrogen or argon and the NO gas were administered. Samples were first saturated with nitrogen or argon, then with NO followed by further nitrogen or argon bubbling to eliminate as much excess NO as possible. The ESR flat cell was then sealed in order to maintain deaerated conditions. NO was delivered to the assay tube at a rate of approximately 1 mL/min for 35

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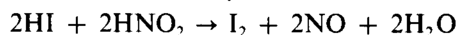
seconds followed by flushing with nitrogen or argon for approximately 3 minutes. Nitric oxide gas (99% purity) was obtained from Matheson Gas Products, Inc. (Fairfield, NJ). Purity was checked by GC-MS and found to 99%. GC-MS was performed using a Hewlett Packard Mass selective detector, model 5970. The GC was equipped with HP-1 column (12 m × 0.2 mm I.D.). $N^{15}O$ gas (99% purity) was obtained from MSD Isotopes Merck Frosst Canada, Inc. (Montreal, Canada).

NO gas was produced chemically according to the following equation:



$$2HI + 2HNO_2 \rightarrow I_2 + 2NO + 2H_2O$$

Sulfuric acid (H_2SO_4, 50%) was slowly added dropwise to a concentrated solution of potassium iodide (KI, 1 part) and sodium nitrate ($NaNO_2$, 2 parts) saturated with N_2 gas. The reaction was carried out in a pyrex tube identical to the one containing the ESR flat cell. The spin trap solution was placed in the tube containing the flat cell. The chemically generated NO was bubbled through this solution via a needle attached to a small section of tygon tubing connected to the side arm of the tube containing the reaction mixture. The sulfuric acid was added to the $KI/NaNO_2$ mixture using a pasteur pipet inserted through the rubber stopper. Immediately prior to adding the sulfuric acid the whole system was saturated with nitrogen or argon. Following NO saturation of the the spin trap solution, the tygon tubing was removed and the needle

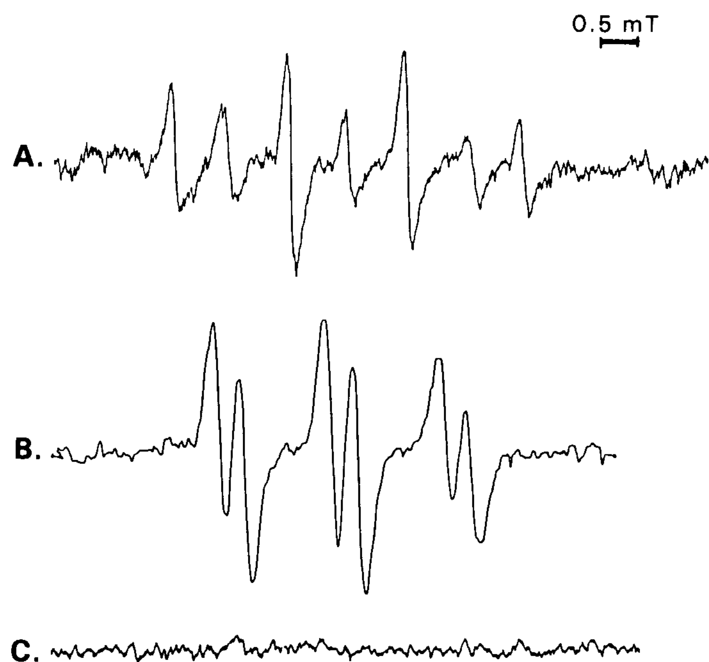


FIGURE 1 ESR spectra obtained when three nitrone spin traps were exposed to NO gas or NO chemically generated. A). aerated solutions of DMPO (0.1 M) in potassium phosphate buffer (50 mM, pH 7.8); the ESR spectrum typical obtained after 30 min reaction started. B). aerated solutions of PBN (50 mM) in potassium phosphate buffer (50 mM, pH 7.8). C). aerated solutions of POBN (50 mM) in potassium phosphate buffer (50 mM, pH 7.8). Spectrometer setting: microwave power 10 mW, modulation amplitude 0.1 mT; receiver gain 1.25 × 10^5.

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was connected to nitrogen or argon stream to eliminate as much excess NO as possible. ESR measurements were made at room temperature with a Varian century series E-109 spectrometer (X-band, 100 KHz field modulation) equipped with a TM_{110} cavity. The magnetic field was set at 333.2 mT, the microwave power was 10 mW and the modulation amplitude was 0.1 mT. The experimental room was kept in darkness throughout the study to prevent photolytic degradation of the spin trap agents.

RESULTS

DMPO

A solution of DMPO (0.1 M, pH = 7.0) in air saturated water exposed to the chemical method of producing NO yields an ESR spectrum originating from two different spin adducts (Figure 1a). One adduct generates a 1:2:2:1 quartet with hyperfine coupling constants $a_N = a_H^\beta = 1.49$ mT and is attributed to DMPO–OH.[10] The other adduct generates a triplet with a hyperfine coupling constant $a_N = 1.5$ mT, and is attributed to a nitroxide. This nitroxide is possibly a dimerization product of DMPO.[6,11] It must be noted that on exposure to NO, the pH of the solution containing the spin trap decreases to pH < 2. This decrease in pH may explain ESR spectra depicted in Figure 1.

The DMPO–OH adduct was obtained in the reaction mixture under air-saturated and deaerated conditions at low pH values (pH ~ 2) but not in deaerated solutions at higher pH values (0.05 M phosphate buffer, pH 8.5). It is unclear by which mechanism the DMPO–OH is formed in deaerated solutions at low pH, however, there is a good explanation for its formation at low pH in the air-saturated solutions. The DMPO–OH adduct has a short lifetime at low pH values,[12] therefore, its persistance in the reaction mixture suggests that it is being formed in a continous manner by mechanisms other than the addition of hydroxyl radicals or superoxide to DMPO. It is possible that the observed DMPO–OH originates from a reaction between nitrogen dioxide (NO_2) and DMPO. NO_2 is a strong oxidant and is formed in the reaction between NO and oxygen.[13] Because this reaction is slow at room temperature, it could explain the continuous formation of DMPO–OH. Evidence for the formation of NO_2 is the color change of the solution to yellow which is characteristic of dissolved NO_2.

PBN

When solution of PBN (50 mM, pH 7.0) were exposed to NO gas or chemically generated NO, no ESR signal was observed under deoxygenated conditions. However, when potassium phosphate buffer (50 mM, pH 7.8) containing PBN (50 mM) under aerobic conditions were subjected to NO gas or chemically generated NO, an ESR spectrum consisting of a triplet of doublets with hyperfine coupling constants of $a_N = 1.6$ mT and $a_H^\beta = 0.435$ mT was obtained (Figure 1b). This species may be assigned to the benzoyl spin adduct of PBN.[14].

POBN

Air-saturated and deaerated solutions of POBN (50 mM, pH 7.0) were tested in the

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presence and absence of chemically generated NO or NO gas, keeping all other conditions unchanged. No spin adducts could be detected (Figure 1c).

MNP

Since MNP is volatile, the experiments were initially carried out in benzene. MNP is known to be more soluble in non-polar solvents than in water. Therefore, it is more probable that a sufficient amount of MNP to trap NO would remain in benzene as compared to water during gas bubbling. In addition, it is important to know whether NO can be trapped in a non-polar environment because if this species is released by endothelial cells it may be done so in the membrane. It is conceivable that following trapping of NO in benzene, the spin trapped adduct could possibly be extracted with water into the aqueous phase as it is known that MNP spin adducts, due to their polarity, are more soluble in water than in non-polar solvents. The experiments in benzene were carried out using the experimental design described for the nitrones. Figure 2a. shows the ESR spectrum obtained following exposure of a deaerated

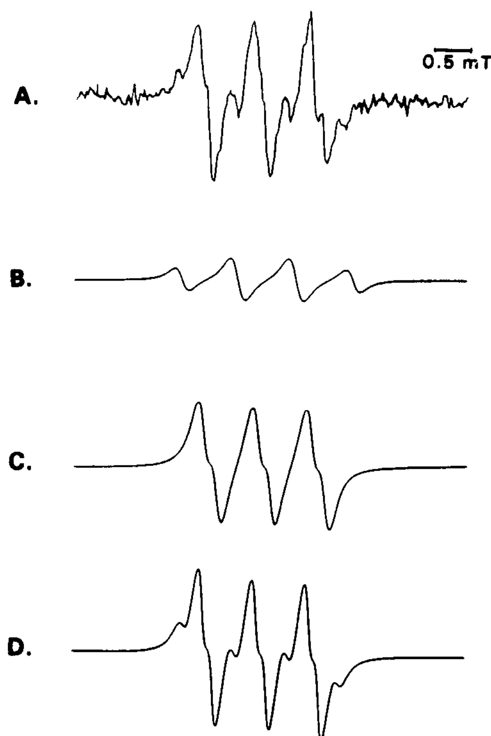

FIGURE 2 Spin adducts of MNP. The spin adduct observed in deoxygenated benzene solutions of MNP (10 mg/ml) upon exposure to NO gas or NO chemically generated. B). Simulated spectrum of a triplet of triplets with hyperfine splittings of $a_N = 0.682$ mT and $a_N^\beta = 0.315$ mT. C). Simulated spectrum of a triplet of doublets $a_N = 0.682$ mT and $a_H^\beta = 0.160$ mT; D). Computer simulated spectrum that best fits the experimental spectrum (A). This spectrum is the addition of spectra B and C by a factor of 0.25 and 0.75, respectively.

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solution of MNP in benzene (10 mg/ml) to NO gas (from commercial sources) or the chemically generated NO. This complex spectrum consists of two overlapping spin adducts with relatively broad lines. One of the spin adducts consists of a triplet of triplets indicating that the unpaired nitroxide electron is interacting with the nucleus of a secondary nitrogen. The experimental ESR spectrum of this spin adduct can be matched by computer simulation (Figure 2b) using line widths of 0.15 mT and hyperfine coupling constants, $a_N = 0.682$ mT and $a_N^\beta = 0.315$ mT. This result suggest that the spin adduct originates from the addition of NO to MNP. It is possible that the broad nature and the overlapping of the ESR lines in this spin adduct, originate from a degree of resonance and electron sharing between the nitroxide group and the NO added to it. The second spin adduct observed in Figure 2a consists of a triplet of doublets indicating that the unpaired nitroxide electron is interacting with hydrogen nucleus. The computer simulation that best matches the experimental spectrum is

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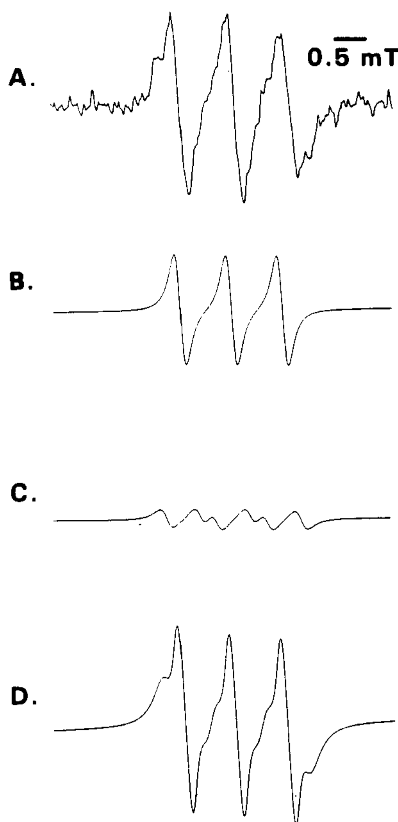

FIGURE 3 Spin adducts of DBNBS. The spin adduct observed when anaerobic solution of DBNBS (50 mM) were exposed to NO gas or NO chemically generated. A). ESR spectrum obtained following rigorous and extensive N_2 saturation of the reaction mixture. B). Simulated spectrum of a triplet using $a_N = 0.959$ mT. C). Simulated spectrum of a triplet of triplets generated using $a_N = 0.950$ mT $a_N^\beta = 0.315$ mT. D). Computer simulated spectrum that can be obtained that best fits the experimental spectrum (A). This spectrum is the addition of spectrum B and C by a factor of 0.36 and 0.64, respectively. Modulation amplitude 0.08 mT and receiver gain 5×10^4, respectively.

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shown in Figure 2c. This theoretical spectrum was obtained using line widths of 0.15 mT and hyperfine coupling constants, $a_N = 0.682$ mT and $a_H = 0.160$ mT. This spin adduct is most likely the reduction product of MNP. Since the reaction was carried out in benzene, the source of protons involved in the reduction of MNP is unclear. However, it is possible that the origin of these protons may be humidity in the NO gas. The addition (Figure 2d) of the computer generated ESR spectra in Figure 2b and 2c shows the overall ESR spectrum that matches the experimental spectrum in Figure 2a.

When the experiments using MNP (2 mg/ml) were attempted in water, the ESR spectrum obtained consists of a broad triplet superimposed on a less intense ESR signal too weak to be identified. The hyperfine coupling constant for the triplet, $a_N = 1.7$ mT, suggests that this spectrum may correspond to ditertiary butylnitroxide (DTBN). This nitroxide is often produced in experiments using MNP.[15,16]

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DBNBS

Deaerated aqueous solutions of DBNBS (50 mM) exposed to NO gas (commercial or chemically generated), yield two superimposed ESR spectra (Figure 3a). The ESR spectrum for one spin adduct consists of a triplet which can be computer simulated (Figure 3b) using line widths of 0.2 mT and hyperfine coupling constant, $a_N = 0.959$ mT. Because better resolution of each line in this triplet was not possible even following vigorous nitrogen saturation, not much can be said at this time with regard to the identity of this spin adduct. The second spin adduct observed in Figure 3a consists of a triplet of triplets indicating that the unpaired nitroxide electron is interacting with nucleus of a secondary nitrogen. The spin adduct can be computer simulated (Figure 3c) with line widths of 0.3 mT and hyperfine coupling constants, $a_N = 0.950$ mT and $a_N^{\beta} = 0.315$ mT. This result suggests that the triplet of triplets originates from the addition of NO to DBNBS. The addition (Figure 3d) of the computer generated ESR spectra in Figure 3b. and 3c. shows the overall ESR spectrum that matches the experimental spectrum in Figure 3a. Similar control experiments as those done for MNP show that no adducts were formed when the pH of solutions of DBNBS were acidified to pH < 2. Furthermore, air-saturated solution of DBNBS exposed to the reaction mixture, generates an ESR signal, similar to the one in Figure 3a. This observation is consistent with previous observations that inorganic radicals produced persistent spin adducts exclusively with nitroso compounds.[17]

^{15}NO

The results obtained in Figures 2 and 3 show that the unpaired nitroxide electron is interacting with the nucleus of a secondary nitrogen. However, because these experiments dealt with predominantly two ^{14}N nitrogen nuclei, nothing could be concluded as to where the nitroxide is located or where the unpaired nitroxide electron spin density is located. Therefore, to address these points and to directly confirm the addition of NO to the nitroso spin traps, the experiments with MNP and DBNBS were repeated using isotopically pure (99%) ^{15}NO gas. In the case of aqueous solutions of DBNBS no additional information was obtained. When aqueous solutions of DBNBS are exposed to ^{15}NO gas the ESR spectrum obtained consists of a broad triplet, $a_N = 0.959$ mT, superimposed on a less intense triplet of triplets with hyper-

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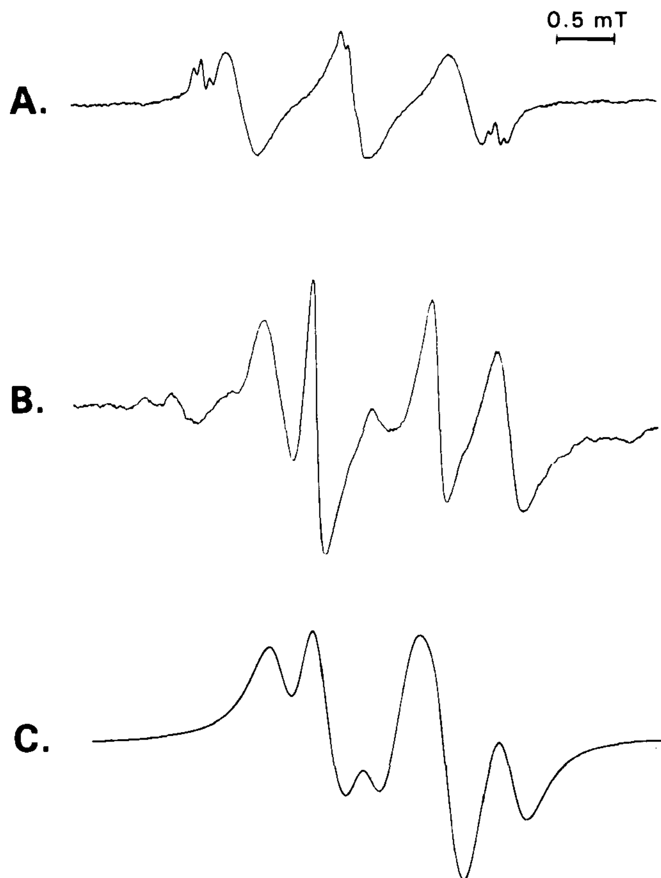

FIGURE 4 The ESR spectrum of ^{15}NO radical adducts generated with DBNBS and MNP. A). The DBNBS adduct obtained when 50 mM of deaerated solutions of DBNBS were exposed to $N^{15}O$. B). The MNP adduct generated when MNP-benzene (10 mg/ml) solutions were exposed to $N^{15}O$. C). Simulation that best fits the experimental spectrum B.

fine coupling constants, $a_N = 1.264\,mT$ and $a_H = 0.071\,mT$ (Figure 4a). The broad triplet is similar to the one obtained in the experiments shown in Figure 3. The triplet of triplets is possibly a radical anion of DBNBS[18] and it is not clear at this time why it was not observed previously in the experiments with DBNBS. However, when MNP

TABLE I
Spectral parameters used to obtain the simulated spectra for each of the two spin adducts shown in Structure I–II

Spin Adduct	Nuclear Spin	HFCC (mT)	Line width (mT)
I	0.5	1.033	0.25
II	1	0.74	0.30
	0.5	0.47	

HFCC: Hyperfine coupling constant.

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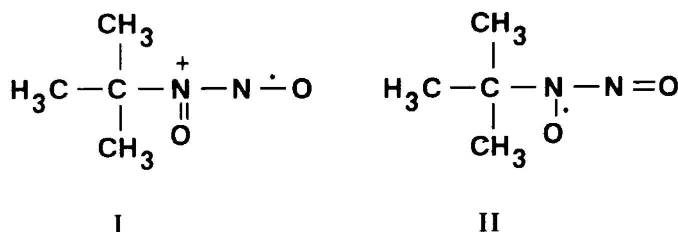
solutions in benzene are exposed to ^{15}NO gas interesting new information regarding the reaction of NO with the spin trap is obtained. The results of these experiments are shown in Figure 4b and Figure 4c. Figure 4b is the ESR spectrum obtained following additions of ^{15}NO gas to an MNP solution (10 mg/ml) in benzene. Figure 4c is the computer generated theoretical ESR spectrum that best matches the experimental spectrum in Figure 4b. The computer generated spectrum (Figure 4c) was obtained by the addition of the simulated spectra corresponding to the two spin adducts shown in Structures I–II. The spectral parameters used to obtain the simulated spectra for



each of these adducts are summarized in Table I. Structure I shows that the unpaired nitroxide electron spin density is located at the ^{15}NO group. In addition, since the ESR spectrum for this adduct (structure I) consists of a doublet, it suggests that the nitroxide is formed on the added ^{15}NO group and that the unpaired electron is close to and interacts only with the ^{15}N nucleus. The formation of the adduct shown in structure I in the experiments using ^{14}NO gas should yield an ESR spectrum consisting of a triplet (not observed) in Figure 2. However, it would be expected that changing from ^{14}NO to ^{15}NO the primary nitrogen hyperfine splitting would be approximately 30% less, for ^{15}N (nuclear spin, $I = 1/2$), than the total splitting observed for ^{14}N ($I = 1$).[19] Furthermore, since the ratio of the magnetogyric ratios for ^{15}N and ^{14}N is $\tau^{15}n/\tau^{14}n = 1.403$, it would also be expected that changing from ^{14}NO to ^{15}NO the primary nitrogen hyperfine coupling constant would increase approximately by a factor of 1.403. Because the hyperfine coupling constant, $a_N = 1.033$ mT, for structure I is approximately 30% less than the total splitting and approximately 1.403 times larger than the hyperfine coupling constant for the spin adducts shown in Figure 2, the results suggest that this adduct may be formed in the experiments shown in Figure 2, but is not observed because of superimposition by the other more predominant spin adducts. Structure II shows that the unpaired nitroxide electron spin density is located at the nitroxide group of the spin trap and can be computer simulated using the parameters given in Table I. The ^{15}N hyperfine coupling constants for this spin adduct are approximately 30% less than the total splitting observed for its ^{14}N counterpart (Figure 2), in addition to, confirming to the rule of magnetogyric ratios. This result is consistent with the spin adduct shown in Figure 2b. which yields a triplet of triplets. These ^{15}NO experiments confirm the addition of nitric oxide to MNP and also show that the unpaired nitroxide electron spin density is not restricted only to the spin trap nitroxide group.

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DISCUSSION

NO gas-phase free radicals were first studied early in the history of ESR spectroscopy.[20] The unpaired electron resides in a π molecular orbital. This gaseous paramagnetic molecule exhibits a feature peculiar to molecules widely separated in space. Its rotational angular momentum, which in the liquid or solid phases is quenched, couples strongly to electronic spin and orbital angular momenta. This coupling leads to a multitude of energy levels inducing a decrease in ESR levels sensitivity due to the division of the intensity among so many ESR lines. Also, the ESR lines are so close together that their resolution suffers.

In the present study, nitrone and nitroso spin traps were examined for their capability as traps for NO or NO-related radicals in cellular systems. Our results indicate that despite the promising features of DMPO and PBN, these traps will not be useful for spin trapping nitric-oxide-related radicals in biological systems and particularly within cells. DMPO and POBN are susceptible to acidic conditions, giving ESR spectra not from free radicals. For instance, the observation of DMPO–OH in the NO reaction mixture does not necessarily reflect the involvement of hydroxyl radicals in the formation of this spin adduct. Neither scavengers of OH radicals nor enzymatic decomposition of the essential precursors by SOD and or catalase are effective in preventing DMPO–OH formation in spin trap solutions at acidic pH. Thus, it must be concluded that formation of the DMPO–OH adduct is possible without the existence of hydroxyl radicals.[21]

The spin-trapping efficiency is not a hundred percent for any of the nitrone and nitroso spin traps used, therefore, gas-phase free radical NO molecules are interacting with the spin adduct formed, producing complex broad spectra with poor resolution (Figure 2 and 3). It appears that MNP (a lypophilic nitroso compound) and DBNBS a water soluble, non-volatile, aromatic nitroso spin trap would be the best spin traps for detecting and characterizing nitric oxide-like compounds released from stimulated endothelial cells. Since nitroso compounds are easily reduced, it is imperative that caution be exercised when using these spin traps particularly in biological systems, where redox systems are more active.

Acknowledgements

Dr. Carmen M. Arroyo is grateful to Ms. Donna Bethea for excellent manuscript preparation and to Dr. Lowell D. Kispert, Dr. Peter Gutierez and Dr. Alasdair J. Carmichael for their helpful discussions. Special thanks to JEOL, LTD, Analytical Instruments Division, for permitting the use of their ESPIRIT computer simulation program. This work was supported grants from the NIH 33550 (GMR) and the National Science Foundation DCB 8616115 (GMR).

References

1. P.M. Vanhoutte, (1970) Vascular physiology. The end of the quest? *Nature*, **327**, 459–460.
2. R.M.J. Palmer, A.G. Ferrige and S. Moncada (1987) Nitric-oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature (London)*, **327**, 524–525.
3. L.J. Ignarro, R.E. Byrns, G.M. Duga and K.S. Wood, (1987) Endothelium-derived relaxing factor from pulmonary artery and vein posesses pharmacological and chemical proprties that are identical to those for nitric oxide radical. *Circulation Research*, **61**, 866–879.
4. E.G. Janzen, (1971) Spin trapping. *Accounts in Chemical Research*, **4**, 31–40.
5. E. Finkelstein, G.M. Rosen and E.J. Rauckman (1980) Spin Trapping of superoxide and hydroxyl radical: Practical Aspects. *Archives in Biochemistry and Biophysics*, **200**, 1–16.

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6. E.G. Janzen (1980) A critical review of spin trapping in biological systems. In: *Free Radicals in Biology*, Vol. IV, (W.A. Pryor, ed.) pp. 115–154, Academic Press, New York.
7. P.J. Thornalley, (1986) Theory and Biological Applications of the Electron Spin Resonance Technique of Spin Trapping. *Life Chemistry Reports*, **4**, 57–112.
8. C.M. Arroyo, B.F. Dickens, J.H. Kramer, R.H. Leiboff, G.W. Mergner, I.T. Mak and W.B. Weglicki (1988) Detection and measurement of free radical generation during cardiovascular injury. In: Life Sciences, 49, Oxygen radicals in Biology and Medicine (Simic, M.G. *et al.*, eds) pp 905–910, Plenum Press, New York and London.
9. H.R. Bonnett, R.F.C. Brown, V.M. Clark, I.O. Sutherland and S.A. Todd, (1959) Experiments towards the synthesis of Corrins. Part II. The preparation and reactions of ▲′-pyrroline-1-oxide. *Journal of the Chemistry Society* 2094–2102.
10. E.G. Janzen, Y.Y. Wang, and R.V. Shetty, (1978) Spin trapping with alphapyridyl 1-oxide N-tert-butyl nitrones in aqueous solutions. A unique electron spin resonance for the hydroxyl radical adduct. *Journal of the Americal Chemical Society*, **100**, 2923–2925.
11. R.F.C. Brown, V.M. Clark, M. Lamchen and S.A. Todd (1959) Experiments towards the synthesis of Corrins. Part VI. The Dimerisation of ▲′-pyrroline 1-oxide to 2-(1′-hydroxypyrrolidin-2′-yl)-▲′-pyrroline 1-oxides. *Journal of the Chemical Society*, 2116–2122.
12. A.J. Carmichael, K. Makino and P. Riesz (1981) Quantitative aspects of ESR and spin trapping of hydroxyl radicals and hydrogen atoms in gamma-irradiated aqueous solutions. *Radiation Research* **100**, 222–234.
13. J. Jones (1973) Nitrous Oxide. In: Comprehensive Inorganic Chemistry, 2, (Bailar, J.C., Jr. *et al.* eds.) pp. 316–323, Pergamon Press, Oxford.
14. E.G. Janzen, D.E. Nutter, Jr., E.R. Davis, B.J. Blackburn J.L. Poyer and P.B. McCay (1978) On spin trapping hydroxyl and hydroperoxyl radicals. *Canadian Journal of Chemistry*, **56**, 2237–2242.
15. K. Makino, N. Suzuki, F. Moriya, S. Rokushika, and H.A. Hatano (1981) A fundamental study on aqueous solution of 2-methyl-2-nitrosopropane as a spin trap. *Radiation Research*, **86**, 294–310.
16. C.M. Arroyo, I.T. Mak and W.B. Weglicki (1989) Spin Trapping of free radicals formed during peroxidation of sarcolemmal membranes (SLM). *Free Radical Research Communication*, **5**, 369–376.
17. K.D. Rehorek, H. Hennig, C.M. DuBose, T.J. Jemp, and E.G. Janzen (1990) *Free Radical Research Communications*, **10**, 75–84.
18. A. Samuni, A. Samuni and H.M. Swartz (1989) Evaluation of dibromonitrosobenzene sulfonate as a spin trap in biological systems. *Free Radicals in Biology & Medicine*, **7**, 37–43.
19. H. Kon (1968) Paramagnetic Resonance Study of Nitric Oxide hemoglobin, *Journal of Biological Chemistry*, **243**, 4350–4357.
20. R. Beringer and J.G. Castle, Jr. (1950) Magnetic Resonance absorption in nitric oxide. *Physics Review*, **78**, 581–586.
21. A.R. Forrester, J.M. Hay and R.H. Thomson (1968) In: Organic chemistry of stabe radicals (Forrester, A.R. *et al.* eds) pp. 180–281, Academic Press, London, New York.

Accepted by Prof. J.V. Bannister

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