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Forum Review

Spin Trapping of Nitric Oxide with the Iron-Dithiocarbamate Complex: Chemistry and Biology

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ABSTRACT

This brief review describes chemical and biological aspects concerning spin trapping of nitric oxide (NO) with the iron-dithiocarbamate (Fe-DTC) complex as a spin trap. Knowledge on basic properties of the Fe-DTC complex would help in understanding the applicability and limitation of the Fe-DTC-based NO spin-trapping method when it is employed in viable biological systems. *Antioxid. Redox Signal.* 6, 639–647.

INTRODUCTION

In a paper published in the *Journal of the American Chemical Society* in 1969, Janzen and Blackburn first used the term "spin trapping" to describe the reaction in which a free radical (spin) is trapped by the double bond of a free radical trapping compound (spin trap) (Scheme 1) (33). The resultant compound (spin adduct) is an electron paramagnetic resonance (EPR)-active free radical species, but less reactive and more persistent than the original free radical. The identification of the original free radical can be made based on the EPR spectral parameters (*g*-value and hyperfine splitting constant) of the spin adduct.

The reaction of nitric oxide (NO) with an iron complex to form an EPR-active nitrosyl complex has been known for many years (54). The discovery of the many important roles of NO in biological systems triggered the development of various detection methods, one of which is the use of EPR-active nitrosyl-iron complexes (see Scheme 1). Mordivintcev *et al.* were the first to show that NO production from intact cells can be continuously monitored with an iron-dithiocarbamate (Fe-DTC) complex (56). Later, Komarov *et al.* used the term "spin trapping" for the reaction between NO and Fe-DTC complex (42). Some scientists seem to disagree with the use of the term "spin trapping" for this reaction because the reaction scheme deviates from the original concept of spin trapping.

The very origin of the concept of the NO spin-trapping method may be found in the paper by McDonald *et al.,* who showed that an iron complex with various ligands such as phosphates and catechols formed EPR-active iron-nitrosyl complexes (54). As an increasing number of scientists joined the research on the biological function of NO, the number of papers on NO detection methods has steadily increased. Numerous reviews, book chapters, and monographs have been written on NO spin trapping with the Fe-DTC complex (17, 18, 27, 28, 37–39, 45, 60, 81, 84, 86), and thus we think that writing a similar review would be redundant. One major objective of this short review is to make it clear the known chemical aspects of the NO spin-trapping reaction. To our knowledge, even the question on what is the actual spin-trapping species in Fe-DTC NO spin trapping has not been clearly answered.

UNIQUE CAPABILITY OF NO SPIN TRAPPING WITH IRON COMPLEX

The spin-trapping technique specifically utilizes EPR spectroscopy. Most other methods use colorimetry for the detection of reaction products between NO and probing reagents, for example, in the Griess-reaction assay NO reacts with aminobenzenesulfonic acid to produce a red diazo

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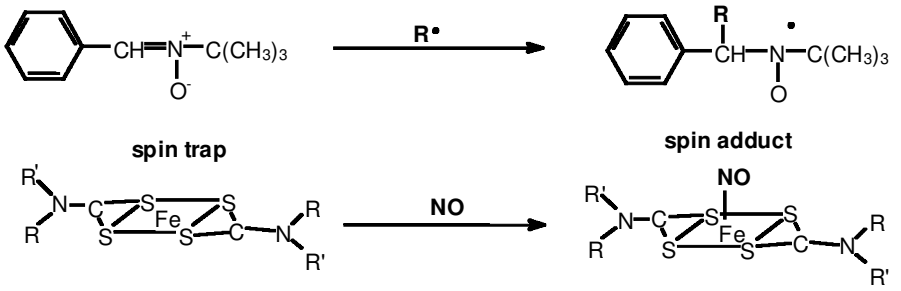


SCHEME 1. Chemical reaction schemes for "conventional" spin trapping (top) and NO spin trapping (bottom).

compound (32). In contrast, the EPR signal obtained from the NO spin adduct shows a signature of the presence of the NO moiety in the spin adduct. When NO (^{14}NO) gas is bubbled in a Fe-DTC spin-trap solution it forms a paramagnetic (EPR-active) spin adduct, and its EPR signal splits into three lines (Fig. 1a) because of the presence of the naturally abundant ^{14}N atom (nuclear spin of 1) in the spin adduct. When ^{15}NO gas is bubbled into the spin-trap solution the EPR signal shows two lines because the nuclear spin of ^{15}N is 1/2 (Fig. 1b). In other detection methods, chemical and physical properties of the original NO molecule would be lost when NO reacts with probing molecules.

The unique capability of EPR NO spin trapping has made it possible to identify the origin of nitrogen and oxygen atoms in enzymatically produced NO. Activated macrophages are known to produce NO from L-arginine and oxygen (51), and the produced NO is readily spin-trapped by Fe-DTC to show a three-line EPR signal. In macrophage NO-generating systems, when L-arginine whose guanidino-nitrogen atoms are substituted with ^{15}N atoms is added to the medium, a two-line EPR signal is obtained, indicating that nitrogen atoms in the guanidine group of L-arginine are the source of the nitrogen atom in NO (45, 46, 51). Switching of the gas over the cell culture plate from natural oxygen (or air) to ^{17}O-enriched oxygen altered the EPR signal because ^{17}O has a nuclear spin of 5/2, indicating that the oxygen atom in the NO molecule came from gaseous oxygen (46). Within an *in vivo* mouse endotoxin shock model, NO produced in the liver was spin-trapped by Fe-DTC and formed a three-line EPR signal. The gradual change of the EPR signal from three lines to two lines occurred after the administration of ^{15}N-enriched L-arginine (41), which again indicated that the source of the nitrogen atom in NO was L-arginine (Scheme 2).

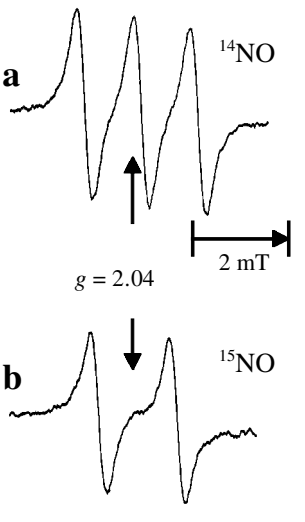
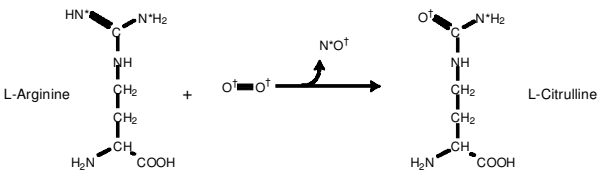


FIG 1. EPR spectra of ^{14}NO-Fe-MGD (a) and ^{15}NO-Fe-MGD (b) in aqueous solution recorded at room temperature.

DTC AND ITS IRON COMPLEX

Solutions of various DTC ions (dithiocarbamic acids) such as those shown in Fig. 2 have found a variety of applications in analytical chemistry, because these compounds form stable complexes with a number of metal ions (8, 9).

N,N-Diethyldithiocarbamate (DETC) (**1**) has been extensively used in quantitative or qualitative analyses of heavy metals such as Ni, Cu, Zn, and Hg because DETC has a high affinity for metal ions. Prior to the finding of the biological roles of NO, the Fe(III)-DTC complex, [Fe(III)$(DTC)_3$] (square bracket denotes coordination compound), and the nitrosyl Fe(II)-DTC complex, [NO-Fe(II)$(DTC)_2$], had attracted considerable interest because of their unique magnetic and electronic properties (8, 9, 13, 18). [NO-Fe(II)$(DTC)_2$] in solution forms a green color and exhibits a three-line EPR signal at room temperature (EPR spectral parameters: g_{av} ~2.04; A_N ~1.27 mT or 12.7 G). In the frozen state, the EPR spectrum shows an axially symmetric signal ($g_\perp$ ~2.037, $g_{||}$ ~2.015). Mordvintchev *et al.* were the first to show that



SCHEME 2. Enzymatic degradation of L-arginine into NO plus L-citrulline, depicting that N and O atoms in the NO molecule come from one of the guanidine nitrogens (marked with *) in L-arginine and the dioxygen molecule (marked with †), respectively.

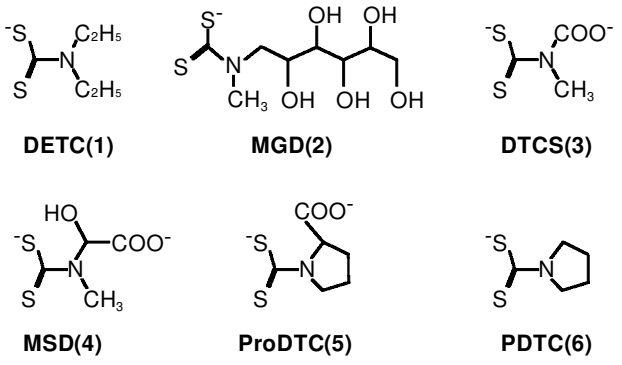


FIG. 2. DTC derivatives used as ligands for Fe-complex NO spin traps: *N,N*-diethyldithiocarbamate (DETC) (1); *N*-methyl-D-glucamine DTC (MGD) (2); *N*-(dithiocarboxy) sarcosine (DTCS) (3); *N*-methyl-L-serine DTC (MSD) (4); L-proline DTC (ProDTC) (5); and pyrrolidine DTC (PDTC) (6).

NO produced from living cells can bind to the Fe-DETC complex to form an EPR-active iron nitrosyl complex (56). In the same study, time courses of NO formation from live macrophages were recorded (56), and since then, the Fe-DTC complex has been widely employed to determine NO generation in cell cultures, tissues, and whole animals (17, 18, 27, 28, 37–39, 45, 60, 81, 84, 86).

A majority of NO spin-trapping studies have employed the iron complexes of DETC, *N*-methylglucamine DTC (MGD), or dithiocarbamoyl sarcosine (DTCS) as spin traps. The Fe-DETC complex is barely soluble in water; therefore an iron salt, *e.g.,* $FeSO_4 \cdot 7H_2O$, and DETC have to be administered via separate routes to living animals (84). Fe-MGD and Fe-DTCS are water-soluble spin traps (42, 96). The presence of hydrophilic groups in the DTC structure helps Fe-MGS and Fe-DTCS become more water soluble. Fe-MGD and Fe-DTCS can be prepared as a solution at the desired concentration or stored as a dry powder. The NO-Fe-DTCS complex is more water soluble (>100 m*M*) than the NO-Fe-MGD complex (<1 m*M*), probably because of negative charges in the DTCS complex (18, 68). Other water-soluble DTCs, illustrated in Fig. 2, have been tested as NO spin-trapping agents in biological systems, but are not widely used (20, 61, 64). The Fe-DETC complex is virtually insoluble in water, but is lipid soluble and permeable through membranes such as the blood–brain barrier (71, 75, 77), and therefore it is suitable for the detection of intracellular and intramembrane NO (28, 84). In contrast, the water-soluble NO spin traps, Fe-MGD and Fe-DTCS, are suitable for detecting cellular NO released to the medium (15, 17, 45, 47). These spin traps can be transported via the circulation system, and thus are suitable for *in vivo* detection (97), but are not permeable in lipophilic organs and tissues (15, 74). The high stability and mobility of NO spin adducts produced from water-soluble Fe-DTC spin traps are also useful as spin probes (18, 93). DETC, MGD, and DTCS are commercially available from Sigma Chemical Co. (St. Louis, MO, U.S.A.), Alexis Biochemicals (San Diego, CA, U.S.A.), and Dojindo Laboratories (Kumamoto, Japan), respectively.

SPIN-TRAPPING MECHANISM, EFFICIENCY, AND SPECIFICITY

Preparation of the Fe-DTC solution for NO spin trapping

In previous studies, various buffers, iron salts [Fe(II) or Fe (III)], negative counter ions (sulfate, chloride, or citrate), and iron/DTC concentration ratios have been used. Preparation of a Fe-DTC solution under aerobic or anaerobic conditions causes a difference at least in the solution color. If the spin-trap solution is prepared under aerobic conditions from DTC and the Fe(II) salt, the solution appears dark brown, but it is pale yellow to colorless when prepared under nitrogen. However, the colorless solution under nitrogen turns brown when air is introduced into the solution. The Fe-DTC spin-trap solution could be produced from an Fe(III) salt and DTC, and in that case the solution color (dark brown) does not depend on the atmosphere. Previous studies of the Fe-DTC complex already revealed that the dark color comes from a Fe(III)-DTC complex, while the Fe(II)-DTC complex is colorless (18, 22, 84, 92).

Is NO spin-trapped by a Fe(II)- or Fe(III)-complex?

High-performance liquid chromatography studies have shown that the Fe(II)-DTC complex reacts with NO to form a NO-Fe(II)-DTC complex as the sole product (Eq. 1) (18, 22):

$$NO + Fe(II)(DTC)_2 \rightarrow NO\text{-}Fe(II)(DTC)_2 \qquad (1)$$

In the formation of the Fe(II)-proline DTC complex, the second-order rate constant of the reaction (Eq. 1) is $1.1 \pm 0.3 \times 10^8\ M^{-1}\ s^{-1}$ (26). Surprisingly, the Fe(III)-DTC complex readily reacts with NO to form a NO-Fe(II)$(DTC)_2$ complex, the same product as in Eq. 1 (20, 22, 78, 82). In coordination chemistry, this reaction is known as reductive nitrosylation, and the same reaction occurs in the nitrosylation of heme proteins and iron-porphyrin complexes (5, 6, 89, 95). Three mechanisms were proposed for the reductive nitrosylation of Fe(III)-DTC complexes, which involve: (1) the dissociation of DTC into a DTC free radical (Eq. 2) (94); (2) two Fe(III)-complex molecules (Eq. 3) (22); and (3) two NO molecules (Eq. 4) (82, 83):

$$Fe(III)(DTC)_3 + NO \rightarrow NO\text{-}Fe(II)(DTC)_2 + DTC\bullet \qquad (2)$$

$$Fe(III)(DTC)_3 + NO \xrightarrow{Fe(III)(DTC)_3} NO\text{-}Fe(II)(DTC)_2 + 1/2[bis(DTC)] \qquad (3)$$

$$Fe(III)(DTC)_3 + 2NO \rightarrow NO\text{-}Fe(II)\,(DTC)_2 + NO_2^- \qquad (4)$$

Mechanism 3 (Eq. 4), which requires two NO molecules, is identical to that of the reductive nitrosylation of Fe(III) hemoproteins. The second-order reaction rate constants of NO

with both Fe(II)$(DTC)_2$ (Eq. 1) and Fe(III)$(DTC)_3$ (Eq. 3) are of the order of $10^8\ M^{-1}\ s^{-1}$, which is as fast as the reaction of NO with O_2-hemoglobin ($3.7 \times 10^7\ M^{-1}\ s^{-1}$) (10) or Fe(II)hemoglobin ($1.5 \times 10^7\ M^{-1}\ s^{-1}$) (24). These three mechanisms are equally justifiable at present. In summary, when the spin-trap solution is prepared and/or used in aerobic conditions, it is very likely that the trapping species is the Fe(III)-DTC complex.

NO spin-trapping efficiency

The important difference in the mechanisms shown in Eqs. 2–4 is the stoichiometric ratio of NO/Fe(III), *i.e.,* 1 in Eqs. 2 and 3 and 2 in Eq. 4. Therefore, the theoretical NO trapping efficiency is 100% for Eqs. 2 and 3 and 50% in Eq. 4. NO spin-trapping efficiencies of the Fe(III)-DTC complexes (DTC = MGD or DTCS) in aqueous solutions are dependent on the kind of buffer used, and range from 40% in phosphate-buffered saline to 95 % in Tris-HCl buffer (both at pH 7.4) (18). Even in the absence of biological components, Fe-DTC complexes are unable to trap all NO produced due to side reactions, therefore the amount of NO calculated from the spin-adduct yield is less than the actual NO produced. In the presence of biological components, NO spin-trapping efficiencies with Fe-DTC complexes would further decrease with the influence of the nature of the medium, such as the content of protein, lipid, or salt (68). Similar to conventional spin trapping with the use of organic spin traps, we believe that the ability to determine the absolute NO concentration using the Fe-DTC NO spin-trapping method is difficult. However, by adopting reasonable control experiments, it should be possible to obtain relative data.

How stable are DTCs and Fe-DTC complexes?

DTCs are strong chelating agents for iron, but the stabilities of Fe(III) complexes have been shown to be much higher than those of Fe(II) complexes (8, 9). However, Fe-DTC complexes may disappear after ligand-exchange reactions with endogenous ligands that have a high affinity to iron, such as glutathione and the imidazole group in the histidine residue, where the effective spin-trap concentration could decrease. We believe that this is one of the reasons that the use of 10–20-fold more DTC than the iron salt, when preparing spin-trap solutions, is beneficial. Free DTCs without iron are not very stable and decompose into carbon disulfide plus secondary amine in aqueous solution. Even in the solid state, the shelf life of DTCs is not so long presumably because of dimerization or an oxidation reaction, and thus reagents should be kept in a dry, cold, nitrogen environment. The half-life of the decomposition of an aqueous DETC solution at ambient condition is 45 min at pH 7, and the decomposition proceeds more rapidly at pH values below 7 (35, 53). In Fe-DETC spin trapping, when the iron salt and DETC are administered *via* separate routes to animals, one should keep in mind that the toxicity of DETC's decomposition products and iron salts may influence NO production pathways. Contradictory results have been presented concerning the effect of exogenous iron on endogenous NO production, *i.e.,* either attenuation or potentiation (23, 43, 49). The differences may be brought about by variation in protocols such as dose timings of iron and DETC.

The NO-Fe(II)$(DTC)_2$ complex reacts with another NO molecule to form the EPR-silent dinitrosyl Fe-DTC complex (34), which may cause a decrease in the EPR signal and affect NO spin-trapping efficiency. Recently, the NO-Fe$(MGD)_2$ complex was demonstrated to react with $O_2^{\cdot-}$ or peroxynitrite to yield EPR-silent species (83). In $O_2^{\cdot-}$-generating systems, this transformation could lead to the loss of NO spin adducts, influencing NO quantifications.

Is NO the sole species trapped by the Fe-DTC spin-trap?

Previous studies demonstrated that Fe-DTC complexes react with NO as well as NO-related compounds, including *S*-nitrosothiols (RSNOs), nitroxyl ion (NO^-), and nitrite (NO_2^-), to yield EPR-active NO complex. RSNOs produce either the nitrosonium ion (NO^+) or NO depending on the media. The reaction products of RSNOs with Fe-DTC were identified as NO-Fe$(DTC)_2$, but the rate constants of the reaction have not been evaluated (58, 80). RSNOs are commonly referred to as cell signaling molecules and NO transporters (29). RSNO formation rates from RSH, NO, and O_2 are low, and the basal level in plasma was reported to be 30–120 n*M* (52). Recently, contradictory results have been reported on the reactivity of the Fe-MGD complex for NO^-. Komarov *et al.* demonstrated that an NO^- donor (Angeli's salt; $Na_2N_2O_3$) reacts with Fe-MGD under aerobic conditions to form a product that shows the same EPR spectrum as NO-Fe-MGD, suggesting that the Fe-MGD spin trap does not discriminate NO from NO^- (44). In contrast, Xia and co-workers demonstrated that the Fe(II)$(MGD)_2$ complex exhibited the EPR spectrum of NO-Fe-MGD upon addition of an NO donor (*S*-nitroso-*N*-acetylpenicillamine), but not with Angeli's salt, while the Fe(III)$(MGD)_3$ complex did show an EPR spectrum after reaction with these NO and NO^- donors (91, 92). These studies suggest that the Fe-MGD complex is capable of distinguishing NO from NO^- when the redox state of iron is adjusted. NO_2^- was shown not to influence Fe-DTC NO spin trapping (58); however, recent reports indicated that the Fe-MGD complex produces NO-Fe$(MGD)_2$ in the presence of NO_2^- after prolonged incubation (62, 79, 85). The reaction of NO_2^- with the Fe-MGD complex exhibits a low rate constant of $4.8\ M^{-2}\ s^{-1}$ (79), and thus proceeds only in the presence of high NO_2^- concentrations, such as 100 μ*M* (79) or 500 μ*M* (85).

The NO_2^- levels in biological fluids are generally lower than the NO_3^- levels (14, 57, 65); for instance, $[NO_2^-]$ = 6.6 ± 11 μ*M* and $[NO_3^-]$ = 34 ± 18 μ*M* in sera of healthy humans (n = 21), $[NO_2^-]$ = 3.4 ± 3.1 μ*M* and $[NO_3^-]$ = 7.6 ± 4 μ*M* in cerebrospinal fluid of healthy humans (n = 10) (14), and $[NO_2^-]$ is ~6 μ*M* and $[NO_3^-]$ is ~260 μ*M* in plasma of endotoxin-treated rats (65). These data suggest that *in vivo* levels of NO_2^- are far below 100 μ*M*; therefore NO-Fe$(DTC)_2$ produced from NO_2^- could be below the EPR detection limit. However, decreases in pH during tissue ischemia may induce the direct reduction of NO_2^- into NO that could be trapped by the Fe-DTC complex (99). Whether NO production is NO synthase (NOS) dependent could be deter-

mined by using NOS inhibitors. If an EPR signal from NO-$Fe(DTC)_2$ is weakened or disappears after the addition or administration of an NOS inhibitor, then the NO production is very likely enzymatic.

Toxicity and pharmacological activity of DTCs

Although the feasibility of continuous and quantitative monitoring of the same biological specimen is a great advantage of the NO spin-trapping technique (45, 56), the addition or administration of exogenous Fe-DTCs or DTCs could cause disturbances to biological specimens. The 50% lethal doses for DETC, MGD, and DTCS in mice were evaluated: (1) for $DETC{\cdot}Na{\cdot}3H_2O$, 1,870 mg/kg (orally) (4) or 1,332 mg/kg (intravenously) (T. Yoshimura, unpublished data); (2) for MGD·Na, more than 2,500 mg/kg (intraperitoneally) (73) and more than 7,000 mg/kg (intraperitoneally) (T. Yoshimura *et al.,* unpublished data); and (3) for $DTCS{\cdot}2NH_4{\cdot}2H_2O$, more than 765 mg/kg (intraperitoneally), and for $DTCS{\cdot}2Na{\cdot}2H_2O$, 1,942 mg/kg (intravenously) (17). There is no report on the toxicity of Fe-DTC complexes; however, rapid intravenous administration of a Fe-MGD solution caused seizures in rats (Y. Kotake *et al.,* unpublished data). $Fe(II)(MGD)_2$ and $Fe(II)(DTCS)_2$ produce less $O_2^{\cdot-}$ and ·OH than chelates of ethylenediamine-*N,N,N,N*-tetraacetic acid (68). Chelating of EDTA to iron has been frequently used to inhibit ·OH production. Overall, the toxicity data suggest that Fe-DTC-based NO spin traps could cause minimum injuries to living organisms and non-significant modulation of NO production when used in the accepted range of concentrations. Thus, continuous NO spin-trapping experiments in living systems should be feasible. The potential therapeutic use of Fe-DTCs as NO-scavenging agents has been demonstrated in animal models (66).

Prolonged monitoring of *in vivo* NO formation using Fe-DTC spin traps could be influenced by the pharmacologic activity of free DTC. Industrial use of DTC analogs ranges from fungicides, herbicides, and insecticides to iron-particle removers, with recent estimates of global consumption on the order of 25,000–35,000 metric tons/year (90). Some DTCs have been clinically tested and shown to be safe and effective in the treatment of human immunodeficiency virus-infected patients (30). Pharmacological activities of DTCs are attributed to their metal-chelating and/or thiol-delivering properties. DETC has been shown to inhibit Cu/Zn-superoxide dismutase activity through the withdrawal of Cu from the protein both *in vivo* and *in vitro* (7, 26, 55). This inhibition may cause an increase in the concentration of $O_2^{\cdot-}$ *in vivo* and a concomitant decrease in the concentration of NO, because NO and $O_2^{\cdot-}$ react in a diffusion-controlled rate to form peroxynitrite, a more toxic species than NO (31). Pyrrolidine DTC (PDTC) (**6** in Fig. 2) and DETC potently inhibit the activation of the transcription factor nuclear factor kappa B (NF-κB) in cells and *in vivo,* and this action is speculated to be through DTCs' antioxidant activity (50, 72). NF-κB regulates gene expression in various physiological processes such as immunity, stress responses, inflammation (2), glial/neuronal function (36, 63), and apoptosis (1, 88). Therefore, the inhibition of NF-κB activity by PDTC or DETC could cause biological consequences, including inhibition of inducible NOS and cyclooxygenase 2 gene/protein expression (3, 11, 59), and gene induction of heme oxygenase-1 (25). Recently, Fe-DTCS and Fe-MGD have been shown to inhibit neuronal NOS-mediated NO production in a cell-free system, but free DTCs did not inhibit the activity (98).

BIOLOGICAL APPLICATIONS OF NO SPIN TRAPPING

Fe-DTC spin trapping has been employed to directly evaluate NO generation from purified NOS (91, 98) and NO quantification in cultured cells such as macrophages, endothelial cells, and neural cells (19, 45, 62) and in the tissues of animal models under various pathological conditions. *Ex vivo* NO detection in resected tissues of target organs is most frequently carried out by using Fe-DTC spin traps (37). In these experiments, animals were treated with Fe-DTC spin traps, tissues were harvested, dissected, and placed into an EPR sample cell (tissue cell), and then EPR signals were recorded. In some experiments, tissues were loaded into a round sample tube, and EPR signals were recorded at liquid nitrogen temperature (71). The Fe-DETC complex is by far the most efficient NO spin trap in brain tissue, because Fe-DETC can pass through the blood–brain barrier (12, 77). The Fe-DETC spin trap has been utilized to determine NO levels in organs other than brain in rats (87). Interestingly, NO-Fe-DETC in tissues can be extracted with chloroform and concentrated to gain better sensitivity (87). Water-soluble Fe-DTC spin traps such as Fe-MGD and Fe-DTCS are well suited for use in water-rich tissues and fluid samples such as blood, bile, and urine (97).

We believe that the less-invasive nature of the Fe-DTC spin trap has made it possible to perform *in vivo* NO measurements (17, 67). Thus, *in vivo* real-time EPR detection of NO has been conducted in various animal models, *i.e.,* in the tails of sodium nitroprusside- and lipopolysaccharide-treated mice with Fe-MGD (41, 42); in the abdominal regions of LPS-treated mice with Fe-DTCS (97), Fe-MGD (16), Fe-DETC (69), and Fe-MSD (61); and in the head region of rat models of sepsis (75) and bacterial meningitis (12, 76) with Fe-DETC.

Two unique approaches have been developed to assess NO production in living animals with the NO spin-trapping technique: (1) EPR imaging of spin adducts and (2) EPR monitoring of spin adducts in bile flow. The EPR imaging approach to visualize *in vivo* NO production was first applied to a mouse endotoxin shock model (96) and later in exogenous isosorbide dinitrate-administered mice (21). Although EPR imaging studies require sophisticated equipment, critical information on the quantity and temporal/spatial distribution of NO in the tissues and organs can be obtained. Presently, the lack of sensitivity seems to prevent this technique from wider application; however, the EPR imaging approach with the use of NO-Fe-DTC as spin probes could be promising.

NO spin adducts produced *in vivo* in various organs eventually reach the liver *via* circulation, which is concentrated and secreted into bile. Fe-MGD administration to a rat lipopolysaccharide model resulted in the appearance of a strong EPR signal of NO spin adducts in bile (70). *In situ* monitoring of the EPR signal from anesthetized rats was

possible by directly introducing bile flow into the EPR cavity (48). This method is suitable for monitoring real-time drug responses of NO formation. Although the spatial information is not available, the bile monitoring method is very sensitive and suitable for semiquantitative time course studies.

SUMMARY

Similar to the conventional spin-trapping method, Fe-DTC-based NO spin trapping could be influenced by numerous factors, especially when it is used in biological systems. The use of ^{15}N-substituted NO or L-arginine provides a unique strength as compared with other photometric NO detection methods. We believe that it is important to understand the chemistry that is involved in the NO spin-trapping reaction, and to ascertain that the spin-trapped NO is the product from the system of interest.

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ABBREVIATIONS

DETC, *N,N*-diethyldithiocarbamate; DTC, dithiocarbamate; DTCS, dithiocarbamoyl sarcosine; EPR, electron paramagnetic resonance; MGD, *N*-methylglucamine dithiocarbamate; NF-κB, nuclear factor kappa B; NO, nitric oxide; NOS, nitric oxide synthase; PDTC, pyrrolidine dithiocarbamate; RSNO, *S*-nitrosothiol.

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