

## NO spin trapping in biological systems

Anatoly Vanin, Alexander Poltorakov

Semyonov Institute of Chemical Physics, Russian Academy of Sciences, Moscow, Russia

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Advantages
4. Mechanisms of formation of paramagnetic MNIC-dithiocarbamate complexes in biological systems
5. Reactions of paramagnetic MNIC-dithiocarbamate complexes with superoxide anions and peroxyntirite
6. On quantitative determination of NO in animal organisms by the iron-dithiocarbamate method
7. Acknowledgment
8. References

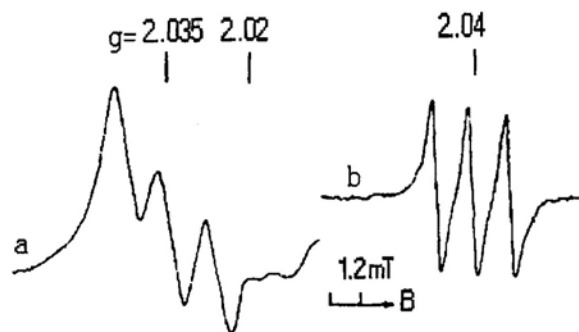
## 1. ABSTRACT

The most recent data on mechanisms of spin trapping of nitric oxide (NO) by iron dithiocarbamate complexes in animal and plant cells and tissues are considered. The rationale is as follows: 1 In the absence of NO in cells and tissues, iron binds primarily to compounds others than dithiocarbamate ligands, e.g., tricarbonic acids. 2. Predominant binding of iron to dithiocarbamate ligands takes place only after its binding to NO, since nitrosylated iron manifests much higher affinity for these ligands than for any non-thiol compounds. 3. Within the composition of mononitrosyl dithiocarbamate complexes, iron exists predominantly in the oxidized ( $\text{Fe}^{3+}$ ) form, i.e., these complexes are originally diamagnetic. Their subsequent single-electron reduction to the paramagnetic, EPR – detectable form is mediated by endogenous or exogenous (e.g., dithionite) reducing agents. 4. Superoxide-mediated transition of paramagnetic mononitrosyl dithiocarbamate iron complexes into EPR-silent state can be accompanied by significant reduction of EPR-detectable complexes. This defect can be overcome through the use of the so-called ABC method. 5. In contrast to hydrophobic complexes fast decomposition of water-soluble mononitrosyl iron complexes in animal organisms testifies to low efficiency of these complexes in determination of NO content in animal cells and tissues.

## 2. INTRODUCTION

In 1984, Vanin *et al.* suggested the use of a bivalent iron complex with diethyldithiocarbamate (DETC) as a trap for nitric oxide (NO) in animal cells and tissues (1). Binding of one NO molecule to this complex results in the formation of a paramagnetic mononitrosyl iron complex with DETC (MNIC-DETC) ( $\{\text{NO-Fe}^{2+}-(\text{DETC})_2\}$ ), which at 77 K generates an anisotropic electron paramagnetic resonance (EPR) signal with the following values of the g-factor:  $g_{\perp} = 2.047$ ,  $g_{\parallel} = 2.022$ ,  $g_{av} = 2.04$ . At room temperature and at  $g = 2.04$ , this signal represents an isotropic triplet of a hyperfine structure (HFS) and splitting at 12.5 G (Figure 1). High values of rate constants ( $\leq 10^8 \text{ M}^{-1}\text{s}^{-1}$ ) for the reactions of iron-dithiocarbamate complexes with NO (2,3) are suggestive of a competition between these complexes and the superoxide anions responsible for the oxidation of free NO molecules in animal cells. The rate of NO binding to iron-dithiocarbamate complexes used at millimolar concentrations exceeds the rate of NO oxidation by the superoxide by the orders of magnitude. High efficiency of using Fe-DETC complexes in NO detection in biological systems and its quantitative determination *in vivo* was confirmed in numerous studies (1, 4-34).

## Nitric oxide, spin trapping, iron dithiocarbamate



**Figure 1.** Typical EPR spectra of NO-Fe<sup>2+</sup>- (DETC)<sub>2</sub> complex in dimethylsulfoxide solution. Recordings were made at 77K (a) or ambient temperature (b) (1).

MNIC-DETC are related to the category of water-insoluble hydrophobic complexes. They are localized in hydrophobic compartments of the cell membrane and are not excreted into the hydrophilic cell environment or the blood stream (4-34). The solubility of DETC molecules in H<sub>2</sub>O is due to the presence in their structure of a negatively charged ionized thiol group. Binding of positively charged Fe<sup>2+</sup> ions to the thiol groups in two DETC molecules results in the formation of an electroneutral water-insoluble Fe<sup>2+</sup> (DETC)<sub>2</sub> complex, while binding of neutral NO molecules to the Fe<sup>2+</sup> (DETC)<sub>2</sub> complex has no effect on the hydrophobic characteristics of the MNIC-DETC complex formed. In 1993, it was suggested to use the water-soluble iron complex with N-methyl-D-glucamine dithiocarbamate (MGD) for NO trapping in biological systems (35). The presence in MGD of a carbohydrate residue provides for high solubility of MGD molecules and their iron complexes in water in the presence and in the absence of NO. More recently, the use of other dithiocarbamate derivatives able to form water-soluble and water-insoluble complexes with iron and to bind NO was proposed by Japanese investigators (30). Water-soluble iron complexes with dithiocarbamates proved to be valuable tools for detecting NO not only in the animal tissue samples and cell cultures, but also in aqueous solutions of NO donors, such as NO synthases (NOS) (35-54).

In this review, we attempted to generalize an extensive body of evidence concerning the use of iron-dithiocarbamate complexes as NO traps in the analysis of biological systems with special emphasis on the advantages and disadvantages of the EPR method. The data available allow reconsideration of the current views on the molecular mechanisms of formation of paramagnetic MNIC with dithiocarbamate derivatives and NO detection in living systems.

### 3. ADVANTAGES

The indisputable advantage of the novel method is the possibility to establish unequivocally the nature of compounds used as NO sources in animal organisms, cell cultures and isolated biochemical systems. This is achieved using a hypothetical NO source, in which the nitrogen

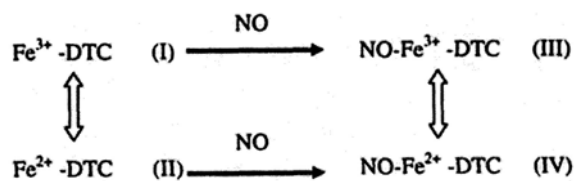
atoms (<sup>14</sup>N) are replaced by the nitrogen isotope, <sup>15</sup>N. If such a substitution is accompanied by a complete substitution of triplet HFS (which in the EPR spectra of MNIC-dithiocarbamate complexes is a result of hyperfine splitting on <sup>14</sup>N nuclei (nuclear spin I = 1)) for doublet HFS (as a result of hyperfine splitting on the <sup>15</sup>N nucleus (I = 1/2)), there are all grounds to believe that the <sup>15</sup>N-labeled compound can indeed produce NO.

This approach proved its worth in experiments designed to investigate the role of L-arginine as a unique source of NO production in animal tissues and cell cultures via a NOS-catalyzed oxidative pathway. The substitution of <sup>14</sup>N for <sup>15</sup>N in the guanidine residue of L-arginine was followed by complete replacement of triplet HFS by doublet HFS in the EPR spectra of MNIC-DETC isolated from mouse liver or macrophage cultures during augmented synthesis of inducible NOS oxide (5, 9, 37, 38, 40). The involvement of the nitrite in NO generation in isolated animal hearts by a nonenzymatic reducing mechanism was established using a similar approach (39). The participation of dioxygen molecules in NO formation catalyzed by NOS was proven using <sup>17</sup>O (O<sub>2</sub>) (I = 5/2) in the experiments with activated macrophages. The additive HFS from <sup>17</sup>O was detected in triplet EPR signal from <sup>14</sup>NO<sup>17</sup>O-Fe<sup>2+</sup>-MGD<sub>2</sub> (38).

The possibility of unique detection of NO in complex systems (e.g., living organisms) both *ex vivo* and *in vivo* is yet another prominent advantage of the iron-dithiocarbamate method. The nature of enzymatic or nonenzymatic systems involved in the generation of NO can be established using specific inhibitors, e.g., NOS inhibitors. Their application, similar to isotopic substitution of L-arginine, made it possible to minimize MNIC-DETC concentrations and provided additional support in favour of the involvement of the L-arginine-NOS-dependent pathway in NO generation in animal cells and tissues (5-7,16,21,37,40-42,48).

The problem of quantitative determination of NO in living systems has not yet been adequately explored and needs unambiguous solution. Apparently, the binding of all NO produced by living organisms to iron-dithiocarbamate traps is impossible in principle, since part of free NO molecules bypass the traps instead of binding to them. Nonetheless, their complete capture is theoretically possible and can be achieved through the use of superhigh concentrations of NO traps in cells and tissues. However, this approach entails significant disturbances in cell metabolism. Recent studies have demonstrated that such efforts are *a priori* unsuccessful, since paramagnetic MNIC containing dithiocarbamate ligands are exposed to the damaging effects of superoxide anions (O<sub>2</sub><sup>-</sup>), which convert them into an EPR-nondetectable form (50). This problem similar to the other pitfalls of using iron-dithiocarbamate complexes as NO traps, will be discussed in the subsequent sections. Here, it is sufficient to remind that the aim of this paper is to revise some commonly held views on the mechanisms of formation of paramagnetic MNIC-dithiocarbamate complexes in biological systems.

## Nitric oxide, spin trapping, iron dithiocarbamate



**Figure 2.** The four possible iron-dithiocarbamate (Fe-DTC) complexes. The oxidized mononitrosyl-iron complexes (III) is diamagnetic, whereas the reduced (IV) complex is paramagnetic ( $S = \frac{1}{2}$ ). The conventional viewpoint of NO trapping is the direct transformation of complex (II) into (IV) by inclusion of a nitrosyl ligand.

### 4. MECHANISMS OF FORMATION OF PARAMAGNETIC MNIC-DITHIOCARBAMATE COMPLEXES IN BIOLOGICAL SYSTEMS

The use of iron-dithiocarbamate complexes as NO traps was first proposed in our publication, which appeared in press as early as 1984 (1). In this study we proposed that the reduced state of ferro-DETC complexes appeared, at least partly, by reducing agents present in animal organisms. By binding to ferro-DETC complexes, NO favors the formation of electroneutral paramagnetic MNIC -  $\{\text{NO}^+\text{-Fe}^+ \text{- (DETC-S)}_2\}$  that can be detected by the EPR method.

At the same time, our more recent data suggest that water-soluble and water-insoluble ferro-dithiocarbamate complexes are both rapidly and virtually completely oxidized to the ferri-form in the air, in cultural media or directly in animal tissues (32). It is noteworthy that water-soluble (but not water-insoluble) complexes are reduced to the ferro-form only in the presence of cysteine. The efficiency of such reduction is very low because of extremely low ( $\leq 100$  microM) concentration of cysteine in living systems (55). Other endogenous reducing agents, e.g., glutathione and ascorbate, are fairly ineffective, since the reduction of the aforementioned complexes by glutathione proceeds at a very low rate, while ascorbate does not reduce them at all (32). These findings suggest that it is the ferri-form of iron-dithiocarbamate complexes that is responsible for the binding of NO at a rate close to the rate of binding of NO with ferro-dithiocarbamate complexes in animal cells and tissues (20,32,56). This reaction results in the formation of diamagnetic  $\{\text{NO}^+\text{-Fe}^{2+} \text{- (DETC-S)}_2\}$  complexes, which themselves do not generate any EPR signals, but can effectively accept electrons from endogenous reducing agents (ascorbate included) to be further converted into an EPR-detectable reduced form (20, 32, 50, 56, 57). Therefore, for biological systems the "clockwise" mechanism of paramagnetic MNIC-dithiocarbamate formation seems to be a more common route (Figure 2 in (32)). It should be noted in this connection that in biological systems the recovery/reduction of nitroso-ferri-dithiocarbamate complexes by endogenous compounds may be incomplete as it can be evidenced from the results of experiments, in which the concentration of paramagnetic MNIC-dithiocarbamate complexes was increased 2-3-fold

after dithionite treatment of animal tissues defrosted in 150 mM HEPES (pH 7.4) (58). This indicates that the use of strong buffers is necessary in order to prevent nitrite reduction to NO by dithionite treatment as it is generally the case with weak buffers, e.g., 15 mM HEPES. Addition of dithionite powder to buffer solutions stimulates the appearance of local low-pH zones, where the nitrite conversion into nitric acid is accompanied by NO release (32,58).

The retention of ferri-dithiocarbamate complexes in animal tissues in the absence of NO also presents a problem. Thus, it was found that some endogenous iron-binding compounds (e.g., citrates, ascorbates, etc.) compete with dithiocarbamates for iron ions up to complete degradation of iron-dithiocarbamate complexes (32,58). This situation changes drastically in the presence of NO, since the affinity of the nitroso-ferri-dithiocarbamate complexes for dithiocarbamates markedly exceeds the affinity of other ligands for iron ions (32,58). As a consequence, dithiocarbamate-containing diamagnetic MNIC retain their activities over sufficiently long periods of time and undergo further reduction to paramagnetic forms under the influence of endogenous compounds.

According to present-day views, iron-dithiocarbamate complexes, especially those involving iron ferri-ions, contain at least three dithiocarbamate molecules (59,60). However, the results of optical analysis of an equilibrium of the  $\text{Fe}^{3+}$ -MGD mixture strongly suggest that these complexes contain only two MGD molecules (32). The stability constants for these complexes ( $\beta_2$ ) are equal to  $1.6 \times 10^7 \text{ M}^{-2}$  (32). Our recent studies have demonstrated that at  $\text{DETC:Fe} \leq 3$  the majority of ferri-DETC complexes contain two DETC molecules (the paper under preparation); their stability constants are by orders of magnitude higher than those for ferri-MGD complexes. At  $\text{Fe}^{3+}:\text{DETC}$  ratio = 1:2, virtually all iron ions appear to be involved in the complex structure. The true reason for the drastic increase in the rate of NO trapping in isolated animal tissues, e.g., in isolated blood vessels, in the presence of water-insoluble colloid iron-DETC complexes formed in aqueous solutions upon mixing of DETC and iron (1:2) thus becomes apparent (61). The concentration of the DETC-iron complexes formed is approximately equal to the concentration of exogenous iron, while all DETC molecules appear to be bound to the complexes. This finding is consistent with the lack of DETC molecules to form paramagnetic DETC complexes with bivalent copper; otherwise, MNIC-DETC signal would be masked by EPR signals (62). Furthermore, the absence of free DETC molecules compensates by the inhibiting effect of the latter towards superoxide dismutase, an endogenous superoxide inhibitor responsible for the oxidation of MNIC-DETC and the resulting increase in the complex yields (61,63).

### 5. REACTIONS OF PARAMAGNETIC MNIC-DITHIOCARBAMATE COMPLEXES WITH SUPEROXIDE ANIONS AND PEROXYNITRITE

It was found that the paramagnetic form of MNIC-MGD complexes reacts with superoxide anions in

## Nitric oxide, spin trapping, iron dithiocarbamate

phosphate buffers (pH 7.4) (50). This reaction proceeds with the second-order rate constant equal to  $3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  and results in the conversion of MNIC–MGD into an EPR-silent form. The latter is reconstituted into paramagnetic complexes in the presence of reducing agents (e.g., sodium ascorbate) added to the reaction solution not later than within 5–7 min after superoxide addition; otherwise the regeneration of the original complex does not take place. It is thought that reaction of the superoxide with NO yields an intermediate peroxynitrite, which remains bound to the complex within 5–7 min. Subsequent isomerization of peroxynitrite into a nitrate and its decomposition with the release of NO are due to degradation and further conversion of MNIC–MGD complexes into ferri-MGD (50). Similar reactions are characteristic for water-insoluble MNIC (50). The results of treatment of MNIC with peroxynitrite suggest that the latter can itself destroy these complexes (50).

The so-called ABC method was devised with a unique purpose to overcome the understated estimation of MNIC–dithiocarbamate concentrations in the presence of the superoxide and peroxynitrite (50). The measurements were performed in two steps. In the first step, an aliquot of MNIC, which additionally fulfilled the function of an exogenous trap for the superoxide and peroxynitrite, was added to animal cells or cell cultures. The binding of endogenous NO was induced by adding bivalent iron and DETC (MGD) to the tested samples. In the second step, aliquots of MNIC and the NOS inhibitor were added to the samples. The intensity of EPR signals (S) was established from the ratio:

$$\begin{aligned} S_1 &= A + B - C && \text{(in the absence of the NOS inhibitor, Exp. No.1)} \\ S_2 &= A - C && \text{(in the presence of the NOS inhibitor, Exp. No.2)} \end{aligned}$$

where A is the signal generated by exogenous MNIC, B is the signal generated by MNIC formed in the presence of endogenous NO and C is the reduction of the MNIC signal resulting from the degradation of the complexes induced by superoxide or peroxynitrite. By subtracting  $S_2$  from  $S_1$ , we obtain the value of B. Under these conditions, the intensity of A should be greater than the value of C in order to minimize the concentrations of the superoxide and peroxynitrite. At the same time, the intensity of A should not be too high with respect to the intensity of B and for animal cells and cell cultures should be selected in the range 1–50  $\mu\text{M}$  (50).

In the paradigm of notations based on the use of conventional methods for NO detection, e.g., the selective use of iron–dithiocarbamate complexes as NO traps, the value of B–C is determined from their EPR spectra. The use of high concentrations of superoxide and peroxynitrite at high values of C results in the degradation of the MNIC formed manifested as a lack of EPR signals.

The ABC method based on the use of iron–dithiocarbamate traps is distinguished for high sensitivity of iron complex assays in the presence of

superoxide anions and peroxynitrite. When NO assays were carried out in cultured endothelial cells by conventional methods, e.g., by the addition of Fe–DETC to cell cultures and the 15-min incubation of MNIC–DETC, the reaction yield was 0.5 nmoles/ $5 \times 10^6$  of cultured cells (data from EPR spectral analysis). For comparison, the yields from the ABC method were 2.5 nmoles (50). In experiments with isolated bean leaves, the difference between experimentally determined values was even more pronounced (64). If the measurements were performed by conventional methods, the rate of MNIC–DETC accumulation in the leaf tissues was increased to 0.9 nmoles/g of wet tissue after 1-hour incubation, whereas in other studies the complexes could not be identified at all. Low yields of MNIC–DETC complexes can be attributed to enhanced production of superoxide anions in bean leaves, which is more characteristic for stress situations e.g., defoliation or incubation in buffer solutions (65). Measurements based on the ABC method revealed that the rate of MNIC–DETC formation in the presence of endogenous NO (the intensity of B) did not change within 1 h and was equal to 20 nmoles/g of wet tissue in all tested samples as can be seen from the difference between the EPR signals corresponding to  $A + B - C$  and  $A - C$ . Inhibition of endogenous NO synthesis in bean leaves was achieved either through addition of a nitrate as the inhibitor of nitrate reductase responsible for this synthesis in plants or deoxyhemoglobin, which competed with Fe–DETC complexes for endogenously produced NO (64).

## 6. ON QUANTITATIVE DETERMINATION OF NO IN ANIMAL ORGANISMS BY THE IRON–DITHIOCARBAMATE METHOD

The damaging effects of superoxide anions and peroxynitrite on MNIC–dithiocarbamate complexes invalidate the assumption that the use of iron–dithiocarbamate complexes as NO traps provides quantitative information about the generation, or, more precisely, the rate of generation of NO in animal organisms. Even if MNIC contain the whole amount of endogenously produced NO, they can partly be converted into the nondegradable form, nondetectable by EPR and thus fall out of the scope of consideration. The key issue in quantitative determination of NO in animal cells by the iron–dithiocarbamate method is to elucidate whether these changes affect a negligibly small part or a major part of the paramagnetic MNIC formed.

The currently available data suggest that in animal cells the overwhelming majority of water-soluble MNIC–MGD complexes undergo irreversible degradation within half an hour, presumably under the action of superoxide anions and peroxynitrite. Therefore, the EPR signals of these complexes detected in animal urine or internal organs provide only qualitative information about NO generation within the first 30 min following administration of MGD–iron complexes as NO traps. This conclusion was based on the results of experiments in which mice and rabbits were injected with 2–3 micromoles of «preformed» MNIC–MGD (66). In the liver and other

## Nitric oxide, spin trapping, iron dithiocarbamate

internal organs, these complexes stimulated the appearance of small ( $\leq 40$  nmoles) concentrations of paramagnetic thiol-containing dinitrosyl iron complexes (DNIC); the content of paramagnetic MNIC–MGD complexes in urine samples did not exceed 1 nmole. Subsequent treatment of animal urine with dithionite increased their content 3–4-fold, apparently at the expense of nitrite formed upon decomposition of MNIC–MGD to NO. The yield of MNIC–MGD increased by more than two orders of magnitude after treatment of urine samples with gaseous NO, which testifies unambiguously to a virtually complete degradation of exogenous MNIC–MGD complexes. The MGD molecules and iron ions released from the complexes were reassembled into MNIC–MGD complexes in the presence of exogenous NO that was detected by the EPR method.

In urine samples of mice treated with bacterial lipopolysaccharide (LPS), the MNIC–MGD content was decreased 5–10-fold concomitantly with inflammation and augmented synthesis of inducible NOS responsible for enhanced production of NO and the superoxide (5,9). A significant contribution to the formation of superoxide was made by oxidase systems triggered upon inflammation. In all probability, enhanced production of superoxide anions is one of the reasons for more efficient degradation of MNIC–MGD complexes in comparison with LPS-untreated animals. The decay of MNIC–MGD complexes in animals affected by inflammation can also be provoked by peroxynitrite; its production is enhanced during augmented synthesis of the superoxide and NO.

In the foregoing sections, we considered the possibility to overcome the damaging effects of the superoxide and peroxynitrite using the ABC method. An alternative approach consists in increasing the content of exogenous water-soluble iron–MGD complexes as nitric oxide traps, which made it possible to achieve dramatic increases in the yields of EPR-detectable MNIC–MGD complexes (49). However, the use of higher concentrations of these complexes is undesirable, since they can provoke toxic effects. Thus, MGD hydrolysis is associated with the formation of a highly toxic compound, viz., carbon disulfide.

The use of water-insoluble MNIC-forming traps, e.g., iron–DETC complexes, localized in hydrophobic compartments of cell membranes is a more promising approach to quantitative determination of NO in animal cells and tissues. An obvious advantage of MNIC–MGD complexes is that they are not excreted from the organism with urine, but are accumulated in the organism. For example, in the mouse liver their concentration does not change for at least one hour (5,67). The content of MNIC–DETC in the livers of LPS-treated mice can reach 1 micromole/g of wet tissue, while their total content in all body organs is commensurate with those of nitrite and nitrate, the oxidation products of endogenously produced NO in the urine of LPS-treated mice to whom iron-dithiocarbamate complexes were not given (67, 68). This prompts a conclusion that specific localization of

MNIC–DETC complexes in hydrophobic zones underlies their effective protection against superoxide-induced damage, since these zones are inaccessible for superoxide anions. These data altogether suggest that the decay of DETC molecules is the only reason for MNIC–DETC degradation.

In conclusion it may be said that MNIC–dithiocarbamate complexes are formed both upon binding of NO to iron–dithiocarbamate complexes and by the reaction of the latter with endogenous S-nitrosothiols and DNIC resulting in the formation of NO depots (1, 27, 52, 69). However, taking into account the extremely small size of NO depots (70,71), the contribution of S-nitrosothiols and DNIC to MNIC production is negligibly small in comparison with NO produced by NO synthases. As regards the interaction of iron–dithiocarbamate complexes with nitrites, which results in the recovery/reduction of the protonated form of the nitrite by these complexes with subsequent formation of MNIC, this process occurs at a very low rate and does not play any conspicuous role in MNIC formation in animal cells and tissues (47).

## 7. ACKNOWLEDGMENT

The work was supported by the Russian Foundation of Basic Researches (Grant 07-04-13506 of c).

## 8. REFERENCES

1. Vanin, A.F., Mordvintcev, P.I. & A.L. Kleschyov: Appearance of nitric oxide in animal tissues. *Studia Biofizica* 102, 135-143 (1984)
2. Paschenko, S.V., Khramtsov, V.V., Skatchkov, M.P., Plyusnin, V.F. & E. Bassenge: EPR and laser flash photolysis studies of the reaction of nitric oxide with water soluble NO trap Fe (II)- proline-dithiocarbamate complex. *Biochem Biophys Res Comm* 225, 577-584 (1996)
3. Fujii, S., Kabayashi, K., Tagawa, S. & T. Yoshimura: Reaction of nitric oxide with Fe (III) complex of N-(dithiocarboxy)-sarcosine: a new type of reductive nitrosylation involving iron (II) as an intermediate. *J Chem Soc Dalton Trans*, 3310-3315 (2000)
4. Mordvintcev, P., Mulsch, A., Busse, R. & A. Vanin: On-line detection of nitric oxide formation in liquid aqueous phase by electron paramagnetic resonance spectroscopy. *Anal Biochem* 199, 142-146, (1991)
5. Kubrina, L.N., Caldwell, W.S., Mordvintcev, P.I., Malenkova, I.V. & A.F. Vanin: EPR evidence for nitric oxide production from guanidine nitrogen of L-arginine in animal tissue *in vivo*. *Biochim Biophys Acta* 1099, 233-237 (1992)
6. Mulsch, A., Vanin, A., Mordvintcev, P. & R. Busse: NO accounts completely for the oxygenated nitrogen

## Nitric oxide, spin trapping, iron dithiocarbamate

species generated by enzymic L-arginine oxygenation. *Biochem. J.* 288, 597-603 (1992).

7. Voevodskaya, N.V. & A.F. Vanin: Gamma-irradiation potentiates L-arginine-dependent nitric oxide formation in mice. *Biochem Biophys Res Comm* 186, 1423-1428 (1992)

8. Mulsch, A., Mordvintcev, P.I., Vanin, A.F. & R. Busse: Formation and release of dinitrosyl iron complexes by endothelial cells. *Biochem Biophys Res Comm* 196, 1303-1308 (1993)

9. Vanin, A.F., Mordvintcev, P.I., Hauschildt, S. & A. Mulsch: The relationship between L-arginine-dependent nitric oxide synthesis, nitrite release and dinitrosyl-iron complex formation in activated macrophages. *Biochim Biophys Acta* 1177, 37-42 (1993)

10. Sato, S., Tominaga, T., Ohnishi, T. & S.T. Ohnishi: Electron paramagnetic study of nitric oxide production during brain local ischemia and reperfusion in the rat brain. *Brain Res* 647, 91-96 (1994)

11. Wizeman, T.M., Gardner, C.R., Laskin, J.D., Quinones, S., Durham, S.K., Goller, N.L., Ohnishi, S.T. & D.L. Laskin: Production of nitric oxide and peroxynitrite in the lung during acute endotoxemia. *J Leukol Biol* 56, 759-768 (1994)

12. Kotake, Y., Tonigawa, T., Tonigawa, M. & I. Ueno: Evaluation of nitric oxide traps: solubility on water, trapping efficiency and spin adduct stability. *Magn Res Med* 6, 106-108 (1995)

13. Hooper, D.C., Ohnishi, S.T., Kean, R., Numagami, Y., Dietzschold, B. & H. Koprowski: Local nitric oxide production in viral and autoimmune diseases of central nervous system. *Proc Natl Acad Sci (USA)* 92, 5312-5316 (1995)

14. Kuppusamy, P., Ohnishi, S.T., Namagami, Y., Ohnishi, T. & J.L. Zweier: Three dimensional imaging of nitric oxide production in the rat brain subjected to ischemia-reperfusion. *J Cerebral Blood Flow Metab* 15, 899-903 (1995)

15. Yoshimura, T., Fujii, S., Yokoyama, H. & H. Kamada: *In vivo* electron paramagnetic resonance imaging of NO-bound iron complexes in a rat head. *Chem Lett* 8, 309-310 (1995)

16. Doi, K., Akaike, T., Horie, H., Noguchi, Y., Fujii, S., Beppu, T., Ogawa, M. & H. Maeda: Excessive production of nitric oxide in rat solid tumor and its implication in rapid tumor growth. *Cancer Supplement* 77, 1598-1604 (1996)

17. Kotake, Y., Tonigawa, T., Tonigawa, M., Ueno, I., Allen, D.R. & C.-S. Lai: Continuous monitoring of

cellular nitric oxide generation by spin trapping with an iron dithiocarbamate complex. *Biochim Biophys Acta* 1289, 362-368 (1996)

18. Akaike, T., Noguchi, Y., Setoguchi, K., Suga, M., Zheng, Y.M., Dietzschold, B. & H. Maeda: Pathogenesis of influenza-virus-induced pneumonia: involvement of nitric oxide and oxygen radicals. *Proc Natl Acad Sci USA* 93, 2448-2453 (1996)

19. Kalyanaraman, B: Detection of nitric oxide by electron spin resonance in chemical, photochemical, cellular, physiological and pathophysiological systems. *Methods Enzymol* 268, 168-187 (1996)

20. Tsushia, K., Tagasugi, M., K. Minakuchi, K. & K. Fukuzawa: Sensitive quantitation of nitric oxide EPR spectroscopy. *Free Rad Biol Med* 21, 733-737 (1996)

21. Setoguchi, K., Takeya, M., Akaike, T., Suga, M., Hattori, R., Maeda, H., Ando, M. & K. Takashashi: Expression of inducible NO synthase and its involvement in pulmonary granulomatous inflammation in rats. *Am J Pathol* 149, 2005-2022 (1996)

22. Wallis, G., Brackett, D., Lerner, M., Kotake, Y., Bolli, R. & P. McCay: *In vivo* spin trapping of nitric oxide generated in the small intestine, liver, and kidney during the development of endotoxemia: a time course study. *Shock* 6, 274-278 (1996)

23. Henry, Y.A., Guissani, A. & B. Ducastel: Nitric oxide research from chemistry to biology: EPR spectroscopy of nitrosylated compounds. Austin Texas USA; Landes, RG (1997)

24. Vanin, A.F. & A.L. Kleschyov: EPR studies and biological implications of nitrosyl nonheme complexes. In *Nitric Oxide in Transplant Rejection and Anti-Tumor Defense* (S. Lukiewicz, J. Zweier, Eds.) Kluwer Acad. Publishers, Amsterdam, 49-82 (1998)

25. Cammack, R., Shergil, J.K., Inaslingh, V.A. & M.N. Hughes: Applications of electron paramagnetic resonance spectroscopy to study interactions of iron proteins in cells with nitric oxide. *Spectrochimica Acta Part A* 54, 2393-2402 (1998)

26. Rosen, G.M., Britigam, B.E., Halpern, H. J. & S. Pou: *Free radicals: Biology and Detection by Spin Trapping*, New York, Oxford University Press, 400-403 (1996)

27. Vanin, A.F.: Iron diethyldithiocarbamate as a spin trap for nitric oxide detection. *Methods Enzymol* 301, 269-279 (1999)

28. Henry, Y. & A. Guissani: Contribution of spin-trapping EPR techniques for the measurement of NO

## Nitric oxide, spin trapping, iron dithiocarbamate

- production in biological systems. *Analysis* 28, 445-453 (2000)
29. Kozlov, A.V., Sobhian, R., Constantino, G., Nohl, H., Redl, H. & S. Bahrami : Experimental evidence suggesting that nitric oxide diffuses from tissue into blood but not from blood into tissue. *Biochim Biophys Acta* 1536, 177-184 (2001)
30. Nagano, T. & T. Yoshimura: Bioimaging of nitric oxide. *Chem Rev* 102, 1235-1269 (2002)
31. Vanin, A.F. , Huisman, A. & E. van Faassen: Iron dithiocarbamates as spin traps for nitric oxide: pitfalls and successes. *Methods Enzymol* 359, 27-42 (2002)
32. Vanin, A., Poltorakov, A., Mikoyan, V., Kubrina, L. & E. van Faassen: Why iron-dithiocarbamates ensure detection of nitric oxide in cells and tissues. *Nitric Oxide: Biol Chem* 15, 295-311 (2006)
33. Charlier, N., Preat, V. & B. Gallez : Evaluation of lipid - based carrier systems and inclusion complexes of diethyldithiocarbamate-iron to trap nitric oxide in biological systems. *Magn Res Med* 55, 215-218 (2006)
34. Vanin, A.F. & E. van Faassen: Mononitrosyl iron complexes with dithiocarbamate ligands: Physico-chemical properties. In *Radicals for Life: The Various Forms of Nitric Oxide* (E.van Faassen, A.Vanin, Eds.) Elsevier Press,383-405 (2007)
35. Komarov, A., Mattson, D., Jones, M.M., Singh, P.K. & C.-S. Lai: *In vivo* spin trapping of nitric oxide in mice. *Biochim Biophys Res Comm* 195, 1191-1198 (1993)
36. Lai, C.-S. & A.M. Komarov: Spin trapping of nitric oxide produced *in vivo* in septic-shock mice. *FEBS Lett* 345, 120- 124 (1994)
37. Komarov, A.M. & C.-S. Lai: Detection of nitric oxide production in mice by spin - trapping electron paramagnetic resonance spectroscopy. *Biochim Biophys Acta* 1272, 29-36 (1995)
38. Kotake, Y., Tonigawa, T., Tonigawa, M. & I. Ueno: Spin-trapping isotopically - labeled nitric oxide produced from (15N)L-arginine and (18O)dioxygen by activated macrophages using a water soluble Fe<sup>2+</sup>-dithiocarbamate spin trap. *Free Rad Biol Med* 23, 287-295 (1995)
39. Zweier, J.L., Wang, P., Samouilov, A. & P. Kuppusamy: Enzyme - independent formation of nitric oxide in biological issues. *Nature Med* 1, 804-809 (1995)
40. Fujii, H., Koszelnik, J. & L. Berliner: Determination and characterization of nitric oxide generation in mice by *in vivo* L-band EPR spectroscopy. *Magn Res Med* 38, 565-567 (1997)
41. Xia, Y. & J.L. Zweier: Direct measurement of nitric oxide generation from nitric oxide synthase. *Proc Natl Acad Sci USA* 94, 12705-12710 (1997)
42. Norby, W.B., Weyhenmyer, J.A. & R.B. Clarkson: Stimulation and inhibition of nitric oxide production in macrophages and neural cells as observed by spin trapping. *Free Rad Biol Med* 22 , 1-9 (1997)
43. Mikoyan, V.D., Kubrina, L.N., Serezhnikov, V.A., Stukan, R.A. & A.F. Vanin: Complexes of Fe<sup>2+</sup> with diethyldithiocarbamate or N-methyl-D-glucamine dithiocarbamate as traps of nitric oxide in animal tissues: Comparative studies. *Biochim Biophys Acta* 1336 , 225-234 (1997)
44. Samouilov, A., Kappusamy, P. & J.L. Zweier: Evaluation and the magnitude and rate of nitric oxide production in biological systems. *Arch Biochem Biophys* 357, 1-7 (1998)
45. Kotake, Y., Moore, D.N., Sang, H. & L.A. Reinke: Continuous monitoring of *in vivo* nitric oxide formation using EPR analysis in billiar flow. *Nitric Oxide: Biol Chem* 3 , 114-122 (1999)
46. Piper, G.M. & C.-S. Lai: Biological evaluation of the nitric oxide - trapping agent, N - methyl - D-glucamine dithiocarbamate-Fe<sup>2+</sup>, as a probe of nitric oxide activity released from control and diabetic rat endothelium. *Jpn J Pharmacol* 80, 359- 370 (1999)
47. Tsuchya, K. , Yoshizumi, M., Houchi, H. & R.P. Mason: Nitric oxide-forming reaction between the iron-N-methyl-D- glucamine dithiocarbamate and nitrite. *J Biol Chem* 275, 1551-1556 (2000)
48. Nakagawa, H., Ikota, N., Ozawa, T. & Y. Kotake: Dose- and time-dependence of radiation-induced nitric oxide formation in mice as quantified with electron paramagnetic resonance. *Nitric Oxide: Biol Chem* 5, 47-52 (2001)
49. Lecour, S., Maupoil, V., Zeller, M., Laubriet, A., Briot, F. & L. Rochette: Levels of nitric oxide in the heart after experimental myocardial ischemia. *J Cardiovasc Pharmacol* 37, 55-63 (2001)
50. Vanin, A.F., Huisman, A., Stroes, E.S.G., de Ruijter-Heijstek, F.C., Rabelink, T.J. & E.E. van Faassen: Antioxidant capacity of mononitrosyl-iron- dithiocarbamate: Implication for nitric oxide trapping. *Free Rad Bio Med* 30, 813- 824 (2001)

## Nitric oxide, spin trapping, iron dithiocarbamate

51. Komarov, A.M. : *In vivo* detection of nitric oxide distribution in mice. *Mol Cell Biochem* 234/235, 387-392 (2002)
52. Tsuchiya, K., Kirima, K., Yoshizumi, M., Houchi, H., Tamaki, T. & R.P. Mason: The role of thiol and nitrosothiol compounds in the nitric oxide-forming reactions of the iron-N-methyl-D-glucamine dithiocarbamate complex. *Biochem J* 367, 771- 779 (2002)
53. Yasui, H., Fujii, S., Yoshimura, T. & H. Sakurai: Spinnokinetics analysis of blood disposition and biliary excretion of nitric oxide (NO)-Fe (II)-N-(dithiocarboxy)sarcosine complex in rats: BCM-ESR and BEM-ESR studies. *Free Rad Biol Med* 38, 1061-1072 (2004)
54. Takahama, U., Hirota, S. & T. Oniki: Production of nitric oxide-derived reactive nitrogen species in human oral cavity and their scavenging by salivary redox components. *Free Rad Res* 39, 737-745 (2005)
55. Meister, A., Anderson, M.E. & Q. Hwang: Intracellular cysteine and glutathione delivery system. *J. Am. College of Nutrition* 5, 137-151 (1986)
56. Fujii, S., Yoshimura, T. & H. Kamada: Nitric oxide trapping efficiencies of water-soluble iron (III) complexes with dithiocarbamate derivatives. *Chem Lett*, 785-786 (1996)
57. Vanin, A.F., Liu, X., Samouilov, A., Stukan, R.A. & J.L. Zweier: Redox properties of iron-dithiocarbamates and their nitrosyl derivatives: implications for their use as traps of nitric oxide in biological systems. *Biochim Biophys Acta* 1474, 365-377 (2000)
58. Vanin, A.F., Bevers, L., Mikoyan, V.D., Poltorakov, A.P., Kubrina, L.N. & E. van Faassen: Reduction enhances yields of nitric oxide trapping by iron- dithiocarbamate complex in biological systems. *Nitric Oxide: Biol Chem* 16, 71- 81 (2007)
59. Pasek, E.A. & D.K. Straub: Tris (N,N-disubstituted dithiocarbamate)iron (IY)- tetra-fluoroborates. *Inorg Chem* 11, 259-263 (1972)
60. de Vries, J.L.K.R., Trooster, J.M. & E. de Boer: Iron (II) complexes with two and three dialkyldithiocarbamate ligands. Mossbauer and electronic spectra. *Inorg Chem* 12, 2730-2733 (1973)
61. Kleschyov, A., Mollnau, H., Oelze, M., Meinertz, T., Huang, Y., Harrison, D. & T. Munzel: Spin trapping of vascular nitric oxide using colloid Fe (II)-diethyldithiocarbamate. *Biochim Biophys Res Comm* 275, 672-677 (2000)
62. Suzuki, Y., Fujii, S., Tominaga, T., Yoshimura, T. & H. Kamada: The origin of an EPR signal observed in dithiocarbamate loaded tissues: Copper (II)- dithiocarbamate complexes account for the narrow hyperfine lines. *Biochim Biophys Acta* 1335, 242-245 (1997)
63. Heikkila, R., Cabbat, F. & G. Cohen: *In vivo* inhibition of superoxide dismutase in mice by diethyldithiocarbamate. *J Biol Chem* 251, 2182-2185 (1976)
64. Vanin, A.F., Svistunenko, D.A., Mikoyan, V.D., Serezhenkov, V.A., Fryer, M.J., Baker, N.R. & C.E. Cooper: Endogenous superoxide production and the nitrite/nitrate ratio control the concentration of bioavailable free nitric oxide in leaves. *J Biol Chem* 279, 24100-24107 (2004)
65. Lamb, C. & R.A. Dixon: The oxidative burst in plant disease resistance. *Annu. Rev. Plant Physiol. Plant Mol Biol* 48, 251-275 (1997)
66. Serezhenkov, V.A., Timoshin, A.A., Orlova, T.R., Mikoyan, V.D., Kubrina, L.N., Poltorakov, A.P., Ruuge, E.K., Sanina, N.A. & A.F. Vanin: Decomposition of water-soluble mononitrosyl iron complexes with dithiocarbamates and of dinitrosyl iron complexes with thiol ligands in animal organisms. *Nitric Oxide, Biol.Chem.* 18 195-203. (2008)
67. Kubrina, L.N., Mikoyan, V.D., Mordvintcev, P.I. & A.F. Vanin: Iron potentiates lipopolysaccharide-induced nitric oxide formation in animal organs. *Biochim Biophys Acta* 1176, 240-244 (1993)
68. Jaeschke, H., Shini, V.B. & A. Farhood: Role of nitric oxide in the oxidant stress during ischemia/reperfusion injuring of the liver. *Life Sci Pharmacol Lett* 50, 1797-1804 (1992)
69. Vanin, A.F., Papina, A.A., Serezhenkov, V.A. & W.H. Koppenol: The mechanisms of S-nitrosothiol decomposition catalyzed by iron. *Nitric Oxide: Biol Chem* 10, 60-73 (2004)
70. Mikoyan, V.D., Serezhenkov, V.A., Brazhnikova, N.V., Kubrina, L.N., Khachatryan, G.N. & A.F. Vanin: Formation of paramagnetic nitrosyl complexes of nonheme iron in the animal organism with the participation of nitric oxide from exogenous and endogenous sources. *Biopfizica (Rus)* English translation, 41, 110-116 (2004)
71. Rodriguez, J., Maloney, R.E., Rassaf, T., Bryan, N.S. & M. Feelish: Chemical nature of



**Nitric oxide, spin trapping, iron dithiocarbamate**

nitric oxide storage forms in rat vascular tissue. *Proc Natl Acad Sci USA* 100, 336-341 (2003)

**Key Words:** NO, iron dithiocarbamate complexes, EPR, Review

**Send correspondence to:** Anatoly Vanin, Semyonov Institute of Chemical Physics, Russian Academy of Sciences, Moscow, Russia, Tel: 7-495-939-7535, Fax: 7-495-651-21-91, E-mail: vanin@polymer.chph.ras.ru

<http://www.bioscience.org/current/vol14.htm>