Bioorganic & Medicinal Chemistry Letters 19 (2009) 6092–6094

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Hydrogen sulfide induces nitric oxide release from nitrite

Loris Grossi *

Dipartimento di Chimica Organica 'A. Mangini', Università di Bologna, I-40136 Bologna, Italy

ARTICLE INFO

Article history:
Received 23 July 2009
Revised 8 September 2009
Accepted 9 September 2009
Available online 12 September 2009

Keywords:
Hydrogen sulfide
Gasotransmitter
Nitrite
Nitric oxide

ABSTRACT

Hydrogen sulfide has recently been considered to have an important role as a gasotransmitter in the cardiovascular system as well as in the central nervous system, but its action seems directly related to the presence of nitric oxide/nitric oxide-derivatives. We report here chemical evidence that emphasizes a prominent role of the hydrogen sulfide as cofactor of NO-derivatives in inducing nitric oxide release.

In the last decade, much attention has been devoted to the role of hydrogen sulfide (H_2S), in vivo, as a possible gasotransmitter,[1–3] and how it affects cardiovascular functions.[4,5] In the light of these stated roles, nowadays, the attention has been focalized on the possible synergy between H_2S and nitric oxide (NO); for example, the positive role of H_2S in improving the NO production from NO-releasers,[6] or the action of NO in inducing an increase in the amount of enzyme responsible of the H_2S production.[7] In fact, for several physiological processes the direct interaction between H_2S and NO is claimed, and hypothesized to lead to the formation of an *S*-nitrosothiol intermediate even if it has never been identified. In support of this, results obtained in vitro in the direct interaction between NO-gas and H_2S or in the incubation of an H_2S-donor, such as the sodium hydrosulfide (NaSH), with different NO-donors have been claimed.[8] The aim of this research has been to prove that the formation of NO in the interaction between the NaSH and the sodium nitrite ($NaNO_2$), an NO-donor, in buffer solutions at pH <7, can take place directly with no involvement of oxidants, as well as the release of NO from NO-derivatives, for example the sodium nitroprusside (SNP).

In our previous study[9] on the interaction between thiol derivatives and the sodium nitroprusside (SNP), we proved that thiol groups induce an Electron Transfer process leading to the formation of the corresponding *S*-nitrosothiol; a mechanism also invoked for metals participating in NO-mediated nitrosation chemistry. In fact, for NO–Fe^{III} (ferric) species with significant nitrosonium ion character, the possibility to react with nucleophiles, for instance thiols, for generating nitrosated nucleophiles, that is, the corresponding *S*-nitrosothiols, and a ferrous species was reported.[10] Moreover, we proved that *S*-nitrosothiols can spontaneously release NO via homolysis.[11] In the light of this evidence the mechanism of NO production from SNP was accounted for.[9] But, in a recent paper[8] on the interaction between NaHS and SNP, it has been reported that only a small quantity of NO can be released unless an oxidant is involved. This was in net contrast with our results,[9] and those in vivo that observed an enhancement, dose-dependent, of the vasorelaxant effect of SNP on rat aortic tissues when NaHS was added.[12]

To settle these discrepancies, a direct method for the NO detection instead of an indirect-method such as the Griess assay, unable to discriminate among different nitrite sources, was necessary. The EPR spectroscopy was able to fulfill this, and then experiments[26] between SNP and NaSH were carried out. The SNP-reduced radical, $[Fe^{II}(CN)_5(NO)]^{3-}$, $a_N = 1.49$ mT and $g = 2.0255$, was the only detectable species, Figure 1a. That confirmed the capability of sulfhydryl-containing molecules to induce the SNP reduction[13] without the intervention of any extra reagent.[14] But, it was still necessary to verify if an oxidant species was needed for inducing NO release from the likely *S*-nitrosothiol intermediate, as hypothesized in the literature.[8,15] Experiments[26] with equimolar amounts of NaHS and SNP, in the presence of an efficient NO trap such as the iron(II) *N*,*N*-diethyldithiocarbamate, [$Fe(DETC)_2$], were carried out. The formation of the corresponding paramagnetic nitrosyl derivative, (Scheme 1), was clearly shown by EPR spectroscopy, [NO–$Fe(DETC)_2$], $a_N = 1.28$ mT and g = 2.039, Figure 1b.

In the light of these results, it is impossible to accept the mechanism reported in the literature. In fact, it is a reducing agent and not an oxidant that can induce such a process. Moreover, the claim of the direct detection of the NO radical by EPR spectroscopy is also unverifiable:[8] experiments with such a goal cannot be conducted for technical reasons.[16] However, it is necessary to underline that

* Tel.: +39 051 2093627; fax: +39 051 2093654.
E-mail address: loris.grossi@unibo.it

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.09.030

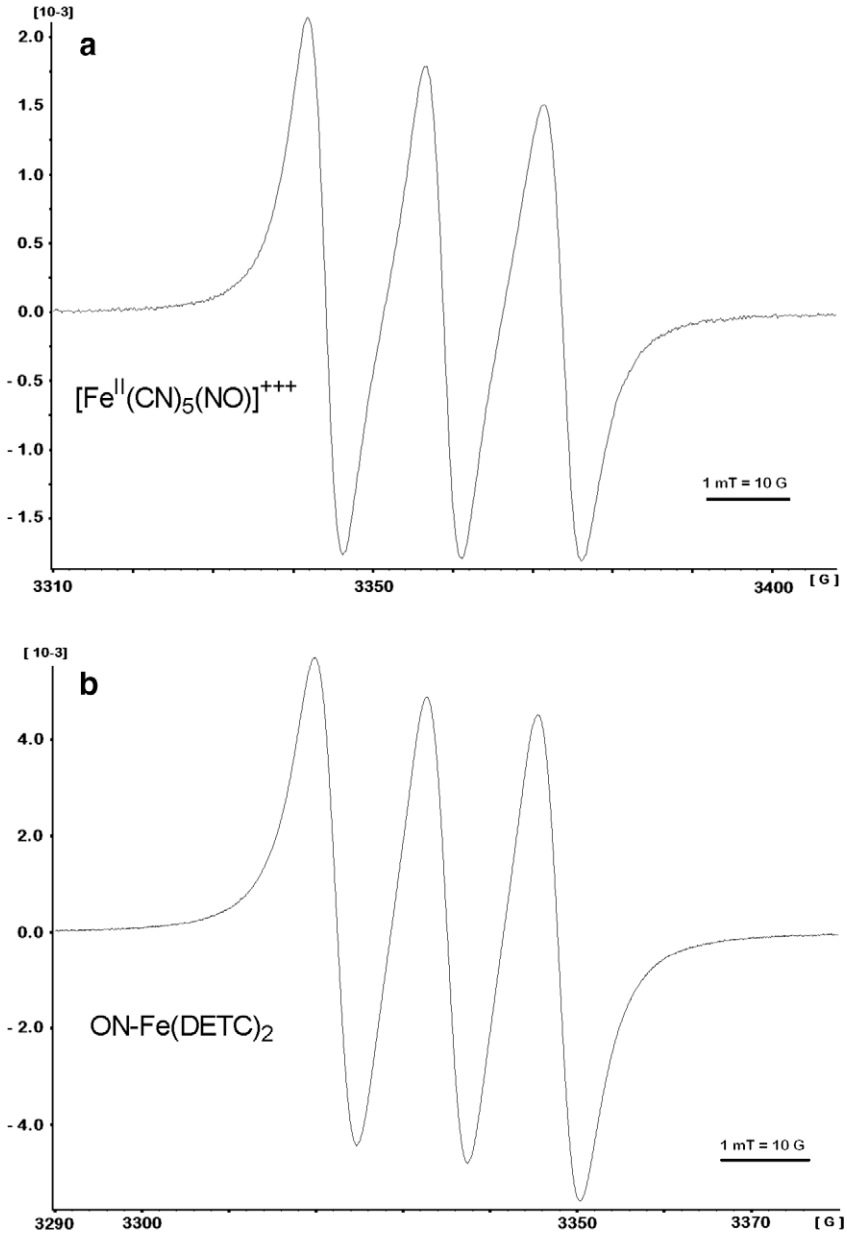
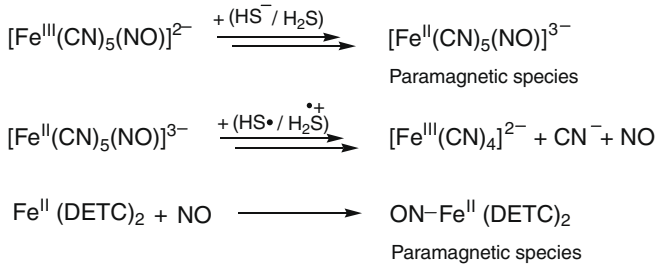


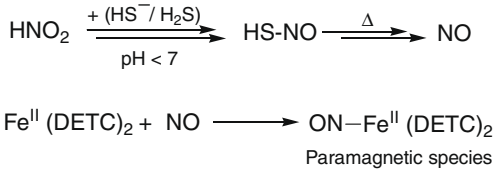
Figure 1. EPR spectra. Scale: 1 mT = 10 G. (a) Radical deriving from the SPN reduction induced by NaHS. (b) Paramagnetic NO adduct to the $Fe(DETC)_2$.



Scheme 1. pH-independent reduction of SNP by HS^- and/or H_2S; trapping of the spontaneous released NO.

the *S*-nitrosothiol intermediate hypothesized by the authors[8] is reasonable, since we have detected the formation of the *S*-nitrosogluthatione (GS–NO) in the interaction between the glutathione and the SNP;[9] but the S–N bond which characterizes these species is known to be very weak, and undergoes a rapid and spontaneous homolytic cleavage.[11] In particular, in the interaction between H_2S and SNP, the expected intermediate should be the thionitrous acid (HS–NO), a well-known unstable molecule.[11,17]

Our experiments stressed the role of the H_2S/HS^- in inducing the NO release from an exogenous NO-releaser, but the key aspect to know was the reactivity of these species in comparison to endogenous NO and/or NO-releasers, that is, mimicking biological conditions. In fact, the reported[18] hypothesis of a direct interaction between NO and H_2S was in net contrast with the chemistry of NO, which allows only the interaction with radical species, or the coordination with metal ions. On the contrary, the interaction between H_2S and NO-derivatives, for instance oxidized species such as the nitrous acid (HNO_2) and/or the dinitrogen trioxide (N_2O_3), could account for the results reported in the literature. In particular, the nitrite, which is considered the main pool of NO in vivo,[19] could be the species involved. To verify this hypothesis, experiments[26] with equimolar amounts of NaHS and $NaNO_2$ were carried out.



Scheme 2. pH-dependent reduction of the nitrite by HS^- and/or H_2S; trapping of the spontaneous released NO.

Unfortunately, no paramagnetic species were detected. Nevertheless, it is known that the nitrite reduction can take place through its *aci*-form,[20,21] (HNO_2), but in these experiments, due to the hydrolysis of both reagents, the pH increases to ca. 10 and, the concentration of HNO_2 becomes very low; consequently the reductive-release of the nitric oxide was hampered Scheme 2.

Experiments were then repeated in buffer solution at pH <7.[22] In these conditions a very intense EPR signal, due to the NO–Fe(-DETC)$_2$ radical, a_N = 1.28 mT and g = 2.039, was immediately detected Figure 1b. Moreover, lengthening the experiment at pH 6.15 for 48 h, a yellow precipitate of elementary sulfur (S_8) was recovered from the aqueous solution.[23] These results definitely demonstrated the straightforward reducing capability of HS^-/H_2S in comparison to the nitrite, and how it depends on the pH of the solution; but, they weakened the hypothesis of H_2S acting as a direct gasotransmitter, a characteristic which had already been claimed to depend on the interaction with NO-releasers.[24]

Actually, the role of H_2S as a gasotransmitter is usually supported by evidence based on final observations, that is, cause-and-effect, but no mechanism of the interaction between H_2S and NO-derivatives has ever been reported. Therefore, a careful examination of the role of H_2S, for example in some vascular diseases such as the hypertension, or its cardio protective effect in ischemic myocardium, as well as its action in septic and endotoxin shock,[25] can lead to a different outcome. In fact, all these pathologies are characterized by blood-acidity, that is, pH-values lower than the physiological, and that creates the right conditions for the H_2S/HS^- to act as reducing agent in comparison to the nitrite, whose acid equilibrium has now fairly shifted towards the *aci*-form, HNO_2, that is, the species able to undergo the reductive-release of NO.

In conclusion, the role of the H_2S as a possible direct gasotransmitter is still far from being proved. In the literature the chemical conditions, in particular the pH of the medium in which the bio-chemical processes occur, have usually not being carefully taken into consideration. On the contrary, it seems definitely conceivable to consider H_2S a cofactor of NO-releasers in inducing the formation of free nitric oxide, the proven neurotransmitter, and the nitrite the species most probably involved in vivo.

Acknowledgment

This work was financially supported by MIUR, Rome, Funds PRIN 2006.

References and notes

1. Qu, K.; Chen, C. P. L. H.; Halliwell, B.; Moore, P. K.; Wong, P. T.-H. *Stroke* **2006**, *37*, 889.
2. Wang, R. *Antioxid. Redox Signal.* **2003**, *5*, 493.
3. Yang, G.; Wu, L.; Jiang, B.; Yang, W.; Qi, J.; Cao, K.; Meng, Q.; Mustafa, A. K.; Mu, W.; Zhang, S.; Snyder, S. H.; Wang, R. *Science* **2008**, *322*, 587.
4. Chen, C. Q.; Xin, H.; Zhu, Y. Z. *Pharmacol. Sin.* **2007**, *28*, 1709.
5. O'Sullivan, S. E. *Br. J. Pharmacol.* **2006**, *149*, 609.
6. Kamoun, P. *Amino Acids* **2004**, *26*, 243–254.
7. Zhao, W.; Zhang, J.; Lu, Y.; Wang, R. *EMBO J.* **2001**, *20*, 6008.
8. Whiteman, M.; Ling, L.; Kosteski, I.; Chu, S. H.; Siau, J. L.; Bhatia, M.; Moore, P. K. *Biochem. Biophys. Res. Commun.* **2006**, *343*, 303.
9. Grossi, L.; D'Angelo, S. *J. Med. Chem.* **2005**, *48*, 2622.
10. Wade, R. S.; Castro, C. E. *Chem. Res. Toxicol.* **1990**, *3*, 289.
11. Grossi, L.; Montevecchi, P. C. *Chem. Eur. J.* **2002**, *8*, 380.
12. Hosoki, R.; Matsuki, N.; Kimura, H. *Res. Commun.* **1997**, *237*, 527.
13. Koenitzer, J. R.; Isbell, T. S.; Patel, H. D.; Benavides, G. A.; Dickinson, D. A.; Patel, R. P.; Darley-Usmar, V. M.; Lancaster, J. R.; Doeller, J. E.; Kraus, D. W. *Am. J. Physiol. Heart Circ. Physiol.* **2007**, *292*, 1953.
14. (a) Actually, the reaction between SNP and thiols was described several decades ago, and used as a test for their identification: Scagliarini, G. Atti V Congr. Naz. Chim. Pure Appl., Rome, 1935; (b) Swinehart, J. H. *Coord. Chem. Rev.* **1967**, *2*, 385.
15. Chvanov, M.; Gerasimenko, O. V.; Petersen, O. H.; Tepikin, A. V. *EMBO J.* **2006**, *25*, 3024.
16. Bershon, M.; Baird, J. C. In *An Introduction to Electron Paramagnetic Resonance*; Benjamin: New York, 1966; p 129.
17. Lai, C.-H.; Li, E. Y.; Chou, P.-T. *Theor. Chem. Acc.* **2007**, *117*, 145.
18. Ali, M. Y.; Ping, C. Y.; Mok, Y.-Y. P.; Ling, L.; Whiteman, M.; Bhatia, M.; Moore, P. K. *Br. J. Pharmacol.* **2006**, *149*, 625.
19. Lundberg, J. O.; Weitzberg, E.; Gladwin, M. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 156.
20. Grossi, L. *EPA/600/R-07/010* (EPA), Washington, DC, 2007, 27, 89.
21. Grossi, L. *J. Med. Chem.* **2008**, *51*, 3318.
22. A control-experiment[26] with $NaNO_2$ and NaSH was conducted in buffer solution at pH 7. The EPR signal due to the NO–Fe(DETC)$_2$ radical was still detectable, but just slightly; it confirmed that the NO-release depends on the concentration of the *aci*-form (HNO_2). A second experiment was conducted in buffer solution at pH 6.4, without adding NaSH, to verify if the NO-release takes place through a reductive process or via a homolytic process. A very weak EPR signal due to the NO–Fe(DETC)$_2$ radical was detectable, but only after more than one hour of N_2 bubbling, that is, trapping all the nitric oxide produced in that elapse of time. Actually, we think this is not a spontaneous NO release but, most probably, a side process induced by impurities. Finally, running an experiment with $NaNO_2$ and NaSH in buffer solution at pH 3 (of course a non-physiological value), a fast production of NO is evidenced.
23. Mahmood, Q.; Zheng, P.; Cai, J.; Wu, D.; Hu, B.; Li, J. *J. Hazard. Mater.* **2007**, *147*, 249.
24. Moore, P. K.; Bhatia, M.; Moochhala, S. *Trends Pharmacol. Sci.* **2003**, *24*, 609.
25. Hui, Y.; Du, J.; Tang, C.; Bin, G.; Jiang, H. *J. Infect.* **2003**, *47*, 155.
26. All experiments were performed at room temperature (~23 °C). To detect the $[Fe^{II}(CN)_5(NO)]^{3-}$ radical, experiments were conducted in water or in buffer solution, (pH 6.40 and 6.15), using an 'H-shaped' sample-tube to keep initially separate the two reactants (both 0.1 mmol). In particular, the water/buffer solution of NaHS and the SNP (powder) were placed in the two branches of the sample-tube, respectively; the solution was deoxygenated by the freeze–pump–thaw technique, and then the sample-tube sealed off. The reactants were then mixed and the sample immediately analyzed by the EPR spectrometer.
The experiments with $NaHS/NaNO_2$, as well as with NaHS/SNP, were conducted both in water and in buffer solution (pH 6.40 and 6.15). In particular, to detect the likely NO released, an apparatus formed by two porous-bottom flasks connected by means of a tygon-tube was utilized. The two flasks were filled with 150 mL of water, or buffer solution, and 100 mL of a CH_2Cl_2 solution of Fe(DETC)$_2$, the NO trap, (0.25 mmol), respectively. To remove the oxygen from both solutions, and keep the apparatus under inert atmosphere, the experiments were carried out under a continuous N_2-bubbling. In particular, the N_2 flowed at first through the aqueous solution and then through the CH_2Cl_2 solution, thus completing also the removal and transportation of the NO formed. The reactants, in equimolar amount (0.2 mmol), were added to the aqueous/buffer solution after at least 40 min of N_2-bubbling. Finally, aliquots of the CH_2Cl_2 solution were tested by EPR spectroscopy.