

Review

Detection of *S*-nitrosothiols in biological fluids: A comparison among the most widely applied methodologies[☆]

Daniela Giustarini^a, Aldo Milzani^b, Isabella Dalle-Donne^b, Ranieri Rossi^{a,*}

^a Department of Neuroscience, Pharmacology Section, Via A. Moro 4, University of Siena, 53100 Siena, Italy

^b Department of Biology, Via Celoria 26, University of Milan, 20133 Milan, Italy

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Abstract

Many different methodologies have been applied for the detection of *S*-nitrosothiols (RSNOs) in human biological fluids. One unsatisfactory outcome of the last 14 years of research focused on this issue is that a general consensus on reference values for physiological RSNO concentration in human blood is still missing. Consequently, both RSNO physiological function and their role in disease have not yet been clarified. Here, a summary of the values measured for RSNOs in erythrocytes, plasma, and other biological fluids is provided, together with a critical review of the most widely used analytical methods. Furthermore, some possible methodological drawbacks, responsible for the highlighted discrepancies, are evidenced. © 2006 Elsevier B.V. All rights reserved.

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Contents

1. Introduction	124
2. Formation and catabolism of <i>S</i> -nitrosothiols	125
3. Mechanism(s) of action of <i>S</i> -nitrosothiols	126
4. From NO to RSNO: what is the yield?	126
5. Quantitative analysis of <i>S</i> -nitrosothiols	126
5.1. General concerns	126
5.2. Sample manipulation and pre-analytical phase	128
5.3. The most common analytical procedures	128
5.3.1. Spectrophotometric detection	130
5.3.2. Fluorimetric detection	131
5.3.3. Chemiluminescence	131
5.3.4. Mass spectrometry	132
5.3.5. High-performance liquid chromatography	133
5.3.6. Other analytical procedures	134
6. Conclusions and future prospects	135
Nomenclature	136
Acknowledgement	137
References	137

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* Corresponding author. Tel.: +39 0577 234198; fax: +39 0577 234208.

E-mail address: ranieri@unisi.it (R. Rossi).

1. Introduction

The function of nitric oxide (NO) in the activation of guanylyl cyclase and in mediating vasorelaxation is now well-established,

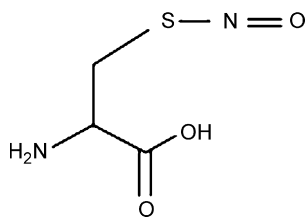


Fig. 1. Chemical formula for *S*-nitrosocysteine, the simplest low-molecular-mass *S*-nitrosothiol.

as also recognized by the award of the Nobel Prize to Furchgott, Ignarro and Murad in 1998 for their discovery about the actions of NO in the cardiovascular system. However, NO and its derivatives may be involved in a much broader range of pathophysiological actions [1]. NO is not detectable per se in human circulation at the basal state because of its short half-life (<0.1 s) in that it rapidly undergoes a series of chemical reactions with various types of biomolecules [2]. However, many storage forms of NO have been postulated to exist in mammalian tissues. Recent studies have focused on the role of intravascular NO-derived molecules that could stabilize NO bioactivity and contribute to blood flow and oxygen delivery [3]. Among these, a pool of derivatives of NO, i.e., *S*-nitrosothiols (RSNOs), where a nitroso moiety is covalently bound to the sulfhydryl group (SH) of proteins or low-molecular-mass (LMM) thiols, has been identified in various tissues (reviewed in Ref. [1]). Chemically, *S*-nitrosothiols are thioesters of nitrite with close analogy with nitrite esters of alcohols (Fig. 1). The evidence that RSNOs possess many of the biological activities of NO itself (e.g., in causing arterial and venous smooth muscle relaxation, in inhibiting platelet aggregation) by activating guanylyl cyclase has stimulated a wealth of research into the biological significance of these molecules. Since RSNOs are relatively stable and release NO under various conditions, they are considered to be a buffering system that controls intra- and extracellular functions of NO increasing its range of actions in terms of time and space [4].

2. Formation and catabolism of *S*-nitrosothiols

S-Nitrosothiols cannot be produced by the simple reaction of NO with free thiol groups. The formation of RSNOs observed in

oxygenated buffers [5] was supposed to be due to the oxidation of NO to dinitrogen trioxide (N_2O_3). This molecule can be produced in environments containing both NO and O_2 , and N_2O_3 is considered a good nitrosating agent, since it reacts with thiols at physiological pH to yield RSNOs and nitrite [1,6]. Differently from the gas phase, the formation of N_2O_3 from autooxidation of NO in aqueous phase is rather unlikely [6,7]. Thus, the mechanism(s) leading to the RSNO formation in vivo are still largely unknown. Nevertheless, in the presence of reduced thiols at mM-concentrations in incubates with nitric oxide synthase (NOS), formation of nM- to μ M-concentrations of RSNOs has been reported [8–10]. Moreover, intravenous infusion of aqueous solution of NO gas was shown to be accompanied by systemic and hemodynamic effects [11], which are mediated by the transport of NO stored as RSNOs, thus further suggesting an in vivo mechanism that can convert a percentage of NO into RSNOs. However, it is unclear what could be the chemical mechanism(s) involved. Among the various possible routes, catalysis due to metal ions in traces, which can serve as electron acceptors, has been hypothesized (Fig. 2) [1]. Alternatively, serum albumin has been shown to catalyze the production of *S*-nitrosothiols by oxidizing, within the protein hydrophobic core, NO to NO^+ , which is then transferred either to the sulfhydryl group of Cys34 of albumin itself or to other thiols (LMM or protein thiols) [4,12].

A controversial theory, in which hemoglobin (Hb) was suggested to be capable of preserving, transporting, and exporting NO activity through the formation of *S*-nitrosohemoglobin (Hb-SNO) as an intermediate, has been proposed for RSNO production in vivo. This theory suggests that NO binds rapidly to the heme group of deoxygenated Hb to form nitrosylhemoglobin (Fe-NO Hb). Once Fe-NO Hb is oxygenated (and undergoes a T to R transition), some of the NO on the heme group is transferred to the β -93 cysteine to form Hb-SNO [13,14]. Upon deoxygenation, some of the NO (actually NO^+) on the cysteine is transferred to erythrocytic glutathione or thiol groups on anionic exchanger (AE1) protein and, finally, it is exported from the red blood cell (RBC) to effect vasodilation, thus actively participating in the regulation of blood flow [15]. Nevertheless, the proposed mechanism seems to have numerous shortcomings, and many researchers in the field were unable to obtain the same results as originally proposed [16–20]. Finally, the lack of an allosterically

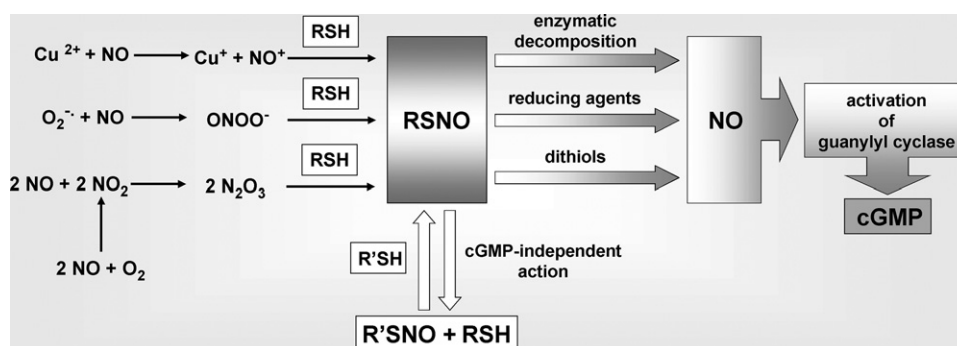


Fig. 2. Summary of proposed main pathways for the formation, decomposition and biological action of *S*-nitrosothiols (RSNOs). N_2O_3 , dinitrogen trioxide; NO_2 , nitrogen dioxide; NO^+ , nitrosonium ion; $ONOO^-$, peroxynitrite; RSH and R'SH, thiols; ($O_2^{\cdot-}$) superoxide anion.

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