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S-nitrosothiols

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Abbreviations

Cys-NO:	S-nitrosocysteine
GSNO:	S-nitrosoglutathione
N_2O_3:	dinitrogen trioxide
NO:	nitric oxide
NO^-:	nitroxyl anion
$RS^\bullet$:	thiyl radical
RSH:	thiol
RSNO:	S-nitrosothiol
RSSR:	thiol disulfide
SNAP:	S-nitroso-N-acetyl-DL-penicillamine

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Abstract:

S-nitrosothiols (RSNOs) are nitrosylated thiols that are supposed to act as a transport system for NO in the body. RSNOs have been studied extensively, but their chemical make-up makes them difficult to isolate *in vivo*. They are not very stable and they cleave homolytically forming a thiyl radical ($RS^{\bullet}$), and releasing $NO^{\bullet}$. They exhibit some antibacterial biological activity, play a role as muscle relaxants and have also exhibited anti-aggregatory effects on platelets. RSNOs have also been detected and other parts of the body, connecting these compounds to NO metabolism. They are believed to be mediators in signal transduction and ion channel regulation. Scientists, however, disagree on how RSNOs are formed *in vivo* and what their specific role is. Even though there is still much to be learned on the chemistry, metabolism and their specific role in the body, RSNOs are being investigated for possible future therapeutic applications.

Introduction:

S-nitrosothiols are a controversial species and, as such, have been the subject of much discussion in the scientific community. Their specific role in the body has been the subject of intensive research, yet scientists still are not sure what the function of S-nitrosothiols is. Some scientists believe that they play a role in the transport and regulation of NO in the body. Other scientists believe that more experimentation is needed to specifically determine their function. S-nitrosothiols are a group of organic compounds with the general formula RSNO where R- can be any protein or amino acid. Tasker and Jones were the first scientists to synthesize them in 1909 [1]. In 1974 it was shown that these compounds exert some antibacterial biological activity [1]. Ignarro *et al*. suggested that RSNOs played a role as muscle relaxants and have also exhibited anti-aggregatory effects on platelets. Stamler and his coworkers were the first to detect these compounds in plasma [1]. Derivatives of RSNOs have been detected in other parts of the body, connecting these compounds to NO metabolism. They are believed to be mediators in signal transduction and ion channel regulation; channels such as cyclic nucleotide-gated and calcium release channels (the latter in dogs only) have been associated with S-nitrosylation of thiol sites [12]. Some of the common RSNOs are GSNO, Cys-NO and SNAP [6].

Chemistry:

The formation of RSNOs is also known as an S-nitrosation reaction. The sulfur center in a thiol is exceedingly nucleophilic, making it a perfect candidate for undergoing nitrosation reactions [2]. Studies show that nitrosation reactions in a cell environment need a steady state level of NO. This steady level can be achieved by diffusion from other cells as well as intracellular formation, which is induced by the presence of extracellular RSNOs [3]. Studies

also show that the metabolism of RSNO in cells demands the presence of cysteine [3]. The formation of RSNOs is not just a simple reversible reaction between a thiol and $NO^{\bullet}$. This direct reaction is extremely slow and the products are disulfide and nitroxyl anion according to reaction **1**:

$$2NO^{\bullet} + 2RSH \rightarrow RSSR + 2NO^{-} + 2H^{+} \qquad \textbf{Reaction 1 [2]}$$

One possible mechanism that has been proposed for the formation of RSNOs is the reaction of NO in O_2 to produce N_2O_3 or some other form of NO_x, which then reacts with a thiol to give the desired RSNO. Nevertheless, it has been shown that the oxidation of NO with O_2 is slow under physiological conditions (3-300 pmol s^{-1} with a NO concentration of 0.1-1.0 μM) [4]. If the RSNOs are formed by the above mechanism, the formation of N_2O_3 is too slow to account for the disappearance of NO in the body [2]. It is believed that membranes (*i.e.* mitochondrial) are utilized in the body to catalyze the formation of N_2O_3 thus enhancing the production of RSNOs [2].

Another mechanism that has been proposed is the reaction of NO with a thiol to create as an intermediate the radical R-S-$N^{\bullet}$-O-H. In the presence of an oxidizing agent (*i.e.* O_2) this intermediate gets oxidized to give RSNO. The overall reaction is shown below [4]:

$$\text{R-SH} + NO^{\bullet} \leftrightarrow \text{R-S-}N^{\bullet}\text{-O-H} \qquad \textbf{Reaction 2}$$
$$\text{R-S-}N^{\bullet}\text{-O-H} + O_2 \rightarrow \text{R-S-N=O} + O_2^{\bullet -} \qquad \textbf{Reaction 3}$$
$$O_2^{\bullet -} + NO^{\bullet} \rightarrow ONOO^{-} \qquad \textbf{Reaction 4}$$
$$\text{R-SH} + 2NO^{\bullet} + O_2 \rightarrow \text{R-S-N=O} + ONO + H^{+} \qquad \textbf{Reaction 5} \quad [4]$$

Under physiological conditions, RSNOs stability varies with different factors (*i.e.* pH, redox state, the R- group on the thiol, and certain metals, in particular Cu^{+}) [1]. RSNOs *in vitro* are decomposed mainly by copper ions (which act as catalysts) to release NO. Reducing agents such as thiols and ascorbate can augment this decomposition [2]. Studies show that the decomposition of RSNOs and formation of NO are greatly mediated by the presence of Cu^{+} ion,

more so than the Cu^{2+} ion (see **Figure 2**) [6]. A general scheme of the decomposition of NO from RSNO is depicted below [5]:

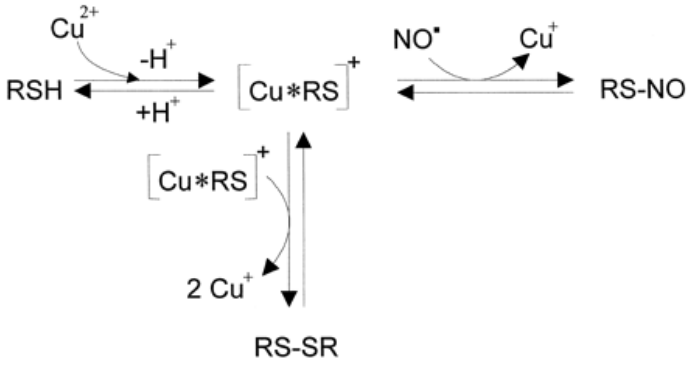

Figure 1. A general scheme of the decomposition of RSNO in the presence of Cu^{+} ions [5].

Below is a graph that relates the formation of NO during the breakdown of GSNO and its relationship to the metal ion present [6]:

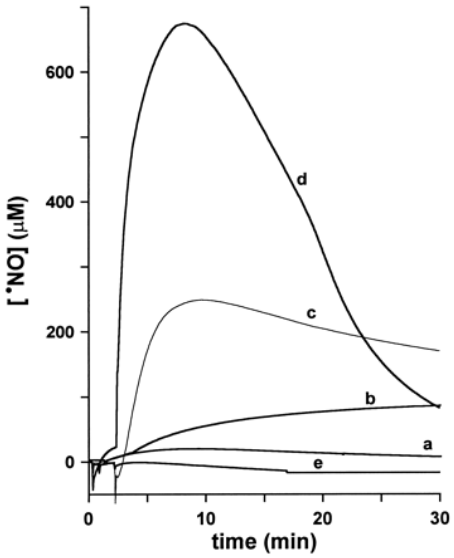

Figure 2. Release of NO during copper-catalyzed decomposition of *S*-nitrosoglutathione. There is a significant increase in the release of NO from the decomposition of GSNO: from GSNO alone (graph a) to GSNO in the presence of Cu^{+} ions (graph d). Graph b corresponds to GSNO in the presence of GSH, graph c corresponds to GSNO in the presence of Cu^{2+} ions [6].

In vivo, RSNOs are stabilized by the fact that there are few metals in a free state to coordinate to the nitrogen and the sulfur, causing them to decompose [1]. RSNOs are also involved in what is called a trans-S-nitrosation reaction, which involves the exchange of an NO group from one thiol to another. The NO group can be transferred from more stable RSNOs,

such as GSNO or SNAP (S-nitroso-N-acetyl-DL-penicillamine), to other thiols like cysteine, forming less stable RSNOs that can decompose further [7].

Other forms of decomposition are known, including enzymatic decomposition, photochemical and thermal decomposition [7]. RSNOs are also believed to act as a buffering system for NO uptake and release [5].

How do RSNOs get incorporated into the cell? Studies have shown that it is not just a simple $NO^{\bullet}$ release, but requires some type of a receptor on the cell surface [3]. It seems that this uptake is mediated by the presence of cystine. One possible mechanism involves the reduction of cystine to cysteine, which then reacts with GSNO to produce S-nitrosocysteine (L-CysNO). L-CysNO is then transported into the cell by the L-amino acid transport system [11]. Shown below is a schematic mechanism of how RSNOs are transported into the cell [3]:

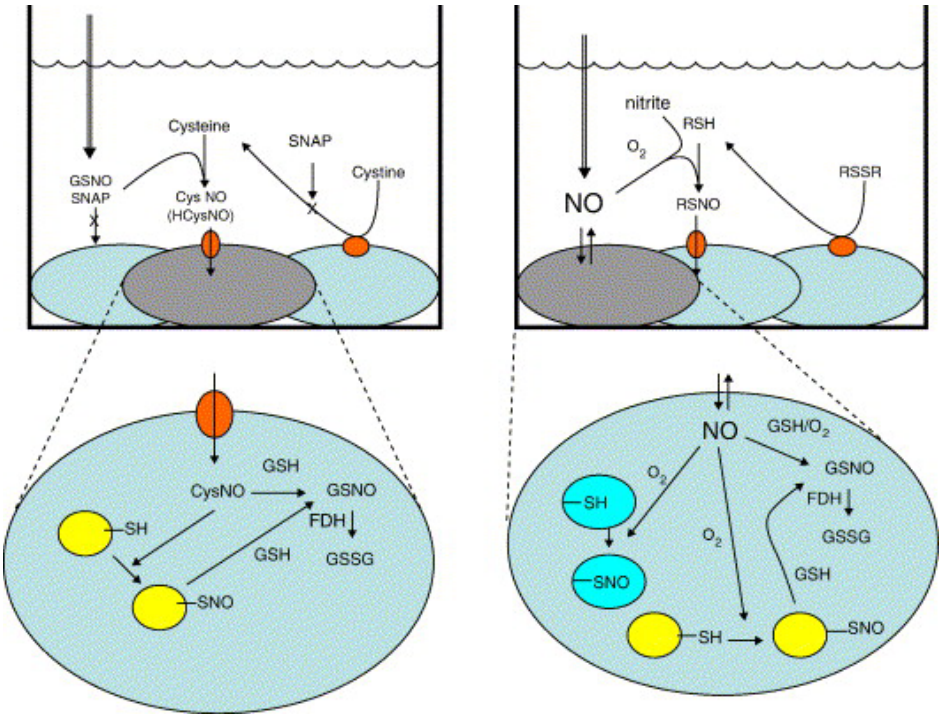

Figure 3. The transport and metabolism of nitric oxide and *S*-nitrosothiols in cells. (Left) CysNO and HCysNO are transported into cells via the L-AT, and perhaps other systems; SNAP and GSNO cannot enter cells and require conversion before uptake is possible [3].

In a UV- visible spectrum, S-Nitrosothiols have a peak that corresponds to ≈ 350 nm. The detection of these compounds by spectrophotometry is difficult, especially in physiological mediums, because their extinction coefficients are low (see **Table 1)** [1].

Table 1. (adapted from [1])

Nitrosothiol	$\lambda_{max\ 1}$ (nm)	$\lambda_{max\ 2}$ (nm)	$\varepsilon_{335\ nm}$ (M^{-1} cm^{-1})	$\varepsilon_{545\ nm}$ (M^{-1} cm^{-1})
S-nitrosocysteine	335	544	503	14.9
S-nitrosohomocysteine	331	545	606	16.7
S-nitrosoglutathione	335	544	586	17.2
S-nitroso-*N*-acetylpenicillamine	335	591	519	$\varepsilon_{591\ nm}$ = 7.0

Other methods have been developed such as photolysis and chemiluminescence along with electrochemical methods utilizing HPLC and capillary electrophoresis [1].

Detection:

The detection of RSNOs is difficult for two reasons. First, they are not very stable in visible light and decompose easily. Second, they are produced at such low concentrations that the detection method has to have a very low detection limit. One of these methods, called the biotin switch assay, was first developed by Jaffrey *et.al.* and has been utilized in order to detect S-nitrosated proteins *in vivo* [8]. The first step to this process is to get rid of endogenous thiols that would interfere. This is done by using methylmethanethiosulfonate (MMTS). The second step involves the reduction of the RSNO with ascorbate to a simple thiol and the third step would be to mark the formed thiol with biotin, which can be identified by an anti-body biotin [8]. Another method that has been utilized is spectrophotometry. RSNOs usually absorb between 320-360 nm and also at about 545 nm [1]. Refer to **Table 1**.

The drawbacks of this method are the low sensitivity (0.1-10 mM detection limits) and also that the RSNOs can decompose by irradiation with UV-Vis light [1]. Fluorometric detections of RSNOs have been developed as well. RSNOs are reacted with $HgCl_2$ and generate

HNO_2 (nitrous acid). HNO_2 proceeds to react with 2,3-diaminonaphthalene to produce a fluorophore. The signal difference is then measured and the concentration of the original RSNO is deduced [10].

Chemiluminescence in conjunction with photolysis has also been used. This method is becoming preferable because of its low detection limits. (detection limits are on the order of about 10^{-10} M) The RSNO sample undergoes photolysis and produces NO and the thiyl radical $RS^{\bullet}$ [1]. NO reacts with ozone to produce O_2 and an excited $NO_2^{\bullet}$ radical, which emits radiation and relaxes to its ground state. This radiation is captured by a photomultiplier tube and can be analyzed [1]. To make sure that RSNO is the only source of $NO^{\bullet}$ present, addition of Hg^{2+} ions is utilized. The Hg^{2+} ions react with RSNO to release NO. NO release measurements can be taken with and without Hg^{2+} present and the concentration of RSNO can be deduced [1]. Below is shown a standard calibration curve for the detection of an RSNO (specifically GSNO) in the presence and absence of Hg^{2+} ions [1]. Other methods used include: capillary zone electrophoresis, electrochemistry and electron paramagnetic resonance.

Applications:

Very few RSNOs are commercially available. The difficulty in synthesizing them as drugs rests in their rapid degradation in the presence of copper ions. Their potential applications are numerous and rely on their ability to release NO where needed. One potential application can be the inhibition of platelet deposition. It has been shown that poly-SNO-albumin has effectively bound itself to areas of injury and inhibited platelet deposition [9]. RSNOs are being investigated as replacement drugs for organic nitrates that are used to treat diseases such as ischemic heart disease and hypertension. The advantage of RSNOs is that the products formed after their decomposition are not harmful to the body. Low concentrations of RSNOs can be

used in contrast to organic nitrates [12]. RSNOs have been shown to relax smooth muscles in bronchia. In studies with asthma patients, RSNO levels were found to be lower than normal. They could be used as a potential treatment for patients with acute asthma [9]. RSNOs have also been shown to play a role in the immune system in the body against viruses and bacteria. Another application may be their use in curing bacterial or viral infections [9]. Care should be taken however, because there are side effects to treatment with RSNOs. Some of them include: hypotension and hemorrhage, altering the function of proteins that have thiol groups such as proteins in cell cycle and DNA repair mechanisms [9].

Conclusion:

RSNOs are thought of as facilitators of NO transport in the body. They have some biochemical activity in the body, but their chemical instability makes them difficult to study. Their exact mechanism of formation, NO transport and the effects they have in vivo are still under investigation. Scientists remain hopeful in the possibilities of future therapeutic applications of RSNOs.

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