Mechanism of Nitric Oxide Release from S-Nitrosothiols*

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S-Nitrosothiols have many biological activities and have been suggested to be intermediates in signal transduction. The mechanism and products of S-nitrosothiol decomposition are of great significance to the understanding of nitric oxide (NO) biochemistry. S-Nitrosothiols are stable compounds at 37 °C and pH 7.4 in the presence of transition metal ion chelators. The presence of trace transition metal ions (present in all buffers) stimulates the catalytic breakdown of S-nitrosothiols to NO and disulfide. Thiyl radicals are not formed as intermediates in this process. Photolysis of S-nitrosothiols results in the formation of NO and disulfide via the intermediacy of thiyl radicals. Reduced metal ion (e.g. Cu+) decomposes S-nitrosothiols more rapidly than oxidized metal ion (e.g. Cu2+) indicating that reducing agents such as glutathione and ascorbate can stimulate decomposition of S-nitrosothiol by chemical reduction of contaminating transition metal ions. Transnitrosation can also stimulate S-nitrosothiol decomposition if the product S-nitrosothiol is more susceptible to transition metal ion-catalyzed decomposition than the parent S-nitrosothiol. Equilibrium constants for the transnitrosation reactions of reduced glutathione, either with S-nitroso-N-acetyl-L-cysteine or with S-nitroso-L-cysteine indicate that S-nitroso-glutathione formation is favored. The biological relevance of S-nitrosothiol decomposition is discussed.

S-Nitrosothiols are compounds with the generic structure of RSNO. Under appropriate conditions these compounds decompose to liberate nitric oxide (NO) and the corresponding disulfide (1). It has been suggested that the formation and decay of low molecular weight S-nitrosothiols, such as S-nitroso-glutathione (GSNO) and S-nitrosocysteine (CySNO), may represent a mechanism for the storage or transport of 'NO (2, 3). According to this proposal, S-nitrosothiols are synthesized chemically by reaction of NO with thiol. Subsequently, these compounds are transported or diffuse to the site of action. Decomposition of the S-nitrosothiol then leads to NO release and the corresponding biological effect. This hypothesis is mainly speculative and remains to be rigorously tested. Little is known about the reaction of NO with glutathione (GSH) in vivo; however, the direct reaction of GSH with NO does not generate GSNO but forms glutathione disulfide and nitroxy anion (NO°-) (4, 5). GSNO is formed only if 'NO is oxidized, by reaction with oxygen, to form NO2 and N2O3 (6). As intracellular oxygen concentrations at the tissue level are in the range of 10–20 μM (7) and as the rate of NO oxidation is proportional to the squared power of the NO concentration (8), it is likely that the oxidation of NO by oxygen in vivo is a slow and insignificant process (4). Evidence for the formation of S-nitrosothiols from endogenous NO remains scarce (9). Nevertheless, nitrosylation of protein thiols has been implicated in the NO-dependent regulation of many enzymes, including protein kinase C (10) and glyceraldehyde-3-phosphate dehydrogenase (11). It has been reported that normal human serum contains S-nitroso-serum albumin (12, 13) which has been proposed to act as an endogenous regulator of vessel tone (14).

Although the physiological relevance of S-nitrosothiols remains to be established, these compounds have been used as donors of NO (1, 15, 16). The most commonly employed compounds are GSNO and S-nitroso-N-acetyl-DL-penicillamine (SNAP) (Fig. 1A). Such compounds have been shown to have diverse and remarkable biological effects. For example, SNAP is a potent vasodilator (1) and low concentrations of GSNO have been shown to afford significant protection to the ischemic myocardium (17). It is generally assumed that S-nitrosothiols decompose by homolytic cleavage of the S-N bond (Reaction 1).

\[ RSNO \rightarrow RS + NO \]

**REACTION 1**

This process generates 'NO and a thyl radical, RS(18). However, this assumption has not been effectively tested under physiologically relevant conditions. It has been established that S-nitrosothiols are sensitive to both photolytic (19, 20) and transition metal ion-dependent breakdown (21) but are stable in the presence of transition metal ion chelators in the dark. The biological activity of S-nitrosothiols may not be exclusively dictated by the release of NO as the chemistry of these compounds is complex. S-Nitrosothiols have also been shown to form NO , which under appropriate conditions can lead to the formation of either nitrosamine (4, 5, 22) or peroxynitrite (4, 23). S-Nitrosothiols can also undergo nitrosation (NO°-) transfer to other cellular thiols by a process referred to as transnitrosation (24).

In this study we have investigated the mechanism of decomposition and transnitrosation reactions of S-nitrosothiols using electron spin resonance (ESR), optical spectroscopy, high-performance liquid chromatography (HPLC), and electrochemical methods.

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†The abbreviations used are: GSNO, S-nitroso-glutathione; CySH, reduced cysteine; CySNO, S-nitroso-cysteine; DMPO, 5,5-dimethyl-1-pyrroline N-oxide; DMPG; spin adduct of GS- with DMPO; DMPG; Scys; spin adduct of SCys with DMPO; DMPG/S-NAP, spin adduct of S-NAP with DMPO; DTPA, diethylenetriaminepentaacetic acid; ESR, electron spin resonance; GSH, reduced glutathione; GSNO, oxidized glutathione; GS, glutathione-derived thyl radical; NAP, N-acetyl-L-cysteine-derived thiyl radical; GSH, glutathione-derived thyl radical; SNAP, S-nitroso-N-acetyl-L-cysteine; SNAP, S-NAP, S-nitroso-L-cysteine-derived thiyl radical; HPLC, high pressure liquid chromatography; ESR, electron spin resonance; G, gauss.
Thiyl Radicals from S-Nitrosothiols

EXPERIMENTAL PROCEDURES

Materials—GSH, reduced cysteine (CySH), oxidized glutathione (GSSG), N-acetyl-α-l-penicillamine (NAP), diethylthreitol, and n-propylamine (DTPA) were obtained from Sigma. DMPO was purified using activated charcoal as reported previously (25). Copper(II) sulfate and copper(I) chloride were obtained from Fisher. GSNO and CySNO were synthesized according to the method of Hart (26). SNAP and nitronyl nitroxide (2-p-carboxyphenyl-4,4,5,5-tetramethylimidazol-1-oxide 1-oxyl) (NNO) were synthesized according to the published procedures (27, 28).

ESR Measurements—ESR spectra were recorded at room temperature on a Varian E109 spectrometer operating at 9.5 GHz and employing 100 kHz field modulation. Samples were prepared in the nitrogen glove box and taken up in a 100-μl capillary (Corning), which was sealed at both ends with MiniSeal (Baxter). The capillary was placed in a 4-mm quartz tube, which was then placed inside the ESR cavity. All samples were prepared in the dark and irradiated, when required, with visible light (λ > 408 nm) inside the ESR cavity. Data were collected using the VIKING software, developed at the Medical College of Wisconsin, and simulated with the ESR software developed by David Duling from the Laboratory of Molecular Biophysics, NIEHS, National Institutes of Health, Research Triangle Park, NC.²

Irradiation Procedures—Photolytic decomposition of GSNO was performed in 8-ml glass vials. Samples were irradiated while stirring with light from a xenon arc lamp source (ILC Technology, Sunnyvale, CA) after passing through a copper(I) sulfate solution (100 g/liter) and a long pass filter of λ > 408 nm. Aliquots were removed for GSSG measurements. Samples for ESR studies were prepared in glass capillaries and were irradiated inside the ESR cavity.

HPLC Methods—Separation of GSNO and GSSG was performed on a Hewlett-Packard 1050 series HPLC system equipped with UV-vis detection. The mobile phase was 0.05% trifluoroacetic acid and methanol (94:6) with a flow rate of 0.75 ml/min. The stationary phase was an analytical C18 reversed phase column (Partisil ODS-3, 5-μm particle size, Whatman, Hillsboro, OR). UV absorption was used to detect GSNO and GSSG (λ = 220 nm).

NO Detection—NO was detected directly using a NO electrode (World Precision Instruments Inc., Sarasota, FL) in a thermostated oxygen-electrode chamber (Yellow Spring Instruments, Yellow Springs, OH) modified by the addition of a glass window for irradiation purposes. The NO electrode was stabilized at 37°C in PBS before use. The presence of NO was confirmed using NNO, which rapidly scavenges NO to form the corresponding imino nitroxide (29).

Kinetic Analysis of Transnitrosation Reactions—Transnitrosation reactions between SNAP and either GSH or CySH were monitored at 37°C in PBS containing DTPA (100 μM) by following the change in absorbance at 234 nm. The initial rate of the transnitrosation reaction was measured using an analytical C18 reversed phase column (Partisil ODS-3, 5-μm particle size, Whatman, Hillsboro, OR). UV absorption was used to detect SNAP, the initial rate of decay of SNAP.

\[
\frac{d[SNAP]}{dt} = -k_1[SNAP][RSH]
\]  

[SNAP]₀ and [RSH]₀ represent the initial concentrations of SNAP and RSH. A similar series of experiments using NAP and either GSNO or CySNO was conducted to determine the rate constant for the reverse reaction (k₂). Equilibrium constants (K) were calculated from the ratio of the forward and the reverse rate constants.

RESULTS

Detection of Thiyl Radicals during the Decomposition of S-Nitrosothiols—The chemical structures of the thiols and S-nitrosothiols used in this study are shown in Fig. 1A. In order to measure the production of thiyl radicals from the decomposition of S-nitrosothiols, these compounds were mixed with DMPO at 37°C and ESR spectra were obtained. GSNO, SNAP, and CySNO, in the dark, gave no ESR-detectable DMPO spin adducts (Fig. 2, A, C, and E, respectively). Irradiation of a mixture of GSNO and DMPO with visible light (λ > 408 nm) gave a four-line ESR spectrum (Fig. 2B), that could be simulated (Fig. 2B, dotted line), using similar values for the nitrogen and hydrogen hyperfine coupling constants (\(a_h = 15.2\) G and \(a_N = 16.3\) G). This spectrum is characteristic of the glutathione-derived thiyl radical (GS) adduct with DMPO (DMPO/SG) (18). The chemical structures of thiyl radical adducts of DMPO are shown in Fig. 1B. Irradiation of a mixture of SNAP and DMPO gave a six-line spectrum characteristic of trapping of N-acetyl-α-l-penicillamine-derived thiyl radical (SNAP) with DMPO (DMPO/SNAP), which could be simulated using the hyperfine coupling constants (\(a_h = 15.3\) G and \(a_N = 17.7\) G) (Fig. 2D). Irradiation of CySNO in the presence of DMPO also gave a six-line ESR spectrum of spin adduct of CySNO-derived thiyl radical (CySy) with DMPO (DMPO/CySy) (\(a_h = 15.1\) G and \(a_N = 17.2\) G) (Fig. 2F). The spectral parameters of DMPO/SG, DMPO/SNAP, and DMPO/CySy adducts are characteristic and distinguishable.

The spectra in Fig. 2 were collected in the absence of transition metal ion chelators and under these conditions the S-nitrosothiols decomposed in the dark with relative rates CySNO > SNAP > GSNO (Ref. 30 and data not shown). The observation that DMPO thiyl radical adducts were not formed in the dark suggests that transition metal ion-induced decomposition of S-nitrosothiols does not proceed through a thiyl radical intermediate (Fig. 2).

Effect of DMPO on GSSG Formation during Light- or Transition Metal Ion-induced Decomposition of S-Nitrosothiols—To further investigate the intermediacy of thiyl radical, GSNO was allowed to decompose both in the dark and under irradiation, in the presence and absence of DMPO, and the extent of conversion of GSNO to GSSG was monitored. DMPO has been shown to react rapidly with thiyl radicals (\(k = 10^7\) m⁻¹ s⁻¹) (31, 32). If thiyl radicals are formed, it is expected that they will react with DMPO and reduce the yield of GSSG.

Photolytic and transition metal ion-mediated decomposition

²This software is available via the World Wide Web at http://lmb.niehs.nih.gov/LMB/.
Decomposition of GSNO in the dark was a slow process. The kinetics of GSNO decay and GSSG formation are shown in Fig. 3. Irradiation of GSNO resulted in the stoichiometric conversion of GSNO to GSSG (Fig. 3A). Photolytic decomposition for 15 min with light ($\lambda > 408$ nm) for HPLC analysis.

DMPO (100 mM) had no effect on the formation of GSSG. decomposition of GSNO under irradiation was more rapid ($t_{1/2}$ = 8 min) (Fig. 4B) and also resulted in the formation of GSSG. DMPO (100 mM) did not affect GSNO decomposition but resulted in a 50% reduction in GSSG formation. At higher concentrations of DMPO (200 mM), an 80% reduction in the yield of GSSG was observed during photolytic decomposition of GSNO (Table I). High concentrations of DMPO are required in order to compete with the diffusion-limited dimerization of GS. Addition of Cu$^{2+}$ (2 $\mu$M) resulted in a more rapid decomposition of GSNO. Under these conditions, DMPO/GSNO was not observed by ESR and DMPO (200 mM) did not affect the yield of GSSG (Table I). These results indicate that photolytic decomposition of GSNO occurs through a G$^\bullet$ intermediate, whereas dark, transition metal ion-mediated decomposition does not.

Effect of Cu$^{2+}$ and Cu$^+$ on NO Formation during GSNO Decomposition—Previous studies have indicated that the decomposition of S-nitrosothiols is catalyzed by Cu$^{2+}$ by a mechanism that does not involve Cu$^{2+}$/Cu$^+$ interconversion (33). Fig. 5 shows that addition of Cu$^{2+}$ to GSNO resulted in a rapid release of ‘NO. However, addition of Cu$^+$ resulted in much greater stimulation of ‘NO release, which was inhibited by DTPA, a transition metal ion chelator (data not shown). These results indicate that the binding of copper ions to GSNO is a prerequisite for enhanced decomposition, as has been described for other S-nitrosothiols (33). Both Cu$^{2+}$ and Cu$^+$-induced ‘NO release were antagonized by NNO, and ESR analysis indicated that, under these conditions, NNO was partially converted to imino nitroxide confirming the production of ‘NO (data not shown). Interestingly, the addition of GSH to GSNO, in the absence of DTPA, stimulated the contaminating transition metal ion-dependent release of ‘NO (Fig. 5). In the presence of DTPA, GSH-stimulated GSNO decay does not result in ‘NO production (4).

These results are consistent with the proposal that Cu$^{2+}$/Cu$^+$ redox cycling mechanism is not responsible for GSNO decomposition. However, reduction of Cu$^{2+}$ to Cu$^+$ by GSH, or other reducing agents, will accelerate GSNO decay. Decomposition of GSNO by Cu$^+$ may occur by the mechanism shown in Reactions 3 and 4. This represents a catalytic redox cycle for Cu$^+$, which will not occur with Cu$^{2+}$ unless additional reducing agents are present.

The $t_{1/2}$ of GSNO has been measured to be 159 h (30). However, this value has an absolute dependence on the amount of contaminating metal ions in the system.

**Fig. 3.** The effect of DMPO on GSSG formation during photolysis of GSNO. GSNO (250 $\mu$M) was incubated in phosphate buffer (200 mM) in either the absence (A) or the presence (B) of DMPO (100 mM). Aliquots were removed before (solid line) and after (dotted line) irradiation for 15 min with light ($\lambda > 408$ nm) for HPLC analysis.

**Fig. 5.** Spectra of DMPO/SG, DMPO/S-NAP, and DMPO/CySNO. A, GSNO (1 mM) was incubated in phosphate buffer (200 mM) in the presence of DMPO (100 mM) in the dark; B, same as A but on irradiation ($\lambda > 408$ nm); C, SNAP (1 mM) was incubated in phosphate buffer (200 mM) in the presence of DMPO (100 mM) in the dark; D, same as C but on irradiation ($\lambda > 408$ nm); E, CySNO (1 mM) was incubated in phosphate buffer (200 mM) in the presence of DMPO (100 mM) in the dark; F, same as E but on irradiation ($\lambda > 408$ nm). Spectrometer conditions: microwave power, 5 milliwatts; modulation amplitude, 1 G; time constant, 0.128 s; gain, 2.5 x 10$^4$; scan range, 100 G; scan time, 120 s. The dotted line in spectra B, D, and F are computer simulations of the respective spectra using the parameters given in the text.
Transnitrosation between S-nitrosothiols and thiols—Transnitrosation between thiols and S-nitrosothiols has been implicated in the biological activity of S-nitrosothiols (34, 35). Previous determinations of the kinetic and thermodynamic parameters for these reactions have used extremely high concentrations of both thiol and nitrosothiol (0.5–10 mM) (24, 34, 35). The reasons for using high concentrations of S-nitrosothiols are: (i) most S-nitrosothiols have similar UV-visible spectra and (ii) the $\Delta \varepsilon_{\text{max}}$ for the transnitrosation reaction is less than 100 M$^{-1}$ cm$^{-1}$. SNAP, on the other hand, has an additional absorbance peak at 228 nm, and transnitrosation between SNAP and other thiols can be monitored at 234 nm with a $\Delta \varepsilon_{\text{max}}$ of approximately 3000 M$^{-1}$ cm$^{-1}$. Fig. 6A shows the spectral changes that occurred upon addition of GSH (100 mM) to SNAP (100 mM) in the presence of DTPA. As transnitrosation proceeded the decay of absorbance at 234 nm was accompanied by an increase in absorbance at 200–220 nm. A sharp isosbestic point at 222 nm was observed, indicating that decomposition of S-nitrosothiol was not occurring. The $\Delta \varepsilon$ for this change was measured to be 3040 M$^{-1}$ cm$^{-1}$ at 234 nm from the maximum change at a high concentration of GSH (500 mM). The initial rate of SNAP decay, using a range of GSH concentrations (Fig. 6B), was used to calculate a rate constant for this reaction (Table II), assuming a reversible second-order reaction (Reaction 5).

\[
\text{SNAP} + \text{GSH} \rightleftharpoons \text{SNOS} + \text{NAP}
\]

**REACTION 5**

A parallel set of experiments were performed using GSNO (100 $\mu$M) and NAP (0, 100, 200, 300, 400, and 500 $\mu$M) (Fig. 6B and data not shown) as the reactants to determine the rate constant of the reverse reaction (Table II). The ratio of these two numbers gave an equilibrium constant of 18.6, indicating that GSNO formation is favored. This is in contrast to Meyer et al. (34), who measured $K_{\text{eq}}$ to be 1.13 for this reaction. A similar set of reactions, using SNAP and CySH for the forward reaction and CySNO and NAP for the reverse reaction, gave the rate constants shown in Table II. The calculated equilibrium con-
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Fig. 6. Change in UV-vis absorption spectra during transnitrosation between SNAP and GSH. A, the UV-vis absorbance spectrum, between 200 and 260 nm, of a mixture of SNAP (100 μM) and GSH (100 μM), in phosphate buffer containing DTPA (100 μM) at 37°C, was recorded every 2 min. B, SNAP (100 μM) and GSH (a, 0 μM; b, 50 μM; c, 100 μM; d, 150 μM; e, 200 μM; f, 250 μM; g, 500 μM) were incubated in phosphate buffer at 37°C in the presence of DTPA (100 μM). Transnitrosation was monitored by following the decrease in absorbance at 234 nm. The reaction of GSNO (100 μM) with NAP (100 μM) was also monitored at 234 nm (- - -). The concentration of SNAP was calculated using Δε = 3,040 M⁻¹ cm⁻¹.

Table II

Kinetic and thermodynamic parameters of transnitrosation reactions

| Reactants    | k₁     | k⁻₁     | K*  |
|--------------|--------|---------|-----|
| SNAP/GSH     | 9.09 ± 0.78 | 0.53 ± 0.13 | 18.6 |
| SNAP/CySH    | 20.63 ± 3.15 | 3.15 ± 1.11 | 6.46 |
| GSNO/CySH    | ND     | ND      | 0.35b |

* K* = k₁/k⁻₁.

b Calculated from K*SNAP/CySHU/UK(SNAP/GSH).

tant of 6.46 indicates that the formation of CySNO is favored in this reaction. This is larger than that measured by Meyer et al. (34), who reported an equilibrium constant of 2.2 for this reaction. The equilibrium constant (KGC) for the reaction between GSNO and CySH was calculated to be 0.35 from the relationship KGC = KSG/KSC, where KSG is the equilibrium constant for the reaction between SNAP and GSH and KSC is the equilibrium constant for the reaction between SNAP and CySH (Table II). This indicates that GSNO formation is thermodynamically favored in this reaction system.

Fig. 7. The transnitrosation reaction between SNAP and GSH monitored by photolysis/spin-trapping. A, SNAP (1 mM), DMPO (100 mM), and DTPA (100 μM) in phosphate buffer, irradiated with visible light; B, same as A, but containing GSH (1 mM) and after 20 min of dark incubation; C, same as B, but irradiated after 60 min of dark incubation; D, solution containing DMPO and GSH irradiated for 60 min. Spectrometer conditions: microwave power, 5 milliwatts; modulation amplitude, 1 G; time constant, 0.128 s; gain, 2.5 × 10⁶; scan range, 100 G; scan time, 120 s. The dotted lines in spectra A–C are computer simulations of the respective spectra using the parameters given in the text.

The observation that photolysis of S-nitrosothiols generates thiyl radicals (Fig. 2) indicates another method by which transnitrosation reactions can be monitored. Photolysis of a mixture of a thiol and an S-nitrosothiol in the presence of DMPO, after a period of dark incubation, will give a "snapshot" of the transnitrosation reaction. Fig. 7 shows the transnitrosation reaction between SNAP and GSH as monitored by ESR. Computer simulations indicate that the presence of DMPO/SG and DMPO/S-NAP is sufficient to account for the spectra shown in Fig. 7. A solution of SNAP gave the ESR spectrum of DMPO/1a-SG and DMPO/S-NAP immediately after photolysis. However, if the solution was incubated with GSH in the dark for 20 min before photolysis, the ESR spectrum observed was a mixture of both the DMPO/1a-SG and the DMPO/S-NAP spin adducts. After 60 min of dark incubation, the DMPO/1a-SG spectrum dominated. These results are consistent with the optical data indicating that GSN0 formation is favored during transnitrosation (Table II).

The transnitrosation reaction between GSH and CySNO (Reaction 6) was monitored by ESR as shown in Fig. 8.

CySNO + GSH ⇌ GSNO + CySH

Reaction 6

Incubation of CySNO (500 μM) with GSH (500 μM) in the presence of DMPO resulted in no ESR signal under anaerobic conditions (Fig. 8A); however, upon irradiation of the solution,
it immediately (within the dead time of sample preparation and ESR analysis which is about 2 min) gave only the DMPO/•OH signal. In the dark, no signal was detected (Fig. 8B). Incubation of GSNO with CySH in the presence of DMPO resulted in no ESR signal under anaerobic conditions (Fig. 8C); however, upon irradiation of the solution (after incubation in dark), it gave only the DMPO/•OH signal (Fig. 8, D and E). This indicates that the transnitrosation reaction between CySNO and GSH is rapid and the equilibrium of Reaction 6 favors GSNO formation.

In the absence of transition metal ions or in the presence of metal ion chelators, the transnitrosation reaction is unlikely to be responsible for the release of •NO from S-nitrosothiols as both the reactant and product S-nitrosothiols are stable. However, in the presence of contaminating transition metal ions, transnitrosation may accelerate •NO release. This is shown for the reaction between GSNO and NAP (Fig. 9). In the presence of DTPA and in the dark, GSNO did not generate •NO and addition of NAP had no effect. In the absence of DTPA, however, GSNO generated a small amount of •NO, which was dramatically enhanced by the addition of NAP. Irradiation of GSNO, in the presence of DTPA, gave a much greater release of •NO, which was unaffected by NAP. However, in the absence of DTPA, the addition of NAP resulted in enhanced production of •NO. Although the equilibrium position of the transnitrosation reaction between GSNO and NAP favors GSNO formation (Reaction 5), SNAP is more susceptible to transition metal ion-catalyzed decomposition (21). SNAP decomposition will pull the equilibrium in Reaction 5 to the left, resulting in enhanced •NO release. It is also possible, as discussed earlier (cf. Fig. 5), that thiol-dependent metal ion reduction is responsible for the accelerated release of •NO.

**DISCUSSION**

Metal Ion- and Light-induced Decomposition of S-Nitrosothiols—The decomposition chemistry of S-nitrosothiols has been discussed widely in the literature (36–40). It has been shown that the biological effects of these compounds do not correlate with the rate of •NO release in solution (30), suggesting either selective metabolism or direct action of some of the S-nitrosothiols used in this study. Mathews and Kerr (30) highlighted the fact that the rate of decomposition of S-nitrosothiols in the experimental buffer cannot be used as an indicator of biological activity. The factors affecting S-nitrosothiol decomposition in solution include light, temperature, pH, and contaminating transition metal ions (19, 20, 37). In the absence of light and transition metal ion contamination, S-nitrosothiols are stable at physiological pH and temperature. In most reported measurements of S-nitrosothiol decomposition, the agent responsible for the breakdown of these compounds is the variable metal ion content of the buffer (21). This is exemplified by the half-time of SNAP, which has been variously measured as 1.15 h (30) and 4.6 h (1). The amount of free redox-active metal ion in each experimental system can vary between different batches and preparations of chemicals and buffers. Tissue preparations may also contribute to the total metal ion content.

The decomposition of S-nitrosothiols by photolysis is well understood. UV-visible light causes a homolytic cleavage of the sulfur-nitrogen bond, resulting in the release of •NO and a thyl radical (19). It is often assumed that the decomposition of these compounds in the dark also occurs through homolytic, and a thyl radical intermediate (21, 30, 36–40). We show here that this is not the case. We observed no evidence of thyl radical formation during decomposition of GSNO either by contaminating metal ions or by exogenously added Cu$^{2+}$ ions. Moreover, DMPO, which is an efficient thyl radical trap (31, 32), did not affect the yield of GSSG during the dark decomposition of GSNO but inhibited GSSG formation during photolytic decomposition. The precise mechanism of transition metal ion-induced decomposition of S-nitrosothiols is unknown, and it is likely that the cleavage of metal ions by thyl and thiol disulfides may complicate the kinetics of this process (39–41). The observation that Cu$^{+}$ is significantly more active than Cu$^{2+}$ implies that reducing agents such as glutathione and ascorbate will accelerate the transition metal ion-dependent decomposition of S-nitrosothiols by chemical reduction of contaminating transition metal ions. Additional studies are required to fully understand the transition metal ion-dependent decomposition chemistry of S-nitrosothiols.

Boese et al. (42) have shown that the S-transnitrosation reaction between serum albumin and dinitrosyl-iron complex
exhibits a direct characteristic ESR signal. It is conceivable that a combination of both the photolytic spin-trapping meth-
ology presented here and direct ESR of dinitrosyl-iron com-
plexes can be used to assess the interaction between *NO* and
proteins.

Biological Relevance of Transnitrosation Reactions—An-
other important reaction of S-nitrosothiolks, which has been
implicated in the biological activity of GSNO, is transnitroso-
tation (27). This reaction consists of NO− transfer from an S-
nitrosothiol to a thiol. This may become biologically signif-
ant if the thiol is a protein cysteiny residue and such modification
leads to altered enzyme or receptor activity. In the case of the
N-methyl-L-aspartate receptor, transnitrosation to a protein
cysteiny residue inhibits receptor activity and thus inhibits
the excitotoxic response (43). This may be the only case where
transnitrosation to protein is protective, as cysteinyl modifica-
tion is more likely to result in cellular ion imbalance, via
inhibition of plasma membrane channels (44), or enzyme inhibi-
tion has been suggested that glyceraldehyde-3-phosphate
dehydrogenase, protein kinase C, and glutathione peroxidase
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Biologically relevant
S-nitrosothiol
reactions are possible. It has been suggested that the enzyme
γ-glutamyl transpeptidase is responsible for GSNO decompo-
sition (45). This enzyme enhances the decomposition of
GSNO forming S-nitrosocysteinylglycine and glutamate. 4
NO production is dramatically enhanced upon the addition of
γ-glutamyl transpeptidase to GSNO, as S-nitrosocysteinyl-
glycine is more susceptible to transition metal ion-dependent
decomposition than GSNO. However, DTPA completely
inhibited the NO release by preventing the decomposition
S-nitrosocysteinylglycine.

Conclusions—In this study we conclude the following. (i)
Biologically relevant S-nitrosothiols are stable in the dark in
the presence of transition metal ion chelators. (ii) Photolytic
decomposition of S-nitrosothiols generates NO and the corre-
spanding thyl radical. (iii) Metal-ion catalyzed (dark) decom-
position of S-nitrosothiols generates NO and disulfides with-
out the intermediary of thyl radicals. (iv) Transnitrosation
from a stable thiol to an unstable thiol may facilitate NO
release in the presence of contaminating metal ions. (v) Reduc-
ing agents, such as ascorbate and thiols, may additionally
accelerate metal ion-dependent decomposition as the reduced
forms of the contaminating metal ions (e.g. Cu+) are more
active at promoting the decomposition of S-nitrosothiols than
their oxidized counterparts. (vi) In the absence of contaminat-
ing metal ions, thiols enhance S-nitrosothiol decomposition to
yield NO− and not NO−.

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