The Chemistry of S-Nitrosothiols

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Introduction

S-Nitrosothiols (thionitrites), are much less well-known than are their oxygen counterparts, the alkyl nitrites, partly due to their relative instability generally. Examples were characterized, however, as early as 1909, and their known chemistry until 1983 has been reviewed.[1] Interest in S-nitrosothiols (henceforth called RSNOs) has been regenerated since ca. 1990, following the spectacular series of discoveries involving the in vivo synthesis of nitric oxide and its control of a range of physiological functions.[2] There are two reasons for this: (a) RSNOs have been detected in vivo, and are believed to be involved in some of the reactions of NO (possibly as a store or transport mechanism for NO), and (b) RSNOs have a potential medical use as NO donors, for the treatment of blood circulation problems for example. It seems an appropriate time, therefore, to review the chemistry of this interesting and largely unknown class of compounds, particularly with reference to their possible reactions in vivo.

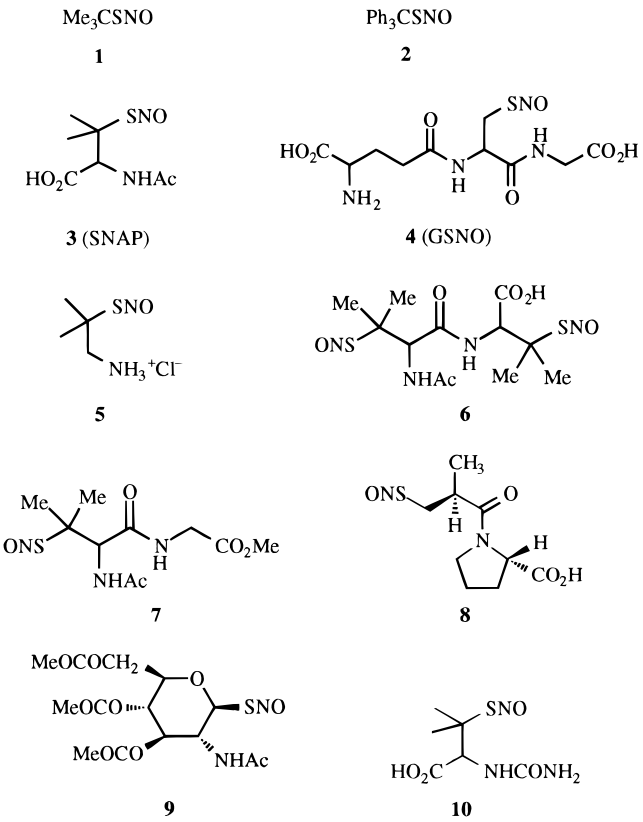
Synthesis

The easiest and most convenient route to RSNO formation is by the nitrosation of thiols. This is readily achieved in water (or mixed aqueous solvents) using nitrous acid as outlined in eq 1,[3] although any reagent acting as a carrier of NO^+ will, in principle, suffice. *tert*-Butyl nitrite in

$$RSH + HNO_2 \overset{H^+}{\rightleftharpoons} RSNO + H_2O \qquad (1)$$

organic solvents and N_2O_4 have been used successfully. Equation 1 is effectively irreversible, contrasting with the related reaction with alcohols leading to alkyl nitrite formation, although very small concentrations of thiol remain at equilibrium; this has important consequences for decomposition of RSNOs to give NO (see later). Nitric oxide itself in the pure state does not react with thiols, but does so readily in the presence of even traces of oxygen,[4] when it is likely that the reactive species is N_2O_3.

Isolation of RSNOs in the pure state has been achieved in a relatively small (but increasing) number of cases. Some examples which have been characterized (not a complete list) are shown as structures **1**,[1] **2**,[1] **3**,[5] **4**,[6] **5**,[7] **6**,[8] **7**,[9] **8**,[10] **9**,[11] and **10**,[11] together with a range of S-nitroso-1-thiosugars.[12] Most of these are solids which are indefinitely stable at room temperature. By contrast some, e.g., N-acetyl cysteine methyl ester, are stable only at low temperatures, and others decompose during extraction. Many experiments with RSNOs have been carried out using solutions prepared by thiol nitrosation in mildly acidic solution.



Physical Properties

In general, isolated RSNOs are green, red, or pink in color. The UV−visible spectra show bands in the 330−350 nm region (ϵ around 10^3 M^{-1} cm^{-1}, $n_o \rightarrow \pi^*$) and also at 550−600 nm (ϵ around 20 M^{-1} cm^{-1}, $n_N \rightarrow \pi^*$). The spectra have been analyzed and the transitions assigned. These absorbances are often used to monitor the decomposition of RSNOs. In the infrared spectra, the stretching (1480−1530 cm^{-1}) and bending frequencies of the N−O bond have been identified, as has the C−S bond-stretching frequency (600−730 cm^{-1}). Both ^1H and ^{13}C NMR spectra have been analyzed.[7] There is a downfield shift of both α-proton and α-carbon resonances upon nitrosation of thiols. The crystal structure of S-nitroso-N-acetyl-penicillamine, **3** (SNAP), has been obtained.[5]

Lyn Williams was born in Wales in 1936 and educated at University College London when the late Professor Sir Christopher Ingold was the head of the department. He completed a Ph.D. with the late Professor Peter de la Mare, holds a D.Sc. from the University of London, and has been at the University of Durham, where he is currently Professor of Chemistry (and former Chairman), for the bulk of his career. He has worked in mechanistic organic chemistry with problems in alkene additions, aromatic rearrangements, nitrosation reactions, and more recently nitric oxide chemistry with particular reference to its importance in vivo.

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Reactions

(a) Thermal and Photochemical Decomposition. RSNOs decompose on heating, or in some cases on standing, to give the corresponding disulfide and initially NO (eq 2), which in the presence of oxygen is oxidized to NO_2. The

$$2RSNO = RSSR + 2NO \quad (2)$$

thermal reaction probably involves homolysis of the S−N bond. The same reaction occurs photochemically, which has been more studied. Irradiation of S-nitrosoglutathione (**4**, GSNO) at either absorption maximum (340 or 545 nm) results in NO release in a process which is approximately first-order.[13] Results are consistent with the mechanism outlined in eqs 3−6, where after homolysis the thiyl radicals react with GSNO to give GSSG and further NO, or with oxygen to give the peroxy radical, which also reacts with GSNO to give GSSG and NO.[14]

$$GSNO = GS^{\bullet} + NO \quad (3)$$

$$GS^{\bullet} + GSNO = GSSG + NO \quad (4)$$

$$GS^{\bullet} + O_2 = GSOO^{\bullet} \quad (5)$$

$$GSOO^{\bullet} + GSNO = GSSG + NO + O_2 \quad (6)$$

The EPR spectra of thiyl radicals have been recorded during photolysis.[15] The cytotoxic effect of GSNO on leukemia cells was found to be enhanced by photolytic irradiation and diminished by addition of oxyhemoglobin (a trap for NO), strongly suggesting that NO is the cytotoxic agent.[13]

(b) Decomposition in Solution. (i) Effect of Cu^{2+}. It is of more relevance to the in vivo situation to consider the "spontaneous" decomposition of RSNOs in solution at physiological pH. Many groups have shown that the same products are formed as in the thermal/photochemical decomposition, i.e., as in eq 2. The final "nitrogen" product is usually NO_2^-, unless stringent precautions are taken to eliminate oxygen from the solution, when NO can be detected more or less quantitatively. Independently prepared solutions of NO in water at pH 7.4 also yield quantitative yields of NO_2^-, probably by oxidation, further reaction with NO_2, and hydrolyis of N_2O_3, as outlined in eqs 7−9.[16] This implies that the reaction in eq 8 competes effectively with that of NO_2 hydrolysis.

$$2NO + O_2 = 2NO_2 \quad (7)$$

$$NO_2 + NO = N_2O_3 \quad (8)$$

$$N_2O_3 + 2OH^- = 2NO_2^- + H_2O \quad (9)$$

Kinetic studies of the decomposition of RSNOs in solution initially yielded erratic and irreproducible results, until we showed in 1993[17] that the reaction is catalyzed by copper ions, often present adventitiously (in varying concentrations) in the buffer solutions. The effect is clearly seen in Figure 1 with increasing [added Cu^{2+}]. [18] Added EDTA (the well-known metal ion chelator) effectively stops the reaction. Later it was shown[19] that the true reagent is Cu^+, generated by thiolate reduction of Cu^{2+} [20] (eq 10). Reaction then occurs between Cu^+ and RSNO (eq 11), regenerating Cu^{2+} and RS^-, and releasing NO.

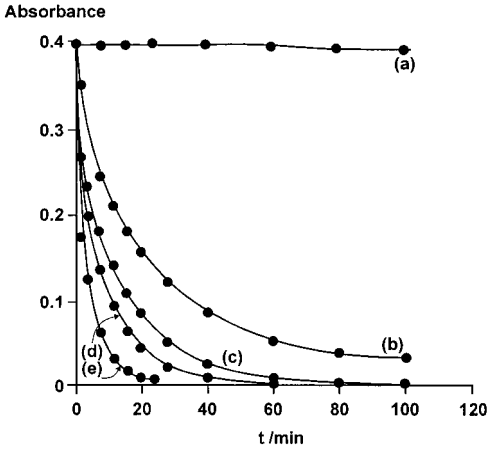


FIGURE 1. Effect of [Cu^{2+}] on the decomposition of SNAP with (a) EDTA, (b) no added Cu^{2+}, (c) 5 μM Cu^{2+}, (d) 10 μM Cu^{2+}, and (e) 50 μM Cu^{2+}

$$2Cu^{2+} + 2RS^- = 2Cu^+ + RSSR \quad (10)$$

$$Cu^+ + RSNO = Cu^{2+} + RS^- + NO \quad (11)$$

In the presence of the specific Cu^+ chelator, neocuproine, decomposition of RSNOs was progressively halted, and the Cu^+ complex was formed quantitatively, detected by its characteristic UV−visible spectrum. In the presence of neocuproine or EDTA, only the thermal reaction occurs, which for most RSNOs at 25 °C is negligibly slow; for example, the half-life for the decomposition of S-nitrosocysteine in solution at 25 °C in the presence of EDTA is ca. 55 h.

Free hydrated Cu^{2+} is not the only possible source of Cu^+. We have shown[21] that Cu^{2+} bound to amino acids, peptides, and proteins is also accessible for thiolate ion reduction to Cu^+, leading to NO production. This means that there is a viable pathway in vivo for NO production by the Cu^+-promoted reaction, even when, as in the human body, the concentration of free (hydrated) Cu^{2+} is very low.

We found no catalytic activity from added Zn^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+}, or Fe^{3+}, although there was some indication of a small catalytic effect by Fe^{2+}. Another decomposition pathway, leading to the thiol and nitrous acid, involves loss of NO^+ rather than NO and occurs with Hg^{2+} and, to a lesser extent, with Ag^+. The Hg^{2+} reaction has been developed to determine RSNOs analytically. Mechanistic features of the reaction have been established,[22] but will not be discussed further here.

(ii) Effect of RS^-. If our interpretation is correct, then decomposition rates by the copper pathway should be very dependent upon the [RS^-], the reductant for Cu^{2+}. The equilibrium constants for RSNO formation in mildly acidic solution (eq 1) have been measured[23] for a number of different structures and are around 10^5−10^6 M^{-1}. This

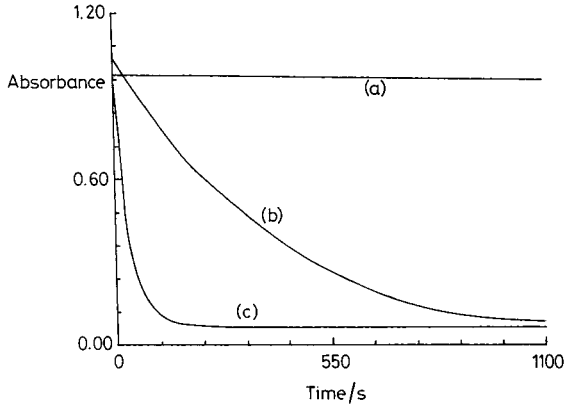


FIGURE 2. Effect of [RS$^-$] on the decomposition of SNAP prepared in situ with (a) excess HNO_2, (b) equimolar HNO_2 and RSH, and (c) excess RSH.

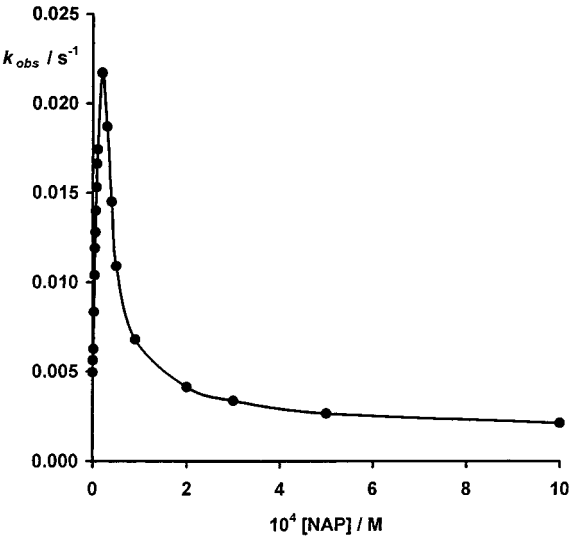


FIGURE 3. Rate constants for the decomposition of SNAP in the presence of added N-acetyl penicillamine (NAP).

ensures that there is always a low [RS$^-$] present at pH 7.4 (equilibrium eq 1 is frozen when the pH is raised), to effect Cu^{2+} reduction. Both RS$^-$ and Cu^{2+} are regenerated (eq 11), and so are only required in catalytic amounts. When RSNOs are generated in situ with an *excess* of nitrous acid, the [RS$^-$] is lowered to such a level that decomposition is effectively halted, [24] presumably since no Cu^+ is generated. It is thus possible to stabilize RSNO solutions by lowering *either* the [Cu^{2+}] by addition of EDTA, *or* the [RS$^-$] by generation of RSNO in situ with a reasonable excess of HNO_2, to drive eq 1 to the right. The latter effect is shown dramatically in Figure 2. Clearly, a whole range of half-lives can occur with intermediate [RS$^-$] values. This explains the very wide range of values quoted in the literature (ranging from a few minutes to several hours) for, e.g., SNAP decomposition.

The effect of added thiols is even more complex than hitherto outlined. For many systems, addition of the same thiol used to generate the RSNO in low concentration does increase the decomposition rate by increasing the [Cu^+]. However, further increase of [added RS$^-$] results in a progressively decreasing rate constant. The turnover point for SNAP occurs at $\sim 1 \times 10^{-4}$ M (see Figure 3).[19] At these

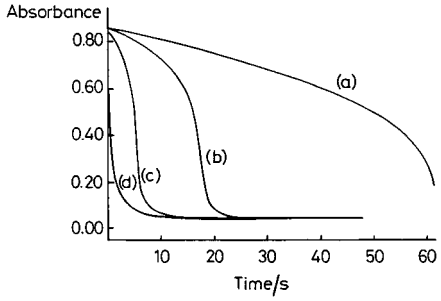
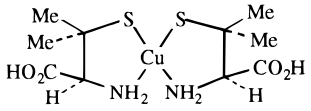


FIGURE 4. Decomposition of S-nitroso penicillamine prepared in situ with (a) excess penicillamine (0.16 mM), (b) excess penicillamine (0.08 mM), (c) equimolar reactants, and (d) excess HNO_2 (0.09 mM).

higher concentrations, it is believed that thiolate complexation of Cu^{2+} occurs, rendering the copper less available for reduction. For other thiolates this effect is very much more pronounced, e.g., with penicillamine, a well-known copper chelator (penicillamine is used to chelate excess Cu^{2+} in the body in the treatment of Wilson's disease). Consequently, even quite low concentrations of penicillamine in S-nitrosopenicillamine solutions act primarily as a copper chelator, thus stabilizing the RSNO, at least for some time, during which Cu^+ is being generated from the low [Cu^{2+}], resulting in rather unusual S-shaped absorbance−time plots (see Figure 4), with quite substantial induction times.[24] At *very* low [RS$^-$], this complexation effect, which probably involves two thiolate ions to one Cu^{2+} ion[20,25] (structure **11**), is much less pronounced, allowing reduction to Cu^+ to occur and RSNO decomposition to take place. Reduction and com-



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plexation of Cu^{2+} must have different stoichiometries. In some cases, oxidation of the thiol occurs in solution on standing, so that some quite dramatic effects can occur when the standing time of the RSNO/RSH solutions is allowed to vary. Addition of a thiol structurally different from RSNO is discussed in the next section.

No thiyl radicals were detected by EPR in the copper-mediated decomposition of RSNOs (contrasting with the photochemical reaction), and addition of an efficient trap for thiyl radicals did not affect the RSSR yield;[15] these findings are in keeping with the mechanistic interpretation.

In principle, any reducing agent capable of Cu^{2+} reduction should initiate RSNO decomposition. We have found[24] that ascorbic acid in the concentration range 1×10^{-6}−2×10^{-5} M is able to fulfill this function. Indeed, the absorbance−time plots for the decomposition of SNAP at different [ascorbate] (when the thiolate concentration was made negligibly low) were found to be similar to those obtained when added thiolate was used as the reductant. It is more likely that, in vivo, ascorbate is a more probable reducing agent than thiolate ion, given their likely relative concentration levels. However, ascorbic acid induces a

Table 1. Values of k^a (M^{-1} s^{-1}) from Eq 12

RSNO	k
S-nitrosopenicillamine	67 000 ± 2000
S-nitrosocysteamine	65 000 ± 1300
S-nitrosocysteine	24 500 ± 500
SNAP	20 ± 1
S-nitroso-N-acetyl cysteamine	~0
S-nitroso-N-acetyl cysteine	~0
GSNO	~0
S-nitrosothioglycolate	300 ± 5
methyl S-nitrosothioglycolate	~0

[a] With no added RSH.

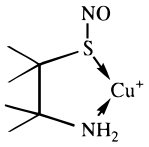
further reaction at higher concentrations (also leading to NO formation), which will be discussed in the next section.

Recently, unusual results have been reported concerning the reactions of RSNOs with *high* concentrations of thiols, particularly the reaction of GSNO in the presence of GSH. As the added thiol concentration is increased, the yield of NO decreases, to be replaced by ammonia as the main "nitrogen" product, together with smaller amounts of nitrous oxide.[26] These findings have been subseqently supported by other workers.[27,28] The "organic" product remains the disulfide GSSG. The kinetics of these reactions show the expected pseudo-first-order behavior, with a first-order dependence on the excess thiol concentration.[28−30] There is no dependence on added copper ions, and the rate constants are unchanged by EDTA addition. There is also not a large dependence of the reactivity upon structure, although a full structure−reactivity analysis has not been attempted. The pH−rate profile shows the reactive species to be the thiolate anion, and the electron-releasing effect of the two methyl groups in penicillamine appears to contribute to its greater reactivity than that of cysteine. The chemistry of the reactions leading to ammonia and nitrous oxide is clearly complex, but a rationale has been given by Singh and coworkers,[26] involving the formation of N-hydroxysulfenamide and other intermediates. The case for HNO as an intermediate (leading to N_2O formation) has also been argued recently.[27] The intracellular concentration of GSH can be as high as 10 mM, but even at this concentration, the half-life of the reaction with GSNO to give ammonia is several hours.

(iii) Reaction Mechanism. For many RSNO structures, the rate equation shown below (eq 12) holds. This should

$$\text{rate} = k[Cu^{2+}][\text{RSNO}] \qquad (12)$$

give some indication of the structure−reactivity relations within RSNOs, but does not take into account the different effects of the nature and concentration of the low concentration of thiols present in each case. Nevertheless, we have found[31] some very large reactivity differences between some RSNOs, as measured by the values of k (eq 12). Some are shown in Table 1. The S-nitroso derivatives of penicillamine, cysteamine, and cysteine are all very reactive, whereas N-acetylation reduces the reactivity massively, in some cases to effectively zero. Similarly, the reactivity of the carboxylate S-nitroso thioglycolate is very much attenuated by esterification. These results led us to suggest that intermediates are involved in which Cu^+ is *bidentately* coordinated. This could occur via five- or six-membered rings involving coordination at the sulfur or the nitrogen atoms, as shown in structures **12** and **13**.



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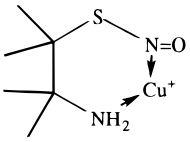

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Similar intermediates can be envisaged for the carboxylate. Electron transfer could then occur within these intermediates, releasing RS^-, NO, and Cu^{2+}. The low reactivity of GSNO and other RSNOs can then be ascribed to the inability of Cu^+ to bind bidentately, due to the absence of a suitably situated amino group. This view is supported by the recent finding that the nitrosothiol from the dipeptide CysGly (where there is a suitable NH_2 group) is ca. 10^4−10^5 times more reactive than both GSNO (GluCysGly) and GluCys.

In some cases, zero-order kinetics occurs, suggesting rate-limiting Cu^+ formation. In other cases, reaction can cease at incomplete conversion. This is particularly noticeable with the Cu^{2+}-catalyzed reaction of the sugar derivative **9** (which is overlaid by a significant thermal reaction). Progressively higher conversion is obtained by increasing the Cu^{2+} concentration. This has been interpreted in terms of complexation of Cu^{2+} by the product disulfide. The same effect occurs in the decomposition of GSNO in the presence of increasing amounts of added disulfide GSSG, and there is spectral and other evidence for the existence of GSSG−Cu^{2+} complexes.[32] For the less reactive RSNOs such as GSNO, there is a significant difference in reactivity pattern between reactions carried out anaerobically and those where oxygen is present. The results are consistent with a sequence where there is a competion for Cu^+ by RSNO (eq 11) on one hand and oxygen (leading to Cu^{2+} formation) on the other.

Reactions with Nucleophiles

RSNO compounds generally react with nucleophiles (Y^-) at the nitroso nitrogen atom, generating NOY, which may be the stable products or which may react further, the other product being the thiolate ion (eq 13), which may oxidize in the air to the disulfide. Many other −NO-

$$\text{RSNO} + Y^- = RS^- + \text{NOY} \qquad (13)$$

containing compounds,[3] notably alkyl nitrites, N-nitrosamines, and N-nitrososulfonamides, react similarly as electrophilic nitrosating agents. A wide range of nucleophilic species has been shown to be effective, and all the experimental evidence points to a direct reaction where NO^+ is transferred to the nucleophilic center, without its appearance as a free entity. The range of nucleophiles examined kinetically in reactions with RSNOs thus far

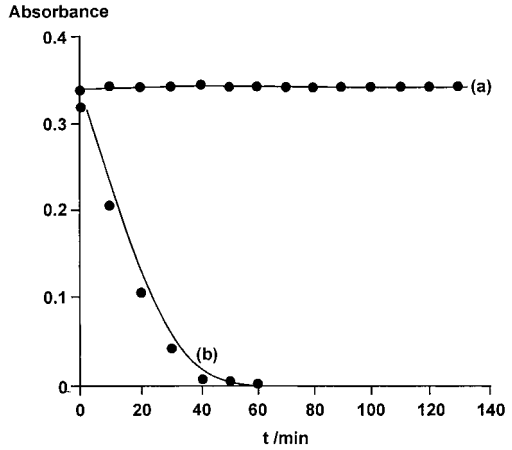

FIGURE 5. Decomposition of GSNO (0.4 mM) (a) without added cysteine and (b) with added cysteine (1.0 mM).

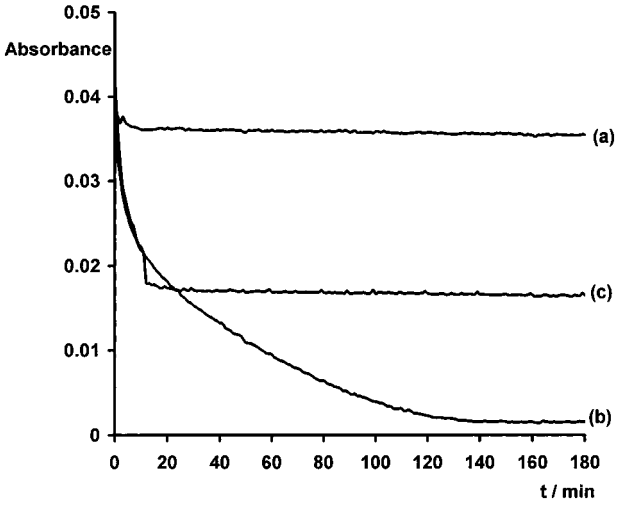

FIGURE 6. Absorbance−time plots for the decomposition of GSNO (2 mM) with added Cu^{2+} (0.1 mM) and ascorbate (0.2 mM) at pH 7.4, with (a) excess EDTA over the [Cu^{2+}], (b) no added EDTA, and (c) EDTA added after 14 min.

includes $R'S^-$ [33] (transnitrosation), $RR'NH$, NH_2NH_2, NH_2-OH, N_3^-, SO_3^{2-}, S_2^{2-}, H_2O_2,[11] and ascorbate.[34] As expected, the secondary amines yield the stable nitrosamine products. The two reactions of interest in connection with NO release (the reactions with $R'S^-$ and ascorbate), and also the reactions with H_2O_2 and superoxide, will now be discussed in more detail.

(i) Exchange Reaction with Thiolate. Early experiments where RSNOs reacted with R′SH[1,35] showed that exchange of the NO group takes place, since both products of decomposition, RSSR and R′SSR′, as well as the mixed disulfide, RSSR′, were formed. Later, equilibrium and rate constants were measured for the overall exchange process (eq 14) using differential optical spectrophotometry.[36] Equilibrium constants have also been measured from the

$$RSNO + R'SH \rightleftarrows RSH + R'SNO \qquad (14)$$

ratio of the rate constants for the forward and reverse reactions.[15] The agreement between the two approaches, however, is not good. More detailed kinetic studies[33] showed that the reactive species is the thiolate ion, $R'S^-$. These rapid exchange reactions were measured kinetically, in some cases, using the stopped-flow technique with a large excess of R′SH, thus driving the reaction in one direction. Transnitrosation does *not* involve prior NO formation by the copper-catalyzed process, since the former is very much faster and is unaffected by Cu^{2+} or EDTA addition.

If a relatively unreactive (toward NO formation) nitrosothiol is treated with a thiol, the "new" RSNO formed by transnitrosation might, for structural reasons outlined earlier, be much more reactive toward NO formation. This provides a more rapid alternative pathway to NO formation. So, when GSNO is reacted with cysteine, S-nitrosocysteine is formed, which decomposes rapidly by the copper-catalyzed pathway.[18] This is illustrated in Figure 5 by absorbance−time plots for the decomposition of GSNO in the presence of increasing cysteine concentrations.

(ii) Reaction with Ascorbate. In the earlier section it was shown that ascorbate can act as a reducing agent for Cu^{2+}, and is effective in the decomposition of RSNOs in the absence of RS^-. However, two recent papers (see ref 34) show that ascorbate promotes NO production from GSNO in blood plasma, even in the presence of metal ion chelators. This prompted us to examine this system further. We find[34] that there are, in fact, *two* separate reactions of ascorbate with RSNOs generally, both of which lead to NO formation: (a) when ascorbate acts as a reducing agent for Cu^{2+} (a well-known reaction), which is dominant at low [ascorbate] (typically up to $\sim 1 \times 10^{-4}$ M), leads to *disulfide* formation, and is totally halted by the addition of EDTA, and (b) when ascorbate acts as a nucleophile and undergoes electrophilic nitrosation by RSNO, leading to NO and *thiol* formation; this reaction is not affected by the presence of Cu^{2+} or EDTA, and is dominant at higher [ascorbate] (typically 1×10^{-3}−1×10^{-2} M). These results are shown graphically in Figures 6 and 7. Figure 6 shows the reaction of GSNO (measured at 545 nm) with ascorbate (2×10^{-4} M) in the presence of added Cu^{2+}, (a) with added EDTA, (b) without added EDTA, and (c) with EDTA added after 14 min. It is clear that EDTA stops the reaction virtually completely, whether added at the start of the reaction or after about a half-life. Conversely, Figure 7 shows the increasing rate of reaction (measured at 340 nm) as the ascorbate concentration is increased from 1×10^{-3} to 1×10^{-2} M, all in the presence of EDTA. There is also no effect when the EDTA is added after a half-life instead of at the start of the reaction. The increasing absorbance toward the end of the reaction in trace d is due to a further reaction of the product dehydroascorbic acid (a known reaction). Under these conditions, there is a good first-order dependence on [ascorbate] for a range of RSNOs. The overall reaction is probably as in eq 15. Nitric oxide was deter-

$$2RSNO + \text{ascorbate} = 2RS^- + 2NO + \text{dehydroascorbic acid} \qquad (15)$$

mined, using the NO electrochemical probe, in 80−90% yield from *both* reactions, i.e., at high and low [ascorbate],

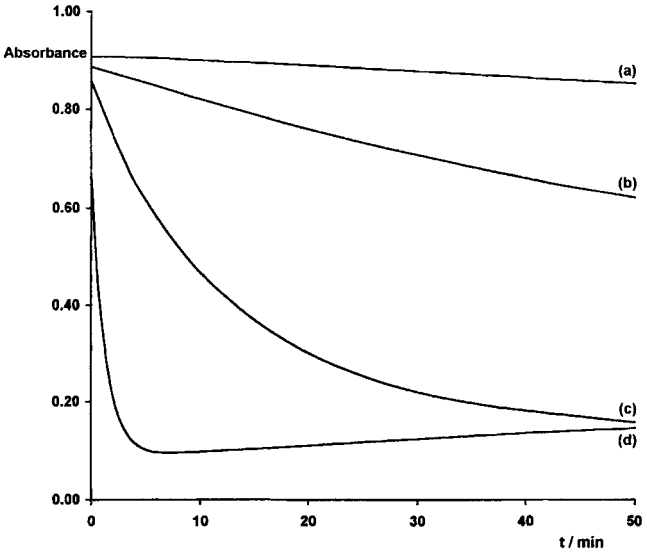

FIGURE 7. Absorbance−time plots for the decomposition of S-nitroso penicillamine with added EDTA, with (a) no ascorbate, (b) added ascorbate (0.1 mM), (c) added ascorbate (1.0 mM), and (d) added ascorbate (10 mM).

and the thiol product was found to be essentially quantitative, using Ellman's reagent, only from the reaction at the higher [ascorbate]. At pH 7.4, the ascorbate monoanion is the likely reagent.

(iii) Reaction with H_2O_2. RSNOs react with hydrogen peroxide via the hydroperoxide anion, yielding the peroxynitrite anion (eq 16).[11] The product is stable at ~pH

$$RSNO + HOO^- = RS^- + ONOO^- + H^+ \qquad (16)$$

12, but isomerizes to nitrate anion (via peroxynitrous acid) rapidly at lower pH values, so that at pH 7.4, even though the RSNO spectrum disappears, no absorbance due to peroxynitrite is observed At this pH, the reaction is probably too slow to be implicated biologically.

(iv) Reaction with Superoxide. Recently[37] it has been shown that rapid RSNO decomposition can be brought about by superoxide generated from xanthine/xanthine oxidase. The measured rate constant is many orders of magnitude smaller than the diffusion-controlled reaction of superoxide with NO (to give peroxynitrite), and RSNOs may thus provide a storage place in biological systems for NO, protecting it from rapid decomposition by superoxide.

Biological Properties

RSNOs generally show many of the same biological properties as does NO itself. These include vasodilation of veins and arteries and inhibition of platelet aggregation.[2] A reasonable assumption is that RSNOs decompose in vivo to generate NO, and several possible pathways have already been discussed. It is generally believed that there is no correlation between the in vitro decomposition rates of RSNOs and their biological activity,[38] but the in vitro studies failed to take into account the potentially substantial effects on the reactivity of the presence of Cu^{2+} (or Cu^{2+} complexes), RS^-, $R'S^-$, and ascorbate and therefore are of no value. It has been suggested that other factors, e.g., catalysis of NO formation by enzymes or by vascular membrane, are important in vivo, which may not have a copper dependence, but at this stage the mechanisms are not established. There is a vast and rapidly growing literature in this area, and there are a number of review articles available, e.g., ref 38.

RSNOs have been detected as naturally occurring species, notably GSNO, in animal and human airways (leading to bronchodilation), and also as S-nitroso proteins (from proteins containing the cysteine residue) in blood plasma. Indeed, it has been claimed that the bulk of the nitric oxide circulates in blood plasma as the S-nitroso derivative of serum albumin, which has been isolated, purified, and characterized.[39] S-Nitroso proteins are believed to be much more stable to NO loss than is S-nitrosocysteine (probably for the same reason which confers stability on GSNO and SNAC in solution). It is a reasonable hypothesis that they can deliver NO after a transnitrosation reaction with a low-molecular-weight thiol such as cysteine, and there is experimental evidence to support this.[40]

There are, however, clear indications that copper does play a part in the in vivo reactions of RSNOs. For example, it has been found that both anti-platelet aggregation[41] and vasodilation responses[42] brought about by GSNO and SNAP are reduced in the presence of a specific Cu^+ chelator. The cytotoxicity of SNAP (from excess NO production) is also enhanced in oxygen-depleted situations, consistent with there being a competition between SNAP and oxygen for reaction with Cu^+ (leading to reoxidation to Cu^{2+} in the latter case).[43] It may well be that the recently discovered pathway to NO from ascorbate and RSNO is of some relevance in vivo.

The S-nitroso derivative of hemoglobin (of a cysteine residue in the β-chain) has been identified in vivo.[44] It is suggested that the interconversion between the Fe−NO and S−NO forms allows a mechanism for the vasodilatory action of NO, and possible control of blood pressure, although the chemistry of the rearrangement is not established.

Already GSNO is being administered medically in clinical trials. Because of its very powerful anti-platelet aggregation property, it has found a use during coronary angioplasty operations, where it can be used to reduce clotting at a dose where the vasodilation effect is small. In addition, GSNO has a beneficial effect in the treatment of a form of preeclampsia suffered by some pregnant women. A novel S-nitroso glyco-amino acid has demonstrated potential as a slow-release NO donor drug.[45]

There is currently a vast amount of work taking place, investigating other possible therapeutic uses for RSNOs, based on their biological properties as NO donors, notably as vasodilators, possibly to replace the much-used glyceryl trinitrate in the treatment of angina, and also in the search for a treatment for male impotence. The generation of NO by photolysis of RSNOs has also much potential.

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Note Added in Proof

Very recent work from our laboratories[46] has shown that the rate of NO release from GSNO is very dependent on its initial concentration. Table 1 gives the reactivity of GSNO in the absence of added thiol as ~0, which is true for reaction measured spectrophotometrically at a concentration of $\sim 1 \times 10^{-3}$ M. However, when the reaction is followed at lower concentrations, using the NO probe, reaction is much quicker and quantitative (half-life ~35 s at 1.6×10^{-6} M with added Cu(II) = 1×10^{-5} M). This explains why GSNO and probably other RSNOs are such effective NO donors at low concentrations. The reduction in reactivity at higher concentrations almost certainly arises by complexation of Cu(II) by GSNO itself.

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