Decomposition of *S*-nitrosothiols by mercury(II) and silver salts

Helen R. Swift and D. Lyn H. Williams*

Department of Chemistry, Durham University, South Road, Durham, UK DH1 3LE

Rate measurements have been obtained for the reaction of a number of *S*-nitrosothiols (RSNO) in water with mercury(II) salts. Reaction is first order in both reactants, and the products are nitrous acid and the corresponding thiol–Hg^{2+} complex. Decomposition is *ca.* 10^3 faster when mercury(II) nitrate, rather than mercury(II) chloride is used as the source of mercury(II). This is consistent with the fact that whereas the nitrate salt is fully ionised in water, the chloride exists almost entirely in the undissociated molecular form. There is very little variation in the rate constant with RSNO structure. Silver ion reacts similarly, although less rapidly, and the kinetic order with respect to [Ag^+] is *ca.* 2. The results are discussed in terms of a mechanism involving rate-limiting attack by water, at the nitrogen atom in the mercury(II) (or silver) ion complex of the *S*-nitrosothiol, and are contrasted with the corresponding reactions of *S*-nitrosothiols with Cu^+, which generate nitric oxide.

S-Nitrosothiols, or thionitrites (RSNO) have been the focus of much attention in very recent times, because it is now believed that they play an important role *in vivo* associated with the amazing functions controlled in the body by nitric oxide (NO).[1] Generally, RSNO compounds show similar biological properties (notably of vasodilation and inhibition of platelet aggregation) to NO itself, and it may well be that decomposition to NO is a pre-requirement for activity. RSNO compounds are also under scrutiny as possible therapeutic reagents to alleviate angina and other blood circulation problems. In fact, they could replace glyceryl trinitrate, which has been prescribed for over a century but which suffers a major drawback in that it induces a tolerance in some patients. A number of RSNO species have been detected in the body, and it has recently been suggested that the formation of *S*-nitroso haemoglobin, and subsequent loss of NO, may be important reactions in the control of blood pressure.[2]

S-Nitrosothiols break up thermally[3] and photochemically[4] to give the disulfide and initially NO, as in eqn. (1). The same

$$2RSNO \longrightarrow RSSR + 2NO \quad (1)$$

overall reaction occurs in aqueous buffer solutions, but it has been shown recently[5,6] that this reaction is brought about by copper ions. Often there is enough Cu^{2+} in the distilled water/buffer components to bring about reaction. In the presence of metal ion chelators, such as EDTA, decomposition is virtually halted. The true reagent is Cu^+ formed by reduction with thiolate [(eqns. (2) and (3)], or in principle by any reducing agent.

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

$$Cu^+ + RSNO \longrightarrow Cu^{2+} + RS^- + NO \quad (3)$$

Both Cu^{2+} and RS^- are regenerated and can be present in catalytic quantities. Reactivity is structure dependent, the most reactive *S*-nitrosothiols being those which can complex Cu^+ strongly bidentately, *e.g.* at a -NH_2 group, or a -COO^- group, in addition to the nitroso group. It has also been shown[7] that these reactions occur, although a little more slowly, when the copper source is Cu^{2+} bound to peptides and proteins. Since in the body copper exists in these forms, the experiments show that, in principle, NO release from RSNO compounds is a feasible process *in vivo*.

Apart from some indication of reaction with Fe^{2+}, no other metal ions tested[5] (we looked at Zn^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+} and Fe^{3+}) were effective in the decomposition of *S*-nitrosothiols, except mercury(II) ion. This reaction has been known for some time, principally in its application to an analytical procedure for the quantitative determination of thiols,[8] but has never been investigated mechanistically. In particular, it is of interest to establish whether there are similarities with the copper reaction, and specifically to determine whether the initial product is nitric oxide. Unless water is very carefully deoxygenated, dilute solutions of NO give quantitative yields of nitrous acid or nitrite anion, depending on the pH of the solution.[9] We also decided to look at the reaction with silver ion, since the report of the mercury(II) ion reaction[8] also mentions that silver ion will also effect the hydrolysis of *S*-nitrosothiols to generate nitrous acid.

Results and discussion

(a) Reaction products

When *S*-nitroso-*N*-acetyl penicillamine (SNAP) **1** was treated with a 20-fold excess of mercury(II) nitrate in mildly acid solution nitrous acid was detected spectrophotometrically, and determined quantitatively using the Griess test, as 98 ± 1%. Similarly 97 ± 1% nitrous acid was measured from the reaction of *S*-nitrosothiomalic acid, **2**, under the same conditions. For the corresponding reaction with silver ion, nitrous acid was again detected directly spectrophotometrically, but the quantitative Griess test was hampered by interference from silver ion. Both Hg^{2+} and Ag^+ reactions were also carried out with solutions which had been thoroughly purged with nitrogen gas. The electrochemical NO-probe was unable to detect any measureable amount of NO. This shows that nitrous acid does not, in these cases, derive from NO, in marked contrast to the reactions of *S*-nitrosothiols with Cu^+. Another major difference is that whilst the copper reaction occurs with catalytic quantities of copper ions, the mercury reaction appears to be stoichiometric; thus no discernable reaction occured when RSNO was in 50-fold excess over Hg^{2+}. This is to be expected, given the enormous values (>10^{30} dm^3 mol^{-1})[10] for the stability constants for 1:1 complexes of thiols generally with Hg^{2+}. Spectral scans of the reaction products from both the Hg^{2+} and Ag^+ reactions showed the complete disappearence of the broad band centred around 350 nm, characteristic of RSNO, but were virtually identical with those generated from the thiol and Hg^{2+}/Ag^+. We can confirm the earlier[8] report of the reaction products, *i.e.*, as given in eqns. (4) and (5).

$$RSNO + Hg^{2+} + H_2O \longrightarrow RSHg^+ + HNO_2 + H^+ \quad (4)$$

Table 1 Values of the first-order rate constant for reaction of ***S***-nitrosocysteamine **3**, as a function of $[HgCl_2]$

$[HgCl_2]/10^{-3}$ mol dm^{-3}	k_o/s^{-1}
2.0	2.87 ± 0.04
3.0	4.39 ± 0.06
4.0	5.84 ± 0.08
5.0	7.48 ± 0.05
6.0	9.26 ± 0.06

Table 2 Values of k [eqn. (6)] for a range of ***S***-nitrosothiol structures in reaction with $HgCl_2$

RSNO	$k/10^{-3}$ dm^{3}mol^{-1}s^{-1}
S-Nitroso-***N***-acetylpenicillamine (SNAP) **1**	60.8 ± 2.7
S-Nitrosocysteine **6**	5.50 ± 0.15
S-Nitrosoglutathione (GSNO) **4**	3.86 ± 0.10
S-Nitroso-***N***-acetylcysteine **7**	3.56 ± 0.06
S-Nitroso-***N***-acetylcysteamine **8**	2.72 ± 0.01
S-Nitrosocysteine ethyl ester **9**	1.75 ± 0.05
S-Nitrosocysteamine **3**	1.50 ± 0.01
S-Nitrosohomocysteine **10**	1.46 ± 0.01
S-Nitrosocaptopril (SNOCAP) **5**	1.29 ± 0.04

$$RSNO + Ag^+ + H_2O \longrightarrow RSAg + HNO_2 + H^+ \quad (5)$$

(b) Kinetic studies with mercury salts

Rate constants were obtained by following the disappearence of the absorbance at *ca*. 350 nm due to the RSNO species. Our first experiments were carried out with mercury(II) chloride as the source of mercury(II) cations. Typical reaction conditions were, [RSNO] *ca*. 2×10^{-4} mol dm^{-3}, $[HgCl_2]$, 2–6 $\times 10^{-3}$ mol dm^{-3} and perchloric acid 0.1 mol dm^{-3}. Good first-order behaviour was always found, and the measured first order rate constant k_0 gave a good first-order dependence upon $[HgCl_2]$. Typical results are given in Table 1 for the reaction of *S*-nitrosocysteamine **3**. All of the results fit the rate law eqn. (6),

$$\text{rate} = -\text{d}[RSNO]/\text{d}t = k[RSNO]\,[HgCl_2] \quad (6)$$

and values of the second-order rate constant k are given in Table 2, for a range of RSNO structures. It is evident that there is not much of a structure–reactivity dependence; the greatest effect seems to be the acceleration resulting from the presence of the 1,1-dimethyl substituents in SNAP **1**.

When mercury(II) nitrate was used as the Hg^{2+} source we found the reactions to be very much faster, so much so that even with the stopped-flow method it was not possible to obtain rate constants under pseudo-first order conditions, as we did with mercury(II) chloride. Instead we worked with equal concentrations of RSNO and $Hg(NO_3)_2$, and found good second-order behaviour. Table 3 shows the values obtained. Again, there is very little difference in the k values over the structure range given. The reaction of SNAP was too fast to measure even by the stopped-flow method, under these conditions. There is clearly a large (*ca*. 10^3) difference in reactivity between the two mercury(II) salts, over a range of substrates. In water, mercury(II) chloride exists primarily[11] as the undissociated molecule, with other equilibria involving, *inter alia*, $HgCl^+$ [eqns. (7) and

$$HgCl_2 \longrightarrow HgCl^+ + Cl^- \quad (7)$$

(8)], and, in the presence of relatively high concentrations of

$$HgCl^+ \longrightarrow Hg^{2+} + Cl^- \quad (8)$$

chloride ion, $HgCl_3^-$, *etc*. On the other hand, mercury(II) nitrate dissolves in mildly acid solution to give free (aquated) mercury(II) ion.

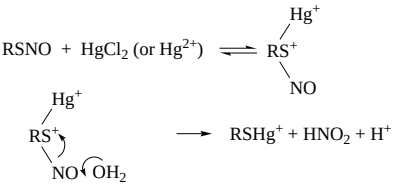
Table 3 Values of k for the reaction of some ***S***-nitrosothiols with $Hg(NO_3)_2$

RSNO	$k/10^{-6}$ dm^{3}mol^{-1}s^{-1}
S-Nitrosocysteine **6**	4.83 ± 0.28
S-Nitroso-***N***-acetylcysteine **7**	2.85 ± 0.11
S-Nitrosoglutathione (GSNO) **4**	2.65 ± 0.06
S-Nitrosocysteamine **3**	2.43 ± 0.05
S-Nitrosocaptopril (SNOCAP) **5**	1.88 ± 0.05
S-Nitroso-***N***-acetylcysteamine **8**	1.34 ± 0.04
S-Nitrosohomocysteine **10**	1.24 ± 0.06
S-Nitrosocysteine ethyl ester **9**	0.58 ± 0.02

Table 4 Values of the first-order rate constant for the reaction of GSNO **4**, with Ag^+ as a function of $[Ag^+]$

$[Ag^+]/10^{-2}$ mol dm^{-3}	$k_o/10^{-2}$ s^{-1}
0.8	0.23 ± 0.01
1.2	0.58 ± 0.01
1.6	1.02 ± 0.05
2.0	1.98 ± 0.07
2.4	3.39 ± 0.28
2.8	5.57 ± 0.13
3.2	7.35 ± 0.29
3.6	10.6 ± 0.3

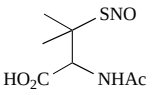
All our kinetic data are compatible with the reaction mechanism suggested by Saville,[8] Scheme 1. The first stage involves the



Scheme 1

rapid and reversible formation of the Hg–sulfur complex, which then allows a water molecule to attack the complex at the nitrogen atom generating nitrous acid. It is to be expected that the equilibrium constant for complex formation is larger when the mercury(II) source is Hg^{2+} rather than $HgCl_2$, since the concentration of Hg^{2+} is much greater in the former case and this difference accounts for the much greater reactivity of the nitrate salt which we find. For *both* salts there was a significant decrease in the measured rate constants with added chloride ion, consistent with the conversion of some free Hg^{2+} to $HgCl^+$ [eqn. (8)]. We were unable to detect a major decrease in the first-order rate constant during reaction of the $HgCl_2$ experiments, but there was a significantly better fit to the first-order rate constant when chloride ion was added, implying that chloride ion released during reaction has a small retarding effect on the reaction rate. Rate reductions in the presence of added chloride have also been reported in the mercury(II) ion promoted hydrolysis reaction of an aryl isothiocyanate.[12] There was no significant change in the rate constants when the acidity was reduced by a factor of two.

The proposed reaction mechanism for the decomposition of *S*-nitrosothiols by mercury(II) ion is similar to that put forward[13] for the acid-catalysed hydrolysis of SNAP. That reaction requires a high acid concentration (typically 2–3 mol dm^{-3} H_2SO_4), because of the low basicity ot the sulfur atom, and also needs the presence of a trap for nitrous acid (*e.g.* sulfamic acid), since the reaction lies well over on the side of RSNO. With the mercury(II) salts, the equilibrium constant for the complex formation is probably larger (due to the well-known affinity of mercury for sulfur sites), although it cannot be very large, since we find no kinetic evidence of 'saturation' at the higher mercury concentrations.



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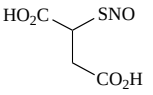

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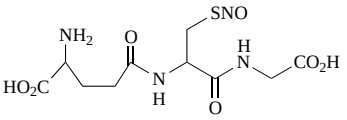

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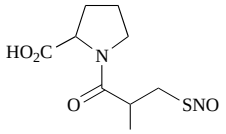

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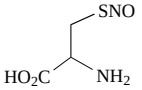

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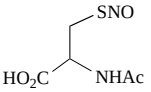

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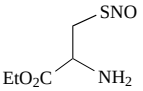

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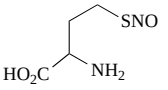

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These reactions are quite different from the recently much studied[14] reactions of RSNOs with Cu^+ (usually generated *in situ* by reduction of Cu^{2+}), which give initially, nitric oxide and the disulfide, rather than nitrous acid and the thiol (as the mercury complex). The copper-catalysed reaction is currently of much interest, ever since the spectacular discoveries were made regarding the synthesis of nitric oxide *in vivo*, and its amazing control over a range of physiological functions.[1] Another major difference between the reactions is the lack of structural dependence in the mercury(II) ion case, whereas in the copper reaction, some of the RSNOs (*e.g. S*-nitrosocysteamine **3**) revealed a large reactivity increase, indicative of bidentate coordination of Cu^+. *N*-Acetylation of many of the cysteine derivatives caused a major ($>10^3$) rate reduction in the copper reaction, which does not occur in the reaction with mercury(II) ion.

The results with SNAP suggest that the 1,1-dimethyl groups have an effect (by virtue of their inductive properties), in increasing the equilibrium constant for complex formation with mercury(II) ion. All of the results point to the fact that in the mercury(II) salt reactions, the mercury becomes bound only to the sulfur atom.

(c) Kinetic studies with silver ion

It is well-known that silver ion also has a strong affinity for sulfur sites, although this affinity, as measured by the stability constants of the thiol complexes, is not as strong as that of mercury(II) ion.[15] Saville[8] had noted that silver ion also effects hydrolysis of RSNOs, so we measured the rates of reaction of some of them. We encountered more problems with the kinetic measurements using silver ion than we did with the mercury salts, as, for many reactants, precipitation of the silver–thiol product occurred during reaction, making kinetic measurements impossible. However, this did not occur with *S*-nitrosoglutathione (GSNO) **4** nor with *S*-nitrosocaptopril (SNOCAP) **5** and so kinetic studies were limited to these *S*-nitrosothiols. We were able to work with $[Ag^+] \gg [RSNO]$, as we did with $HgCl_2$, and again excellent first-order behaviour was found. However, although values of k_0 increased with $[Ag^+]$ in both cases (see Table 4 for GSNO), there was clearly no first-order dependence on $[Ag^+]$. Analysis of the data revealed the fact that the rate is dependent on $[Ag^+]^2$.[5] This is a rather surprising result and a mechanistic explanation is not obvious. It may well be that one or two silver ions are involved in the transition state of the rate-limiting attack by a water molecule. Species such as $RSAg_2^+$ are known,[16] and a pathway for silver-ion promoted hydrolysis of an aryl isothiocyanate involving a di-silver complex with the sulfur atom of the reactant has been proposed,[12] following the finding of a second-order kinetic term in $[Ag^+]$. Since the rate law, and therefore the mechanism, is different for the silver ion reaction, is not possible to make quantitative comparisons with the mercury reactions. However it is clear that over the concentration range studied, the silver reactions are much slower, probably reflecting the lower complexing power compared with mercury(II).

Experimental

All of the thiols and metal salts were commercial samples of the highest purity grade available. The *S*-nitrosothiols were all prepared *in situ*, from the thiol solutions and nitrous acid.[14] A World Precision ISO-NO specific electrode, calibrated with ascorbic acid and nitrous acid, was used to test for nitric oxide, when oxygen was excluded from the solutions by purging with nitrogen. A modification of the Griess test[8] was used for the quantitative determination of nitrous acid.

Kinetic measurements were made spectophotometrically in water containing 0.1 mol dm^{-3} perchloric acid. Measurements were made at *ca*. 350 nm following the disappearance of the absorbance due to the RSNO. Most of the measurements were carried out on an Applied Photophysics SX17MV stopped flow spectrophotometer, using the software packages to obtain values of the rate constants. Quoted values are mean values of at least five determinations, and the standard error was always better than ± 4%.

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