Metal Ion Catalysis in Nitrosothiol (RSNO) Decomposition

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The decomposition of *S*-nitroso-*N*-acetyl D,L penicillamine (SNAP), an NO-donor drug, to give the disulfide and NO is catalysed by trace amounts of Cu^{2+} and Fe^{2+}.

Thionitrites or nitrosothiols RSNO are easily prepared in solution by electrophilic nitrosation of thiols.[1] Only a few however have been isolated and characterised because of their general instability. The most stable[2] appears to be *S*-nitroso-*N*-acetyl D,L pencillamine (SNAP). It has been suggested that thionitrites are implicated in the recently discovered role played by nitric oxide in animal physiology.[3] Certainly SNAP is both a vasodilator[4] and prevents platelet aggregation.[5] It is assumed that this takes place by NO release from SNAP, but attempts to quantify this reaction *in vitro* led to erratic results. We can now demonstrate that this behaviour results from catalysis from trace amounts of metal ions pressent in the systems. This could have profound consequences for any physiological action of thionitrites.

We have followed the decomposition of SNAP, by noting the disappearance of the absorbance at 340 nm, in water and also in a range of aqueous buffers. Under all conditions we get virtually quantitative (>90%) formation of NO_2^- as measured by the diazotisation and azo coupling method, and the disulfide. It is known[6] that in oxygenated water NO is quantitatively converted into nitrite anion with no nitrate formation. The kinetic pattern, however, did not follow a

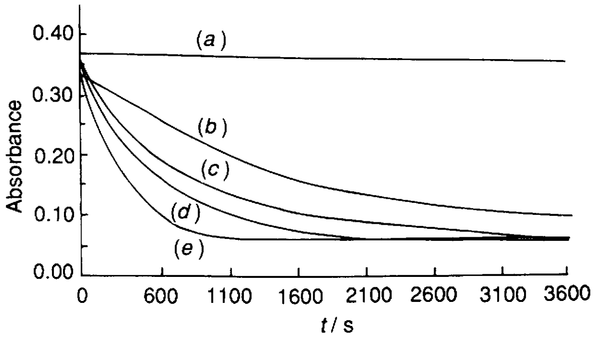


Fig. 1 Absorbance time plots for the decomposition of SNAP (5×10^{-4} mol dm^{-3}), (*a*) no added Cu^{2+}, (*b*) $[Cu^{2+}]$ 1×10^{-5}; (*c*) $[Cu^{2+}]$ 5×10^{-5}; (*d*) $[Cu^{2+}]$ 1×10^{-4} and (*e*) $[Cu^{2+}]$ 5×10^{-4} mol dm^{-3}

simple rate law and half-lives varied from week to week and were different in our two laboratories using the same sample of SNAP. Following a suggestion made at an informal seminar at the Wellcome Research Laboratories at Beckenham we investigated the effect of addition of ethylenediaminetetraacetic acid (EDTA) on the decomposition rates, and found that decomposition was dramatically reduced even in the presence of only 1×10^{-5} mol dm^{-3} EDTA; typically SNAP was effectively stable in solution (at pH 7–8) under those conditions over many hours. Addition of a slight excess of Cu^{2+} (over the EDTA) re-established the decomposition pattern. Fig. 1 shows the effect of increasing the $[Cu^{2+}]$ on the decomposition rate. With added Cu^{2+} the reactions followed first-order kinetics quite closely. We found no significant catalysis by Zn^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+} or Fe^{3+}, whereas Fe^{2+} gave results similar to those found for Cu^{2+} and there was a small degree of catalysis from Ag^+. Clearly our erratic early results were due to trace and variable amounts of Cu^{2+} or Fe^{2+}. Analysis of some of the water sources used showed that the Cu^{2+} content varied from 0.006 to 0.06 ppm compared with 0.2 ppm for tap water. Fig. 2 shows that the use of different water sources with increasing 'natural' Cu^{2+} content results in an increasing rate of decomposition. The reaction is also very pH dependent. The results given in Figs. 1 and 2 are for reactions without added buffers and refer to a pH *ca.* 3. On increasing the pH decomposition rates increase markedly, with a maximum in the region of pH 7. Further work is in progress in this area.

SNAP with added Cu^{2+} at pH 7 in the presence of *N*-methylaniline gave almost quantitative formation of *N*-methyl-*N*-nitrosoaniline. When the experiment was repeated in the complete absence of oxygen, very little *N*-nitroso product formed. These findings are consistent with

$$\begin{array}{ll}
\text{SNAP} & \xrightarrow{Cu^{2+}} \text{NO} + \text{disulfide} \\
\text{NO} + O_2 & \longrightarrow NO_2 \\
NO_2 + \text{NO} & \longrightarrow N_2O_3 \\
N_2O_3 + \text{amine} & \longrightarrow \text{nitrosamine} \\
N_2O_3 + 2OH^- & \longrightarrow 2NO_2^- + H_2O
\end{array}$$

Scheme 1

NO release, oxidation to NO_2, combination with NO to give N_2O_3 which nitrosates the amine, or in the absence of an amine yields NO_2^- (Scheme 1). In the absence of oxygen N_2O_3 cannot be formed and no nitrosation occurs.

A possible interpretation of Cu^{2+} catalysis is given in Scheme 2. Many examples of copper catalysis of organic reactions involve the interconversion $Cu^{I} \rightleftarrows Cu^{II}$. It is

$$\begin{array}{ll}
Cu^{2+} + RS^- & \longrightarrow Cu^+ + RS\cdot \\
Cu^+ + RSNO & \longrightarrow [RSNO\cdot Cu]^+ \\
[RSNO\cdot Cu]^+ & \longrightarrow RS^- + NO + Cu^{2+}
\end{array}$$

Scheme 2

expected that there would be sufficient thiol (and so thiolate) present even in 'pure' SNAP to allow the above to occur.

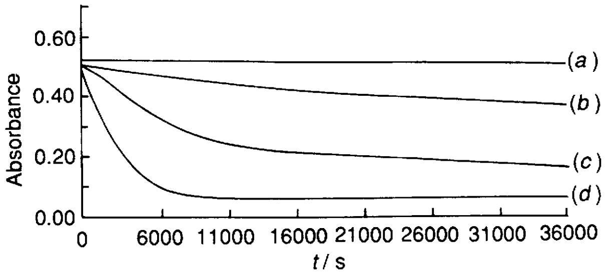
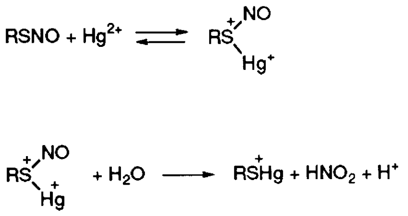


Fig. 2 Absorbance time plots for the decomposition of SNAP (5×10^{-4} mol dm^{-3}), (*a*) with added EDTA (3×10^{-5} mol dm^{-3}), (*b*) in 'new' distilled water ($[Cu^{2+}]$ 0.006); (*c*) in 'old' distilled water ($[Cu^{2+}]$ 0.059) and (*d*) in tap water ($[Cu^{2+}]$ 0.198 ppm)



Scheme 3

Addition of the thiol *N*-acetylpencillamine enhanced Cu^{2+} catalysis. Examples of Cu^{II} catalysis of thionitrite decompositions are known, for example[7] some thionitrites can be used to deaminate arylamines in acetonitrile in the presence of Cu^{II} halide. The pH dependence is very unusual and may involve equilibria involving ligand replacement. For example it is known[8] that the reaction $[Cu(OH_2)_6]^{2+} \rightleftarrows [Cu(OH_2)_5OH]^+ + H^+$ has a pK_a value of 6.8. The pH dependence may also result from the $RS^- + H^+ \rightleftarrows RSH$ equilibrium.

We also find that Hg^{2+} catalyses thionitrite decomposition. This reaction is known[9] and is the basis of an analytical procedure for thiol determination. The Hg^{2+} reactions are very fast (much faster than the Cu^{2+} reactions) and that Hg^{2+} is required in at least stoichiometric quantitites (again different from the Cu^{2+} case) to effect quantitative decomposition. All of our results suggest that the Hg^{2+} reaction releases NO^+, as outlined in Scheme 3, as suggested initially by Saville,[9] and not NO.

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