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Direct NO group transfer from *S*-nitrosothiols to iron centres

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A number of *S*-nitrosothiols react rapidly with the Fe(II) complexes of 2,3-dimercapto-1-propanesulfonic acid (DMPS) and of *N*-methyl-D-glucamine dithiocarbamate (MGD), transferring the NO group directly to the iron centres.

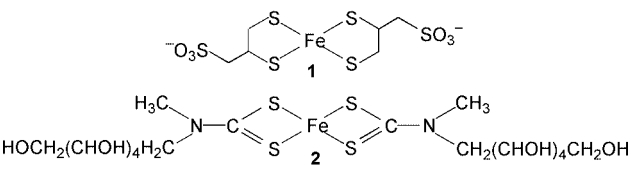
S-Nitrosothiols (RSNO) are now widely believed to be important species in the storage and transport of nitric oxide *in vivo*.[1] Since they generally show the same physiological properties as does nitric oxide itself (notably the induction of vasodilation and inhibition of platelet aggregation[2]), it has been generally assumed that RSNO species act in this way by first releasing nitric oxide. Release of NO from an RSNO can be brought about by at least three routes: (a) a spontaneous thermal reaction, which is too slow at ambient temperatures to be important, (b) a photochemical reaction, which is also unimportant in the absence of appropriate incident radiation, and (c) a rapid copper(II) catalysed process.[3] For reaction (c) it has been shown[4] that decomposition is brought about by Cu^+ generated by thiol reduction of Cu^{2+}. Even low $[Cu^{2+}]$ at the impurity level are effective. Cu^+ can also be obtained by reduction from Cu^{2+} bound to proteins and peptides.[5] Complexation of Cu^+ in biological experiments results in loss of some biological function.[6] Low levels of [RSH] always present in equilibrium with RSNO[7] are sufficient to bring about the reduction. All three decomposition pathways initially generate nitric oxide and the corresponding disulfide, [eqn. (1)] although in the

$$2\mathrm{RSNO} \rightarrow \mathrm{RSSR} + 2\mathrm{NO} \qquad (1)$$

presence of oxygen oxidation of nitric oxide occurs and the final product in aqueous buffer is nitrite ion.

In addition, *S*-nitrosothiols react readily with many nucleophiles transferring the NO group in the NO^+ sense to, for example, amines, thiols and ascorbate[3] directly, without it ever becoming free, *i.e.* they can act as electrophilic nitrosating agents. Many of the biological properties of NO are believed to occur by activation of the enzyme guanylate cyclase, through the binding of NO to a haem iron atom in the enzyme. This leads to elevated levels of cyclic guanosine monophosphate (cGMP) which effects smooth muscle relaxation. It is an interesting and pertinent question as to whether RSNO compounds can achieve this without NO formation, *i.e.* whether they can transfer the NO group directly to an iron atom.

We have chosen initially to look at a simple non-haem iron compound. Iron(II) readily forms a complex $[Fe(DMPS)_2]^{4-}$ with 2,3-dimercaptopropane-1-sulfonate (DMPS) shown as **1**.[8]



This is stable in solution under anaerobic conditions and the solution is red with absorbance maxima at 358 and 509 nm. This iron complex has a strong affinity for NO and has been used industrially as a scrubber of flue gases to remove NO. The nitrosyl complex has been characterised.[9]

The complex is readily prepared in aqueous solution from Fe^{2+} and a two-fold excess of DMPS. Addition of *S*-nitrosoglutathione (GSNO), generated in solution from equimolar quantities of nitrous acid and glutathione, to a solution of **1** at physiological pH 7.4 resulted in the rapid disappearance of the absorbance at 509 nm. The final absorbance spectrum was identical with that obtained when NO gas was reacted with $[Fe(DMPS)_2]^{4-}$ and is taken to be that of $[Fe(DMPS)_2NO]^{3-}$. Kinetic measurements, noting the decreasing absorbance at 509 nm, were made with [GSNO] >> $[Fe(DMPS)_2{}^{4-}]$. Each individual run followed the first-order law and there was also a first-order dependence on [GSNO], leading to a second-order rate constant value k [defined by eqn. (2)] of 24 $dm^3\ mol^{-1}\ s^{-1}$

$$\mathrm{Rate} = k\,[\mathrm{Fe(DMPS)_2}^{4-}]\,[\mathrm{RSNO}] \qquad (2)$$

at 25 °C. The *S*-nitroso derivatives of *N*-acetylcysteine, cysteine, homocysteine and captopril (all generated *in situ*) behaved similarly yielding comparable k values (6.1, 71, 16 and 7.5 $dm^3\ mol^{-1}\ s^{-1}$ respectively). The nitrosyl complex from *S*-nitrosocysteine was identical (spectrally) with that derived from GSNO.

An HPLC analysis of the final solution from the reaction of GSNO with the iron complex showed that the other product of reaction between GSNO and the iron complex was glutathione (GSH) in > 80% yield. This oxidised slowly to give some of the disulfide GSSG on standing in air for several hours. Initial (essentially quantitative) formation of glutathione indicates that the NO group is being transferred as NO^+ rather than as NO. That the same complex is formed by reaction with NO itself means that there must be an oxidation step in that process. It is not unknown in the synthesis of metal nitrosyls for the formal oxidation state of the nitrosyl ligand in the product to be different from that in the reactants.[10]

GSNO also reacts rapidly with the Fe^{2+} complex of *N*-methyl-D-glucamine dithiocarbamate (MGD) shown as structure **2**. The reaction is rapid and much faster than the 'spontaneous' release of NO from GSNO under the same conditions. The Fe^{2+} complex of MGD is EPR active and has been used recently as a trap for NO.[11] Reaction occurs rapidly with NO generated in solution giving the NO complex with a characteristic EPR spectrum. We find that the same UV-Visible spectrum is generated from the reactions of GSNO and NO with the Fe^{2+} complex of MGD. Strangely here in our reaction, the other product is GSSG and not GSH as in the DMPS case. This implies that with the DMPS complex NO is delivered in the NO^+ sense, whereas with the MGD complex it appears that it is in the NO sense. Our principal conclusion is that a direct bimolecular process occurs in the reactions of RSNO compounds with these two iron complexes and there is no prior release of NO. Our product studies also show that this NO-transfer reaction can occur in the NO or NO^+ sense depending presumably on which is the more favoured ligand in the resulting nitrosyl complex. Both reactions are far too rapid for the results to be explained mechanistically in terms of prior breakdown of RSNO to yield NO.

Our results indicate that when considering the reaction of an *S*-nitrosothiol with a biological target, the possibility of a direct

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bimolecular process must be considered. Transfer may occur as either NO or NO^+.

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