Transnitrosation between Nitrosothiols and Thiols

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Transfer of the nitroso group from nitrosothiols to thiols occurs very readily in aqueous solution particularly at pH > ≈8. The results are consistent with attack by the thiolate anion at the nitroso nitrogen atom of the nitrosothiol. Results have been obtained for the reaction of *S*-nitroso-*N*-acetylpenicillamine (SNAP) with thioglycolic acid and also for the reaction of *S*-nitrosocysteine (SNCys) with thiomalic acid. Both reactions showed the same kinetic characteristics. The results are discussed in terms of transnitrosation reactions of nitroso compounds generally, and also in the case of nitrosothiols, in terms of possible *in vivo* transnitrosation and subsequent decomposition of a possibly more unstable nitrosothiol to yield nitric oxide; this may have implications for the mechanism of action of nitric oxide in a range of physiological processes.

Nitrosothiols (or thionitrites) RSNO[1] are generally not as well-known as are the corresponding alkyl nitrites RONO, principally because of their greater instability, particularly with regard to S–N homolytic bond fission, and consequent difficulties in the preparation of pure samples. A number however have been isolated as relatively stable materials, notably *S*-nitroso-*N*-acetylpenicillamine (SNAP) which has indefinite stability in the solid state and *S*-nitrosoglutathione (SNOG). All can be made readily in solution by *S*-nitrosation[2] of thiols using any of the standard carriers of NO^+ such as acidified nitrous acid, dinitrogen trioxide, dinitrogen tetroxide *etc.* Generally nitrosation of thiols is a very rapid process[3] and is in effect irreversible, contrasting with the situation for the corresponding *O*-nitrosation reactions of alcohols. In recent years nitrosothiols have become more a focus of attention in connection with the recently discovered amazing range of biological activity of nitric oxide, synthesised from L-arginine *in vivo*.[4] One aspect of the chemistry of RSNO species is their known ability to release nitric oxide, which may be connected with their known vasodilatory properties and ability to inhibit platelet aggregation. Additionally some workers believe that nitrosothiols are more intimately involved in the biological processes,[5] possibly involving nitrosothiols from tissue-bound thiol groups or from free glutathione.

Loss of nitric oxide from nitrosothiols can occur photochemically[6] or by a recently discovered[7] Cu^{2+}-catalysed reaction where Cu^{2+} is present in catalytic amounts [eqn. (1)].

$$2RSNO \xrightarrow[\text{or } Cu^{2+}]{h\nu} RSSR + 2NO \qquad (1)$$

Under aerobic conditions at pH > 4 the final fate of the released nitric oxide is nitrite anion, presumably after oxidation to NO_2 and subsequent formation of N_2O_3. Under certain circumstances electrophilic nitrosation *e.g.* of added amines[8,9] can occur, presumably again *via* the intermediacy of N_2O_3.

The question arises as to whether nitrosothiols can transfer the NO group directly to a suitable nucleophile, without the intermediacy of nitric oxide. This can readily be achieved with alkyl nitrites particularly with very reactive nucleophiles such as thiolate anions[10] in a reaction which has all of the characteristics [eqn. (2)] of nucleophilic attack by the thiolate

$$\begin{array}{ccc} RONO + R'S^- & \longrightarrow & RO^- + R'SNO \\ {\scriptstyle H^+}\uparrow\downarrow & & \\ R'SH & & \end{array} \qquad (2)$$

anion. The pH–rate constant profile accords with such a mechanism, and reaction is much favoured by the presence of electron-withdrawing substituents in the R group of the alkyl nitrite.

There are indications in the literature that this transnitrosation reaction involving nitrosothiols and thiols does occur. For example in organic solvents, treatment of a nitrosothiol with a thiol[8] leads to the unsymmetrical disulfide [eqn. (3)]

$$RSNO + R'SH \longrightarrow RSSR' \qquad (3)$$

probably *via* an initial transnitrosation process. More recently[11] it has been shown that a reaction occurs in water at pH 7.4 between SNOG and cysteine, leading again to formation of the mixed disulfide.

We have set out in this work to establish whether a direct reaction between nitrosothiols and thiols (or thiolate ions) exists and if so to establish whether such a reaction occurs *via* the prior formation of nitric oxide or whether a direct nucleophilic attack by thiolate ion occurs, as in the case of the corresponding reactions of alkyl nitrites.

Results and Discussion

It is easy to observe a nett transnitrosation reaction spectrophotometrically. For example when SNOG (5×10^{-4} mol dm^{-3}) and *N*-acetylpenicillamine (5×10^{-3} mol dm^{-3}) are mixed in a buffer of pH 7.4, there is a rapid colour change from red to green and the absorbance at 545 nm due to SNOG is replaced by one at 590 nm which is a characteristic absorption of SNAP, which was checked independently with an authentic sample.

Two reactions were examined kinetically using stopped-flow spectrophotometry for most of the experiments but conventional spectrophotometry for the slower reactions. In the first set of experiments we followed the reaction of SNAP with thioglycolic acid ($HSCH_2CO_2H$) over the pH range 6.5–11.7 noting the decreasing absorbance at 600 nm due to SNAP. Since the change in extinction coefficient is quite small at this wavelength we were forced to work with quite high reactant concentrations which necessitated the use of 40% dioxane–water as the solvent. In the second set of experiments we studied the reaction of SNCys with thiomalic acid [$HO_2CCH_2CH(SH)CO_2H$] over the pH range 6.5–12.7 noting the increasing absorbance at 340 nm due to the product nitrosothiol, this time in a completely aqueous medium. Reactions were carried out with $[R'SH]_0 \gg [RSNO]_0$ and good first-order behaviour was

Table 1 Variation of k_0 with [thiomalic acid] in the reaction of SNCys (5×10^{-4} mol dm^{-3}) at pH 7.75

[Thiomalic acid]/10^{-3} mol dm^{-3}	k_0/s^{-1}
5.0	0.084
10	0.162
15	0.334
20	0.460

Table 2 Values of k_0 for the reaction of SNAP (2.0×10^{-2} mol dm^{-3}) with thioglycolic acid (4.0×10^{-1} mol dm^{-3}) as a function of pH

pH	k_0/s^{-1}
6.5	7.1×10^{-3}
9.8	1.09
10.1	6.28
10.8	16.2
11.1	29.2
11.4	43.4
11.7	69.3

Table 3 Values of k_0 for the reaction of SNCys (5.0×10^{-4} mol dm^{-3}) with thiomalic acid (2.0×10^{-2} mol dm^{-3})

pH	k_0/s^{-1}
6.56	0.056
6.95	0.090
7.25	0.148
7.82	0.445
8.65	1.70
9.75	2.52
10.2	2.79
10.4	2.96
12.7	3.06

found throughout. For the reaction of SNCys with thiomalic acid at pH 7.75 the variation of the first order observed rate constant k_0 with [thiomalic acid] given in Table 1, shows that within the experimental error the reaction is first-order with respect to [thiomalic acid]; the results yield a value of 24 dm^3 mol^{-1} s^{-1} for the second-order rate constant at this pH. The details of the variation of k_0 with pH for both reactions are given in Tables 2 and 3. It is clear that k_0 increases with pH, tending towards an upper limit at high pH. The point of inflexion corresponds with the pK_a of the thiol (~10.7 for thioglycolic acid[12]). The S-shaped curve is characteristic of reaction *via* the anion form of an acid *i.e.* the thiolate ion in this case. The reaction parallels those between alkyl nitrites and thiols[10] and is an example of nucleophilic attack by thiolate at a nitroso-nitrogen atom. At this stage no direct comparison between the reactivities of RSNO and RONO can be made but it is to be expected that RSNO would be the more reactive given the better leaving group ability of RS^- compared with RO^-

We can rule out the reaction pathway for transnitrosation to thiols *via* the prior formation of nitric oxide and subsequent oxidation to give an electrophilic nitrosating agent (probably N_2O_3) because the rates of formation of NO from SNAP and SNCys are orders of magnitude smaller[7,13] than are the observed rates of transition. The former reactions occur *via* a Cu^{2+}-catalysed process[7] and we now have substantial rate data on a range of RSNO species.[13] It is not possible that the Cu^{2+} concentration naturally present (*via* the water supply) in our transnitrosation reactions is large enough to allow any sort of competition between direct transition and NO fission. As a check, we carried out some of the transnitrosation experiments in the presence of EDTA (which would complex any Cu^{2+} present). There was no difference in the kinetic behaviour.

Direct transnitrosations are reasonably well characterised for a number of NO containing species. For example alkyl nitrites, apart from their reaction with thiolate anions can bring about direct nitrosation of amines under alkaline conditions[14] and also of alcohols.[15] Similarly it has been shown that a nitrososulfonamide can nitrosate thiolate anions,[16] amines[17] and carbanions[18] in a direct reaction, whilst nitrosamines can transfer directly the –NO group to halide ions, thiourea, *etc.*[19] Earlier we had noted that the protonated form of a nitrosothiol can transfer the –NO group to nucleophiles Cl^-, Br^-, SCN^- and $SC(NH_2)_2$ (ref. 20) but this could only occur at a reasonable rate at high acid concentrations, typically 2 mol dm^{-3} H_2SO_4.

The relative ease with which nitrosothiols can transfer the –NO group to thiolate ions allows the possibility that NO release could arise *in vivo* by the transfer of the –NO group from a relatively stable (*i.e.* to NO loss) nitrosothiol such as SNOG to yield another nitrosothiol which is much more labile towards NO loss by the Cu^{2+} catalysed pathway.

Experimental

Both SNAP and SNOG were prepared and isolated as stable solid materials according to literature procedures.[9,21] Other nitrosothiols were prepared in acid solution from the corresponding thiol and nitrous acid and then used immediately in the transnitrosation experiments after pH adjustment. All other materials were of the highest purity grade available commercially.

Most of the kinetic studies were carried out using stopped-flow spectrophotometry, although some of the slower reactions (at low pH) were monitored using conventional spectrophotometry. For the reaction of SNAP with thioglycolic acid, reaction was followed at 600 nm noting the decreasing absorbance as the colour changed from green to red. Because the extinction coefficients are quite small in this region, rather high concentrations of reactants were used, typically 0.4 mol dm^{-3} thioglycolic acid and 0.02 mol dm^{-3} SNAP. To ensure full solubility of SNAP the solvent was changed from pure water to 40% dioxane–water. In the other case, the reaction of SNCys with thiomalic acid reaction was followed at 340 nm. In this region the absorbance change (an increase) was sufficiently large to use typically concentrations of thiomalic acid of 2.0×10^{-3} and SNCys 5.0×10^{-4} mol dm^{-3} which enabled the reactions to be carried out in pure water solvent. In all cases at least six duplicate kinetic measurements were made; good first-order behaviour (in [RSNO]) was found throughout and the reproducibility of the first-order rate constant k_0 was always better than ±5%. Rate constants were obtained using the normal integrated form of the first-order rate equation on a PC interfaced with the spectrophotometers.

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