The thermal stability of *S*-nitrosothiols: experimental studies and *ab initio* calculations on model compounds

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One factor responsible for the enhanced thermal stability of *S*-nitroso-*N*-acetylpenicillamine (SNAP), compared with *S*-nitroso-*N*-acetylcysteine (SNAC), has been shown, by *ab initio* calculations on model compounds, to be steric interactions in the dimerisation reaction leading to disulfide formation. Studies using DSC and TGA indicate that the two *gem* methyl groups in SNAP do not have a substantial effect on the strength of the –S–NO bond.

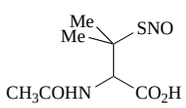
Introduction

One of the many consequences of the discovery of the roles of nitric oxide (NO) in animal physiology[1] has been heightened interest in NO derivatives, particularly *S*-nitrosothiols (RSNO) which decompose[2] with formation of NO and a disulfide. *S*-

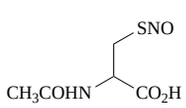
$$2RSNO \longrightarrow RS{-}SR + 2NO$$

Nitrosocysteine was proposed[3] as an alternative to NO during early attempts to identify the endothelium-derived relaxing factor (EDRF).[4] This view has recently been refuted.[5]

The formation of *S*-nitrosothiols by reaction of nitrous acid with the appropriate thiol is readily detected spectroscopically, but most *S*-nitrosothiols are far too reactive to isolate and characterise.[6] There are two notable exceptions to this generalisation: *S*-nitroso-*N*-acetylpenicillamine (SNAP) and *S*-nitro-



SNAP



SNAC

soglutathione (GSNO). The former can be readily obtained as an analytically pure, pink solid which is stable enough for the determination of its structure by X-ray crystallography.[7] In contrast, *S*-nitroso-*N*-acetylcysteine (SNAC) appears to have a halflife of only seconds in aqueous solution.[8] The difference in stability between SNAP and SNAC, in view of their similar chemical structures, has been a matter of interest and speculation. Its origin has been ascribed to the two *gem* methyl groups in SNAP, and we report now an attempt to understand the effect.

Before continuing it is necessary to describe a complication which occurs when *S*-nitrosothiols are in aqueous solution. Our initial efforts to study the kinetics of *S*-nitrosothiol decomposition in solution in which we compared, *inter alia*, the rates of reaction of SNAC, prepared *in situ*, and SNAP were frustrated by erratic and irreproducible results. The explanation was that copper ions are very powerful catalysts of *S*-nitrosothiol decomposition and even the concentration of copper ions in good, distilled water is sufficient to effect reaction.[9] A further complication appeared when it was confirmed that copper(I) ions, rather than the more readily available copper(II) ions, are the effective catalyst. In an extensive study of this effect[10] we proposed that copper(I) ions act by forming a complex with SNAP or SNAC from which NO is readily lost. Calculations to elucidate the sites of Cu^+ complexation in *S*-nitrosothiol are now in progress. In the copper-catalysed reaction the effect of the two *gem* methyl groups could be merely to lower the formation constant of the reactive intermediate complex and thus give to SNAP an enhanced stability. However, careful examination of the results reported in refs. 8 and 10 indicate that in addition to the copper-catalysed reaction there is thermal decomposition, and that SNAP is more stable than SNAC also in the latter pathway to NO release, which becomes dominant at very low copper concentrations (10^{-8} M).

There is EPR evidence[11] that thiyl radicals are formed during *S*-nitrosothiol decomposition and so the mechanism must be a two-step process, eqns. (1) and (2). The two *gem* methyl groups

$$RSNO \longrightarrow RS^{\bullet} + NO \quad (1)$$

$$2RS^{\bullet} \longrightarrow RS{-}SR \quad (2)$$

could affect either of these two steps, (1) by increasing the strength of the –S–NO bond or (2) by influencing the ability of the two thiyl radicals to dimerise. We have addressed both these possibilities, (1) through studies of *S*-nitrosothiol decomposition by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) and (2) by comparing the amounts of steric conflict in the disulfide products formed from dimerisation of cysteinyl and penicillamyl radicals. In previous *ab initio* calculations[12] diethyl disulfide (CH_3–CH_2–S–S–CH_2–CH_3, ESSE) was used as a model compound for the L-cystine disulfide bridge. It was shown that the molecule has six different conformational minima with energies varying within 6.9 kJ mol^{-1} due to small inherent differences in the electronic stabilities, as well as to various amounts of steric conflict between the terminal methyl groups. The scope of the *ab initio* calculations presented here was to establish the level of steric strain in the penicillamine disulfide through calculations using di(*tert*-butyl) disulfide (tBSStB) as a model compound.

Experimental

SNAP[4] and GSNO[13] were prepared by literature methods. The DSC was performed on a Perkin-Elmer DSC 7 and the TGA on a TA Instruments SDT 2960.

All *an initio* optimisations were carried with the GAUSSIAN94 program[14] at the MP2/6-31G*//MP2/6-31G* level of theory, with inclusion of second-order electron correlation effects. Previous calculations on ESSE[12] and ethyl hydrodisulfide (CH_3CH_2SSH, ESSH)[15] indicated that this is an adequate choice for studies of energy minima for the selected compounds. The minimisation of the fully relaxed tBSStB

structure required 49 h of CPU-time on a DEC Alpha 3000/900 computer, and the use of still larger basis sets was not feasible.

The amount of steric strain of a particular molecular conformation is normally estimated by calculating first the energy (absolute or relative) of a conformation with negligible steric interactions and then the energy of the strained conformer, interpreting the difference as a measure of steric conflict. One thus ignores differences in the inherent electronic stabilities, which may, in many cases, be a good approximation. For tBSStB this technique is not possible since the molecule has just one single minimum conformation. Hence, we decided to obtain an *ab initio* estimate in an indirect manner. Calculations for two ESSE minima were also included for comparison with previous results. These are the absolute energy minimum with C–C–S–S and C–S–S–C torsion angles all *gauche*+ (code *GGG*) and the least favourable minimum with the two C–C–S–S torsion angles *gauche*− and C–S–S–C *gauche*+ (code *G′GG′*).

The energetic costs of the molecular deformations observed in the disulfide dimers have been estimated by considering, for each model compound, two different molecular fragments.

Fragment 1

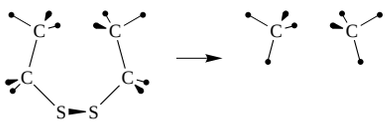
X–S–S–H, X = *tert*-butyl or ethyl. Used to study deformation of the covalent geometry of the alkyl group. The molecular geometry of the alkyl group was kept fixed as obtained in the tBSStB and ESSE disulfides, with only the –S–S–, –S–H bond lengths, the –C–S–S– and –S–S–H bond angles and the –C–S–S–H torsion angle free to refine. The energies were then compared with those of the fully optimized X–S–S–H structures, giving for each the deformation energy Δ1.

Fragment 2

CH_3–S–S–CH_3 (dimethyl disulfide, MSSM). The –S–S– bond lengths, the –C–S–S– bond angles and the –C–S–S–C– torsion angles were fixed as in the respective disulfide, with other parameters free to refine. The energies were then compared with those of the fully optimised CH_3–S–S–CH_3 structure, giving for each the deformation energy Δ2.

When considering molecular deformation energies we refrained from using further single point calculations, since it is our experience that application of other (larger) basis sets than the one used when obtaining the minimum structure invariably leads to an overestimation of the associated energy penalties.

In addition to direct structural modifications of the disulfides studied, there is also a contribution to the total energy from through-space van der Waals' contacts between terminal methyl groups in ESSE (*G′GG′*) and tBSStB. The associated interaction energies were estimated by, in each case, deleting all other S, C and H atoms, except the (CH_3)C atoms which were transformed into H-atoms. This procedure generated a methane dimer, shown for ESSE (*G′GG′*) in Scheme 1, for which the



Scheme 1

interaction energy was obtained from single point calculations at the MP2/6-311++G(2d,p)//MP2/6-31G* level, corrected for basis set superposition error by the full counterpoise correction method of Boys and Bernardi.[16]

A similar method has been used for ESSE (*GGG*) which has a 2.63 Å H···H contact between ethylene groups. In this case all atoms other than the ethylene C-atoms were deleted, the C-atoms being transformed to H-atoms leaving a H_2 dimer. This gives a rather gross approximation of the van der Waals' energy since the H–H bond is not as polar as a C–H bond, and no atoms carry partial charges, but this should not be a serious problem since this is a weak interaction with a small energy contribution.

Table 1 Deformation energies and van der Waals' repulsion (vdW/kJ mol^{-1}) in ESSE and tBSStB structures

Molecule (conformation)	Δ1	Δ2	vdW	SUM[a]
ESSE(*GGG*)	0.09	0.14	1.06	1.38
ESSE(*G′GG′*)	0.42	6.52	1.62	8.99
tBSStB	0.52	9.27	2.37	12.68

[a] 2 × Δ1 + Δ2 + vdW.

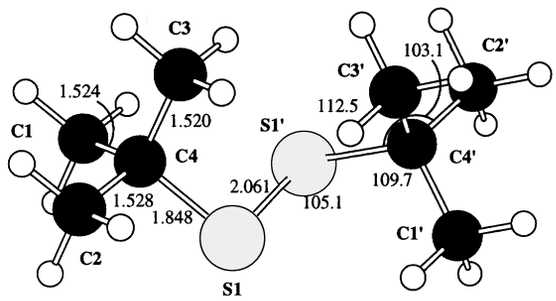
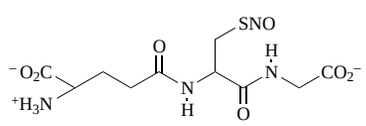


Fig. 1 MP2/6-31G* molecular geometry of the C_2-symmetric tBSStB energy minimum structure with atomic numbering, bond lengths (Å) and bond angles (°). C1 is *gauche*+ to the SS-bridge, C2 is *trans* and C3 is *gauche*−.

Results and discussion

The complications induced by the Cu^+ catalysed pathway can be avoided if we look at the solid state decomposition of *S*-nitrosothiols. However, SNAC cannot be obtained as a solid and so we chose the nearest, stable *S*-nitrosothiol of similar structure, which is GSNO. In view of the greater



GSNO

complexity of this molecule, results must be used with some caution.

When SNAP was subjected to DSC it was found to decompose very cleanly at 148 °C. Use of TGA showed that the change at 148 °C is consistent with loss of NO. Clearly SNAP is a rather stable substance and the general insistence that it should be stored refrigerated is not well founded. With GSNO the results of DSC were a little more difficult to interpret. There is a general drift in the base line which we now ascribe to loss of moisture, but a much sharper heat absorption occurred at 148 °C. Study by TGA confirmed that this change is again consistent with loss of NO. These data suggest that the two *gem* methyl groups have little or no effect on the strength of the –S–NO bond, and that the enhanced thermal stability of SNAP in solution may reside in radical dimerisation.

Results from the *ab initio* calculations are given in Table 1,† with molecular geometry for tBSStB indicated in Fig. 1. Due to the C_2 symmetry of the molecules considered, the total energy of deformation, E_d, is then calculated as $E_d = 2\,\Delta1 + \Delta2 + vdW$, where vdW is the van der Waals' repulsion.

The calculated steric hindrance for tBSStB is 12.7 kJ mol^{-1}. In comparison, the steric hindrance of the ESSE (*GGG*) minimum is very moderate at 1.4 kJ mol^{-1}, which is 7.6 kJ mol^{-1}

† Complete listings of molecular geometries and absolute energies of all molecular fragment studied are available from the authors on request.

Table 2 MP2/6-31G* molecular geometry (Å,°) for various disulfides

Molecule (conformation)	S–S	C–S–S	C–C–S–S	C–S–S–C/H
MSSM	2.054	102.1	—	85.1
ESSH (*GG*)	2.063	102.5	65.7	89.2[a]
ESSH (*G′G*)	2.063	102.3	−69.4	89.2[a]
tBSSH	2.061	104.5	61.0	89.7
			179.3	
			−62.4	
ESSE (*GGG*)	2.056	102.3	68.3	87.2[b]
ESSE (*G′GG′*)	2.064	103.4	−70.9	111.4[b]
tBSStB	2.061	105.1	61.0	113.3
			178.6	
			−62.4	

[a] From ref. 15. [b] From ref. 12.

less than the *G′GG′*. From calculations on ESSH, it has been shown that with positive disulfide chirality a *gauche*− rotamer for the C–C–S–S torsion is inherently more stable than the *gauche*+ rotamer by 0.34 kJ mol^{-1}. With two such torsions in ESSE, the *G′GG′* conformation is tentatively 0.68 kJ mol^{-1} more stable than the *GGG* conformation in the absence of steric conflict. Combining these figures one arrives at a 6.9 kJ mol^{-1} estimate for the conformational energy difference between ESSE in *GGG* and *G′GG′* conformations. The actual energy difference was calculated to 7.3 kJ mol^{-1} at this level of theory. These values are surely sufficiently close to lend credit to the procedure used for calculating steric conflict, and confidence to the 12.7 kJ mol^{-1} estimate for tBSStB.

It is obvious from Table 1 that the strain in tBSStB and ESSE (*G′GG′*) is relieved mainly at the central disulfide bond. Data in Table 2 show that while the S–S bond lengths and C–S–S bond angles change little with dimerisation, the C–S–S–C torsion angles undergo major shifts, from 87.2° in ESSE (*GGG*) to 111.4° in ESSE (*G′GG′*). In ESSE the C–C–S–S torsion angles deviate slightly from the ideal staggered positions, but the *tert*-butyl groups in tBSStB are almost perfectly staggered, forcing a further opening of the C–S–S–C torsion angle to 113.3°. This theoretical value is close to the 113.2° mean value for the C–S–S–C torsion angles of penicillamine disulfide bridges in three crystal structures: *meso*-penicillamine disulfide dihydrate[17] = 119.4°, D-penicillamine disulfide dihydrochloride[18] = 114.7° and [D-Pen^2, D-Pen^5]enkephalin[19] = 105.6° (average of three molecules with closely related conformations in the asymmetric unit).

Conclusions

When a disulfide bridge is formed from cysteine residues, it can adopt a number of conformations. For some of these, steric conflict from close H···H contacts is negligible. A disulfide bridge formed from penicillamine, on the other hand, is inevitably forced into a high energy sterically congested conformation in which the C–S–S–C torsion angle has been opened *ca*. 25° from the values observed in sterically unstrained molecules. This observation may be important, if in the solution decomposition of an *S*-nitrosothiol, step (1) above is an equilibrium

$$RSNO \rightleftharpoons RS^{\bullet} + NO \qquad (1')$$

(1′) and the rate-determining process is thiyl radical dimerisation (2). In solution, in contrast to the situation in the solid

$$2RS^{\bullet} \longrightarrow RS{-}SR \qquad (2)$$

state, NO and the thiyl radical may recombine, and decomposition occurs only with thiyl radical dimerisation.

This study gives insight into one factor to be taken into account in designing *S*-nitrosothiols as NO-donor drugs when thermal stability is a matter of importance. Already GSNO has been used clinically to inhibit platelet aggregation during coronary angioplasty,[20] and other *S*-nitrosothiols are currently under scrutiny.

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