S−N Dissociation Energies of *S*-Nitrosothiols: On the Origins of Nitrosothiol Decomposition Rates

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The importance of nitric oxide (NO) as a potent biological signaling agent has been clearly demonstrated, and considerable evidence exists supporting the role of *S*-nitrosothiol (RSNO) compounds in the uptake, intracellular trafficking, and release of NO groups in biological systems.[1−4] Quantitative understanding of the in vivo transport of NO by *S*-nitrosothiols is vital to the development of new NO therapeutic agents.

The reported stabilities of *S*-nitrosothiols vary widely, primarily as a function of substitution at sulfur. Primary and secondary RSNOs are commonly reported to be highly unstable, with half-lives of seconds to minutes,[5] with a few notable exceptions. On the other hand, many tertiary *S*-nitrosothiols are stable to isolation and long-term storage; *S*-nitroso-*N*-acetylpenacillamine exemplifies such a stable, tertiary RSNO.[6]

The role of S−N bond homolysis in *S*-nitrosothiol decomposition is unclear. Williams has reported the half-life for *S*-nitrosocysteine as 55 h at 25 °C,[3] while conflicting reports of greatly diminished stabilities have also appeared.[5] To address this issue, bond dissociation enthalpies (BDEs) for representative RSNOs of differing substitution have been computed, and rates of unimolecular decomposition have been evaluated experimentally.

Quantum mechanical calculations were carried out on a series of model RSNOs, utilizing the CBS-QB3 methodology of Petersson et al.[7,8] This method is known to predict thermochemical quantities typically to within 2 kcal/mol of experiment. Table 1 lists the predicted S−N bond dissociation energies and free energies of dissociation for representative primary though tertiary *S*-nitrosothiols **1**−**4**, along with vinyl-substituted thiol **5**, a model for *S*-nitrosobenzenethiol **9**.

Table 1. Computed Energetic Parameters (CBS-QB3, kcal/mol) for S−N Bond Dissociation in *S*-nitrosothiols **1**−**5**

S-nitrosothiol	BDE [a]	$\Delta G^{\ddagger}$	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$ [b]	ΔG_{rxn} [c]
CH_3SNO (**1**)	32.4	29.5	30.3	2.8	21.5
CH_3CH_2SNO (**2**)	32.0				21.4
$(CH_3)_2CHSNO$ (**3**)	32.4				21.5
$(CH_3)_3CSNO$ (**4**)	31.3				20.1
CH_2CHSNO (**5**)	23.3				12.5

[a] Enthalpic difference between RSNO and separated radicals at 298 K, 1 atm. [b] cal/mol·K, 298 K, 1 atm. [c] Free energy difference between RSNO and separated radicals at 298 K, 1 atm.

Immediately evident is the similarity in S−N BDEs predicted for the family of RSNOs. With the exception of vinyl-substituted *S*-nitrosothiol **5** (yielding a resonance-stabilized thiyl radical) the predicted BDEs are essentially constant, with values of ca. 31−32 kcal/mol. The calculated BDEs for these RSNOs are somewhat less than those found for the O−O bonds of dialkyl peroxides (ca. 34−39 kcal/mol)[9,10] which possess half-lives with respect to unimolecular O−O homolysis of weeks to years at temperatures of ca. 25−40 °C.[11]

The variational transition state[12] for dissociation of **1**, the position of the free energy maximum along the dissociation coordinate,[13] was located. At this free energy maximum at 298 K, the S−N bond is stretched to a distance of 3.55 Å (from an equilibrium distance of 1.87 Å), $\Delta S^{\ddagger}$ is +2.8 eu (versus +36.6 eu for complete dissociation) and $\Delta H^{\ddagger}$ is 30.3 kcal/mol, 2.1 kcal/mol below the predicted enthalpy of dissociation.

These predicted activation parameters are prohibitively high for spontaneous thermal homolysis to occur under biologically relevant conditions, refuting recent reports suggesting the involvement of homolytic pathways in the decomposition of RSNOs.[4,14−17] From the computed free energy of activation for homolysis of primary species **1**, the predicted half-life with respect to S−N cleavage is 15.3 years at 25 °C, 2.1 years at 37 °C, and 3.7 h at 100 °C.

To test these predictions, rates of S−N homolysis for a series of RSNO compounds **6**−**9** were measured experimentally. Nitrosation of the parent thiols in dry acetonitrile by several methods, including reaction with inorganic nitrosonium salts and organic nitrites, produced RSNOs which decomposed rapidly by an apparently zero-order process. Metal chelators failed to alter the course of the decomposition. Although this decomposition results in the expected disulfide as the sole organic product, the observed kinetic order is clearly inconsistent with that expected for S−N bond homolysis. Rather, this non-first-order decay is likely the

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(1) Stamler, J. S. *Cell* **1994**, *78*, 931−936.
(2) Stamler, J. S. *Curr. Top. Microbiol. Immunol.* **1995**, *196*, 19−36.
(3) Williams, D. L. H. *Acc. Chem. Res.* **1999**, *32*, 869−876.
(4) Al-Sa'doni, H.; Ferro, A. *Clin. Science* **2000**, *98*, 507−520.
(5) Roy, B.; d'Hardemare, A. M.; Fontcave, M. *J. Org. Chem.* **1994**, *59*, 7019−7026.
(6) Carnahan, G. E.; Lenhert, P. G.; Ravichandran, R. *Acta Crystallogr.* **1978**, *B34*, 2645−2648.
(7) Montgomery, J. A.; Frisch, M. J.; Ochterski, J. W.; Petersson, G. A. *J. Chem. Phys.* **1999**, *110*, 2822−2827.
(8) All calculations were performed with the Gaussian 98 program system: Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, Rev. A.9; Gaussian, Inc.: Pittsburgh, PA, 1998.
(9) Pryor, W. A. *Free Radicals*; McGraw-Hill: New York, 1966.
(10) Bach, R. D.; Ayala, P. Y.; Schlegel, H. B. *J. Am. Chem. Soc.* **1996**, *118*, 12758−12765.
(11) Walling, C. *Free Radicals in Solution*; Wiley: New York, 1957.
(12) Tucker, S. C.; Truhlar, D. G. In *New Theoretical Concepts for Understanding Organic Reactions*; Bertran, J., Csizmadia, I. G., Eds.; Kluwer Academic: Dordrecht, 1989; pp 291−346.
(13) Keating, A. E.; Garcia-Garibay, M. A.; Houk, K. N. *J. Am. Chem. Soc.* **1997**, *119*, 10805−10809; Keating, A. E.; Merrigan, S. R.; Singleton, D. A.; Houk, K. N. *J. Am. Chem. Soc.* **1999**, *121*, 3933−3938.
(14) Lü, J.-M.; Wittbrodt, J. M.; Wang, K.; Wen, Z.; Schlegel, H. B.; Wang, P. G.; Cheng, J.-P. *J. Am. Chem. Soc.* **2001**, *123*, 2903−2904.
(15) Lü, J.-M.; Zhu, X.-Q.; Li, Q.; He, J.-Q.; Xian, M.; Cheng, J.-P. *Gaodeng Xuexiao Huaxue Xuebao* **2000**, *21*, 570−572.
(16) Wang, K.; Zhang, W.; Xian, M.; Hou, Y.-C.; Chen, X.-C.; Cheng, J.-P.; Wang, P. G. *Curr. Med. Chem.* **2000**, *7*, 821−834.
(17) The underestimation of BDEs involving heteroatoms by density functional theory with modest basis sets (ref 14) has been documented: DiLiabio, G. A.; Pratt, D. A.; LoFaro, A. D.; Wright, J. S. *J. Phys. Chem. A* **1999**, *103*, 1653−1661; DiLiabio, G. A.; Pratt, D. A. *J. Phys. Chem. A* **2000**, *104*, 1938−1943.

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Table 2. Measured Energetic Parameters for S−N Bond Dissociation in *S*-nitrosothiols **6**−**9**

S-nitrosothiol	E_a[a]	$\Delta G^{\ddagger}$	$\Delta H^{\ddagger}$[b]	$\Delta S^{\ddagger}$[c]
$CH_3(CH_2)_5SNO$ (**6**)	28.9	27.4[d]	28.1	2.2
c-$C_6H_{11}SNO$ (**7**)	31.6	27.1[d]	30.9	11.1
$CH_3CH_2C(CH_3)_2SNO$ (**8**)		26.2[d]		
C_6H_5SNO (**9**)		21.7[e]		

[a] From Arrhenius expression, kcal/mol. [b] From Eyring expression, kcal/mol. [c] From Eyring expression, cal/mol·K. [d] 343.2 K, kcal/mol. [e] 303.2 K, kcal/mol.

result of catalytic decomposition of RSNO by trace substances and byproducts present in the reaction medium.

By contrast, in the presence of a 10- to 100-fold excess of thiol, each RSNO decomposed by a smooth first-order process to produce disulfide and nitric oxide. Accordingly, the decompositions were carried out with excess thiol in dry, degassed acetonitrile.

A solution of thiol (1 M) was prepared and brought to the desired temperature. Nitrosonium tetrafluoroborate was added to bring the solution to between 10 and 100 mM in RSNO, and decomposition was followed by visible wavelength absorbance at 550, 555, 596, and 568 nm for **6**−**9**, respectively. First-order rate constants for decomposition were determined from the semilog plot of absorbance versus time. Arrhenius and Eyring parameters were obtained for **6** and **7** over a range of 60−80 °C. These data yield activation energies of 28.9 ± 0.3 and 31.6 ± 0.8 kcal/mol, with log *A* values of 13.8 ± 0.2 and 15.7 ± 0.5 for **6** and **7**, respectively. Activation parameters are listed in Table 2. The measured Arrhenius preexponential factors are within the range (10^{13}−10^{16} s^{-1}) commonly reported for O−O bond homolysis.[9,11,18]

Rate constants for homolysis were measured at single temperatures for **8** and **9**. Activation free energies of 26.2 ± 0.2 kcal/mol at 70.2 °C for **8** and 21.7 ± 0.1 kcal/mol at 30.2 °C for **9** were obtained. Eyring analyses for **8** and **9** were complicated by significant deviations from linearity at lower temperatures. Additionally, plots of absorbance versus time for **6**−**8** deviate from the expected first-order decay at temperatures below 65 °C. We interpret these observations as the result of competition from various bimolecular processes at temperatures where homolytic decomposition is slow. These observations mandate extreme caution in studies designed to evaluate rates of *S*-nitrosothiol decomposition.

Reports of both stabilizing and destabilizing effects of added thiol on RSNOs have appeared, resulting in a variety of nitrogenous end products and indicative of varying experimental conditions. Gow et al. have reported an acceleration in the decomposition of CysSNO in the presence of added cysteine.[19] Similarly, Singh et al. have reported a significant destabilizing effect of added glutathione (GSH) on GSNO in buffered (pH 7.4) aqueous solution.[20] Product analysis of GSNO decomposition in the presence of added GSH revealed lower oxides of nitrogen, including ammonia and hydroxylamine. In contrast, GSH-induced decomposition of GSNO as reported by Mayer et al.[21] was completely suppressed upon addition of EDTA, suggesting involvement of trace metal or other contaminants introduced or regenerated within the reaction medium.

Under our conditions, the addition of thiol greatly stabilizes *S*-nitrosothiols and simplifies the decay to a first-order process. These nonaqueous, mildly acidic conditions preclude formation of intermediates via nucleophilic addition of thiolate to RSNO. Bimolecular decomposition via nucleophilic displacement of NO^- by thiolate was ruled out by a diminished rate of decomposition at elevated thiol concentration, despite the fact that this process would be pseudo-first-order in *S*-nitrosothiol and zero-order in thiol at high thiol concentrations. Examination of the inorganic products of our RSNO decomposition solutions revealed no significant production of either ammonia or hydroxylamine.

Our stable, persistent solutions of *S*-nitrosothiols demonstrate that under our conditions, added thiol alone does not accelerate RSNO decomposition. Our measured activation parameters should thus correspond to the purely thermal homolytic pathway and represent a lower bound to the homolysis energy.

Primary, secondary, and tertiary *S*-nitrosothiols show remarkably similar rates of homolysis. Alkyl RSNOs **6**, **7**, and **8** decompose with free energies of activation of 26−27 kcal/mol. These solution-phase $\Delta G^{\ddagger}$ values are in good agreement with the predicted $\Delta G^{\ddagger}$ of 29.5 kcal/mol for **1**. Consistent with calculations on **5** and the formation of a resonance-stabilized radical upon S−N homolysis, *S*-nitrosobenzenethiol **9** shows a diminished $\Delta G^{\ddagger}$ of 21.7 ± 0.1 kcal/mol at 30.2 °C. Thus, all aliphatic *S*-nitrosothiols, including those derived from cysteine or other biologically relevant thiols, should demonstrate homolytic stabilities similar to, or slightly less than, those of organic peroxides.

Our results show that thermal homolysis of the S−N bond is not an important contributor to RSNO decomposition under physiological conditions, consistent with a large body of data suggesting the involvement of heterolytic and other decomposition mechanisms.[22] As the effect of solvent on radical processes is generally small, our conclusions should be directly applicable to RSNOs of physiological interest. An important corollary of this conclusion is that other, presumably bimolecular, processes are responsible for *S*-nitrosothiol decomposition and, ultimately, control of RSNO concentration and reactivity in vivo and under normal preparatory conditions. Such processes might include *S*-nitrosothiol dimerization, *S*-nitrosothiol-thiol adducts, and catalytic processes involving metal ions, various oxygen species, and enzymes. The effect of copper on *S*-nitrosothiol stability under nonphysiological, in vitro conditions has been extensively investigated,[3,23,24] whereas RSNOs have been found to be stable in the dark in the presence of metal chelators.[25] The wide variations in stability reported for RSNOs must arise from their differential ability to participate in higher order chemical processes and variations in reaction conditions. Elucidation of these processes and characterization of the reactive intermediates involved in *S*-nitrosothiol decomposition are underway.

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Supporting Information Available: Theoretical data and table of rate constants for homolysis reactions of **1**−**5** and **6**−**9**, respectively (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Reints, W.; Pratt, D. A.; Korth, H.-G.; Mulder, P. *J. Phys. Chem. A* **2000**, *104*, 10713−10720.

(19) Gow, A. J.; Buerk, D. G.; Ischiropoulos, H. *J. Biol. Chem.* **1997**, *272*, 2841−2845.

(20) Singh, S. P.; Wishnok, J. S.; Keshive, M.; Deen, W. M.; Tannenbaum, S. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14428−14433.

(21) Mayer, B.; Schrammel, A.; Klatt, P.; Koesling, D.; Schmidt, K. *J. Biol. Chem.* **1995**, *270*, 17355−17360.

(22) Stamler, J. S. *Current Topics in Microbiology and Immunology* **1995**, *196*, 19−36; Gaston, B.; Drazen, J. M.; Jansen, A.; Sugarbaker, D. J.; Loscalzo, J.; Richards, W.; Stamler, J. S. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 978−984 and references therein; Mathews, W. R.; Kerr, S. W. *J. Pharmacol. Exp. Ther.* **1993**, *267*, 1529−1537 and references therein.

(23) Munro, A. P.; Williams, D. L. H. *Can. J. Chem.* **1999**, *77*, 550−556; Noble, D. R.; Swift, H. R.; Williams, D. L. H. *Chem. Commun.* **1999**, 2317−2318; Dicks, A. P.; Swift, H. R.; Williams, D. L. H.; Butler, A. R.; Al-Sa'doni, H. H.; Cox, B. G. *J. Chem. Soc., Perkin Trans. 2* **1996**, 481−487; Askew, S. C.; Barnett, D. G.; McAninly, C.; Williams, D. L. H. *J. Chem. Soc., Perkin Trans. 2* **1995**, 741−745; McAninly, J.; Williams, D. L. H.; Askew, S. C.; Butler, A. R.; Russell, C. *J. Chem. Soc., Chem. Commun.* **1993**, 1758−1759.

(24) Burg, A.; Cohen, H.; Meyerstein, D. *J. Biol. Inorg. Chem.* **2000**, *5*, 213−217.

(25) Singh, R. J.; Hogg, N.; Kalyanaraman, B. *J. Biol. Chem.* **1996**, *271*, 18596−18603.