

Influence of  $\text{Cu}^+$  on the RS–NO Bond Dissociation Energy of S-Nitrosothiols

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Received: December 10, 2004

Density functional theory methods have been used to investigate the role and effects of  $\text{Cu}^+$  binding to the S and N centers of the –SNO functional group within S-nitrosothiols (RSNOs), on the lability of the NO group. The binding of  $\text{Cu}^+$  to the S center is found to weaken the S–N bond, while the N–O bond is concomitantly strengthened, consistent with the notion that  $\text{Cu}^+$  binding catalyzes NO radical release. In contrast, however, the binding of  $\text{Cu}^+$  to the N center is found to dramatically shorten and strengthen the S–N bond with a concomitant lengthening of the N–O bond, suggesting stabilization of the RSNOs against NO release. Upon solvation, complexes with  $\text{Cu}^+$  bound to the N center are stabilized relative to the corresponding S-bound complexes, though remaining slightly higher in energy. The barriers to interconversion between corresponding isomers were also investigated. Implications for biochemical regulation of NO release from RSNOs are discussed.

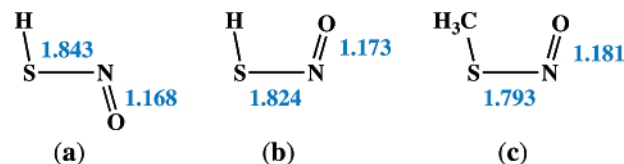
## I. Introduction

S-nitrosothiols (RSNOs) are of increasing interest as they are believed to be used within the body to store and transport the physiologically important species nitric oxide (NO).<sup>1–5</sup> In addition, they have also shown potential as NO-releasing therapeutic agents for the treatment of a variety of diseases.<sup>2</sup> However, too much NO has also been proposed to play a key role in various diseases.<sup>3,4</sup> Thus, central to the biochemical function of RSNOs is an ability to regulate the release of NO. It is widely proposed that metal ions, in particular,  $\text{Cu}^+$ , may play important roles in NO-release regulation.<sup>2,6–9</sup> Indeed,  $\text{Cu}^+$  is known to catalyze the decomposition of RSNOs. For example, their decomposition by the copper-containing enzyme CuZn-superoxide dismutase has been observed.<sup>10</sup> In addition, it has been shown that  $\text{Cu}^{2+}$ -doped polymers can spontaneously generate NO from RSNOs, presumably via a  $\text{Cu}^+$  intermediate.<sup>11</sup> Recently,<sup>8</sup> Toubin et al. investigated select  $\text{RSNO}\cdots\text{Cu}^+$  complexes theoretically and found that complexing  $\text{Cu}^+$  at the S center elongates the S–N bond, thus promoting decomposition. However,  $\text{Cu}^+$  binding at N in the –SNO group has not been examined. In addition, despite increasing insight into NO release, factors that may instead aid in stabilizing RSNOs, an important part of NO regulation, are not well-understood. In this study, density functional theory (DFT) methods have been used to investigate the binding of  $\text{Cu}^+$  to S versus N in RSNOs ( $\text{R} = \text{H}, \text{CH}_3$ ; Scheme 1), in particular, with regard to the lability of the NO group.

## II. Computational Methods

Optimized geometries were obtained using the DFT method B3P86/6-311+G(2df,p),<sup>13–15</sup> as implemented<sup>16</sup> in the *Gaussian 98* and *Gaussian 03* suite of programs.<sup>17</sup> It has previously been shown that, for RSNOs, this level of theory provides reliable

SCHEME 1: Selected B3P86/6-311+G(2df,p) Optimized (gas-phase) Bond Lengths (Å) of (a) *trans*-HSNO, (b) *cis*-HSNO, and (c) *cis*- $\text{CH}_3\text{SNO}$ <sup>12</sup>



structures and RS–NO bond dissociation energies (BDEs).<sup>12</sup> Harmonic vibrational frequencies and zero-point vibrational energies (ZPVEs) were also calculated at this level. Relative energies were calculated at this level with the inclusion of the appropriate ZPVE, vibrational, and entropy corrections. Previously,<sup>18</sup> similar DFT-based approaches have been shown to give reliable relative  $\text{Cu}^+\cdots\text{X}$  binding energies for a variety of ligands (X). This is found to also be the case for the DFT method used in this study (Supporting Information Table S1). General solvent effects were included using the Onsager method,<sup>19</sup> the solvent being water with a dielectric constant of 78.4.

## III. Results and Discussion

$[\text{HSNO}\cdots\text{Cu}]^+$ . Schematic potential energy surfaces (PESs) for the reaction of  $\text{Cu}^+$  with HSNO in gas (blue) and aqueous phases (red) are shown in Figure 1. In the gas phase, the lowest-energy complex is obtained when  $\text{Cu}^+$  binds to the S center to give the nonplanar complex **1**.  $\text{Cu}^+$  is also found to bind to the N center. However, unlike binding to S, when  $\text{Cu}^+$  binds to N, the most stable complex formed is with *cis*-HSNO (**5**), which lies 46.3  $\text{kJ mol}^{-1}$  higher in energy than **1**. The complex formed with *trans*-HSNO (**3**) lies 9.2  $\text{kJ mol}^{-1}$  higher in energy than **5**. Both **3** and **5** are planar. Thus, the geometries of **1** and **3/5** indicate that  $\text{Cu}^+$  interacts via the lone pairs of S and N, respectively. Complexes **1** and **3** are able to interconvert via transition structure (TS) **2**, with a barrier of 89.1  $\text{kJ mol}^{-1}$ , while

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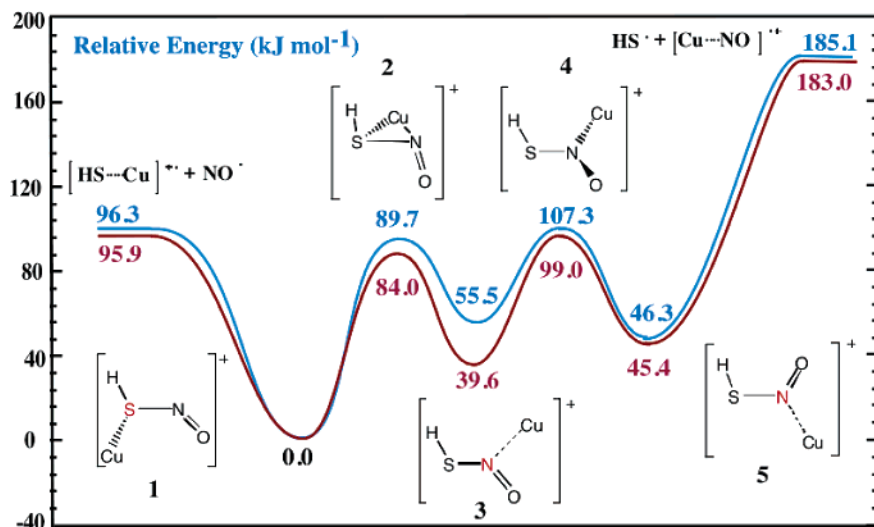


Figure 1. Schematic potential energy surfaces for reaction of  $\text{Cu}^+$  with HSNO in the gas-phase (blue) and aqueous solution (red). See text.

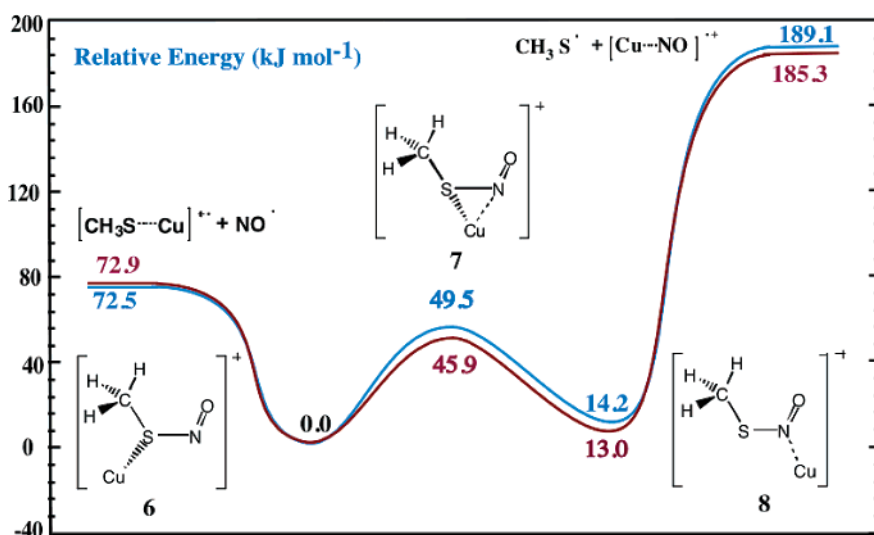


Figure 2. Schematic potential energy surfaces for reaction of  $\text{Cu}^+$  with  $\text{CH}_3\text{SNO}$  in the gas-phase (blue) and aqueous solution (red). See text.

TABLE 1: Optimized S–N, N–O, and  $\text{Cu}\cdots\text{X}$  ( $\text{X} = \text{S}$  or  $\text{N}$ ) Bond Lengths ( $\text{\AA}$ ) of the  $[\text{RSNO}\cdots\text{Cu}]^+$  Complexes ( $\text{R} = \text{H}$ ,  $\text{CH}_3$ )

species		$r(\text{S}-\text{N})$	$r(\text{N}-\text{O})$	$r(\text{Cu}\cdots\text{X})$
1	gas-phase	2.227	1.108	2.163
	solution	2.236	1.107	2.164
3	gas-phase	1.724	1.190	1.940
	solution	1.720	1.196	1.945
5	gas-phase	1.709	1.191	1.940
	solution	1.707	1.194	1.941
6	gas-phase	2.132	1.122	2.166
	solution	2.117	1.125	2.170
8	gas-phase	1.691	1.200	1.930
	solution	1.689	1.202	1.933

3 and 5 can interconvert via TS 4 with a barrier of 51.8  $\text{kJ mol}^{-1}$  relative to 3 (Figure 1). However, of particular interest is that homolytic cleavage of the S–N bond in 1 is predicted to require just 96.3  $\text{kJ mol}^{-1}$ , while the analogous cleavage in 5, assuming no rearrangement back to 1, requires 185.1  $\text{kJ mol}^{-1}$ , an increase of 88.8  $\text{kJ mol}^{-1}$ !

Insight into these observations is gained by examining the effects on the S–N and N–O bonds upon complexing  $\text{Cu}^+$  with HSNO (Table 1). When  $\text{Cu}^+$  binds at S to give 1, the S–N bond lengthens dramatically to 2.227  $\text{\AA}$ , while concomitantly, the N–O bond shortens considerably to just 1.108  $\text{\AA}$  (cf. Scheme

1). That is, the –NO group increasingly resembles free NO, suggesting the promotion of HSNO degradation and NO release by  $\text{Cu}^+$ , as previously suggested.<sup>8</sup> In contrast, however, when  $\text{Cu}^+$  binds at N to give 3 or 5, the opposite effects are observed: the S–N bonds shorten significantly by almost 0.12  $\text{\AA}$ , while the N–O bonds lengthen slightly by approximately 0.02  $\text{\AA}$ ; that is, HS–NO bonding is enhanced.

The inclusion of solvation effects results in slight energetic and structural changes. The interconversion barriers, TS 2 and 4, are reduced slightly by 6–8  $\text{kJ mol}^{-1}$  (Figure 1; red line). Interestingly, the  $\text{Cu}\cdots\text{N}$ -bound complexes 3 and 5 are stabilized relative to the  $\text{Cu}\cdots\text{S}$ -bound complex 1, with the largest effect occurring for 3, which now lies just 39.6  $\text{kJ mol}^{-1}$  higher in energy than 1. Indeed, in solution, 3 is slightly more stable than 5 by 5.8  $\text{kJ mol}^{-1}$ . Structurally (Table 1), the binding of  $\text{Cu}^+$  to HSNO in solution has the same effects as observed in the gas phase, although marginally enhanced. For example, in solution, the S–N bond of 1 is lengthened to 2.236  $\text{\AA}$ , while in 3 and 5, the S–N bonds are marginally lengthened by <0.005  $\text{\AA}$  (cf. Table 1). We note that all complexes show marginally longer  $\text{Cu}^+\cdots\text{X}$  distances in solution relative to the gas phase.

$[\text{CH}_3\text{SNO}\cdots\text{Cu}]^+$ . Schematic PESs for the reaction of  $\text{Cu}^+$  with  $\text{CH}_3\text{SNO}$  in the gas phase and solution are shown in Figure 2. Similar effects are observed upon solvation as noted for

HSNO, and hence, the following discussion is limited to the solvated results, unless otherwise noted.

As for HSNO, the lowest-energy complex is again found when  $\text{Cu}^+$  binds at S in  $\text{CH}_3\text{SNO}$  to give **6**. However, when  $\text{Cu}^+$  binds at N, it forms complex **8** lying higher in energy than **6** by just  $13.0 \text{ kJ mol}^{-1}$ . These complexes, both having the  $\text{CH}_3\text{-SNO}$  moiety in the cis conformation, are able to interconvert via TS **7** at a cost of  $45.9 \text{ kJ mol}^{-1}$ . Direct homolytic cleavage of the S–N bond of **6** requires just  $72.9 \text{ kJ mol}^{-1}$ , while from **8**, it requires  $185.3 \text{ kJ mol}^{-1}$ . Similar to HSNO,  $\text{Cu}^+$  binding to the S center again causes a dramatic lengthening of the S–N bond with concomitant shortening of the N–O bond (Table 1), although the effect in solution is again tempered marginally compared to the changes observed in the gas phase. In comparison,  $\text{Cu}^+$  binding to the N center in  $\text{CH}_3\text{SNO}$  causes a considerable shortening of the S–N bond, while the N–O bond is lengthened (see Table 1). Again, similar solvation effects are noted as previously described for HSNO.

The present results indicate that the interaction of  $\text{Cu}^+$  at the S center in RSNOs lengthens the S–N bond, thus enhancing NO loss as previously suggested.<sup>8</sup> Indeed, the lowest-energy RS–NO bond cleavage occurs via  $\text{Cu}^+$  bound to the S center. Most significantly, however, it is found that the interaction of  $\text{Cu}^+$  with the N center of RSNOs has the opposite effect and, in fact, causes considerable shortening of the RS–NO bond. It is also found that the barrier for interconversion of  $\text{Cu}^+$  bound from N to S is relatively modest. Hence, to stabilize the S–N bond for a period of time, the barrier to rearrangement must be enhanced either chemically or physically. An example of how this may be achieved may come from rhodanese. Upon inhibition by nitrosylation of an active site cysteine (i.e., –SNO formation), it forms the remarkably stable S-nitrosorhodanese.<sup>20</sup> Significantly, because of constraints imposed by the structure of the active site, the –SNO group is spatially close to a neighbouring arginine with which it can interact via its –NO moiety (see Figure S1 in Supporting Information). This results in effects similar to those described already for  $\text{Cu}^+$  binding to the N center and, furthermore, suggests that the effects detailed already may be a general consequence of cation–NO moiety interactions for RSNOs.

#### IV. Conclusions

Density functional theory calculations have been performed to investigate the interactions of  $\text{Cu}^+$  ions with the prototypical S-nitrosothiols (RSNOs) HSNO and  $\text{CH}_3\text{SNO}$ , specifically, with their S and N centers. The most stable complexes involve binding of  $\text{Cu}^+$  to the S center, which results in elongation of

the RS–NO bond as observed previously. In contrast, binding of  $\text{Cu}^+$  to the N center induces a considerable shortening of the RS–NO bond (i.e., strengthening of the RS–NO bond) with a concomitant lengthening of the N–O bond. However, the barrier separating the N-bound and S-bound  $\text{Cu}^+\cdots\text{RSNO}$  complexes is found to be small. Similar structural effects are observed when a model RSNO is allowed to hydrogen bond with a protonated arginine amino acid, thus suggesting that the observed phenomenon of RS–NO bond strengthening may be due to increased electron density being moved from the S to the NO group and, furthermore, provides some insights into methods by which RSNOs may be stabilized in vivo.

**Acknowledgment.** We thank the Natural Sciences and Engineering Research Council of Canada (NSERC), Canadian Foundation for Innovation (CFI), and Ontario Innovation Trust (OIT), and the John and Anne Cristescu Memorial Scholarship (C.B.), for financial support. We also thank SHARCnet and the University of Waterloo for additional computational resources.

**Supporting Information Available:** Optimized geometries,  $\text{Cu}^+\cdots\text{RSNO}$  ( $\text{R} = \text{H}, \text{CH}_3$ ) binding energies (BEs), comparison of experimental and calculated  $\text{Cu}^+\cdots\text{X}$  BEs for model ligands, complete citations for *Gaussian 98* and *03*. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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