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Theoretical Evidence that Cu^I Complexation Promotes Degradation of *S*-Nitrosothiols

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S-Nitrosothiols (RSNOs) are known to be involved in nitric oxide (NO) storage and transport in vivo and are implicated in blood pressure regulation and many other physiological processes.[1−4] Experimental and theoretical values in the range of 25−32 kcal/mol for the homolytic dissociation energy of the S−N bond have been reported recently for a number of RSNO species.[5,6] Given these large bond dissociation energies, NO release via thermal homolysis of the S−N bond is unlikely in vivo. However, previous studies reveal that NO release may be Cu^I-catalyzed,[1,2,7−13] $\mathrm{Cu^I + RSNO \rightarrow Cu^{II} + RS^- + NO}$, where Cu^I is regenerated from RS$^-$ ($\mathrm{2Cu^{II} + 2RS^- \rightarrow 2Cu^I + RSSR}$).[10] In the presence of Cu^I, the reported stability of RSNOs varies widely as a function of R.[2,9] For example, the measured rate constants (Table 1) indicate that the decomposition of *S*-nitrosocysteine (**C**) and its ethyl ester (**E**) is rapid, while that of *S*-nitrosohomocysteine (**H**) is much slower, but *N*-acetyl *S*-nitrosocysteine (**A**) is kinetically stable.[9] In the present communication, the catalytic role played by Cu^I ions in the degradation of these four RSNOs of widely varying kinetic stabilities is investigated by first-principles quantum chemistry calculations. The geometrical and electronic structures of the Cu^I-ligated intermediates and their thermodynamic stabilities are analyzed to elucidate the mechanism of NO release.

Calculations were performed with the *Gaussian 98* (version A.9) suite of quantum chemistry programs at the Becke3 Lee−Yang−Parr (B3LYP) level of hybrid density-functional theory[14−16] combined with the 6-31G* basis set[17] for geometry optimization and the 6-311+G(2f,2d,2p) basis set[18] for higher-level electronic structure and energy calculations. Previous theoretical studies[19,20] showed that this model chemistry yields binding energies for Cu^I−L (L = H_2O, NH_3, cysteine) in good agreement with experimental values.[21] The calculated gas-phase S−N bond energies for the unligated RSNOs are 27.3 (**C**), 30.6 (**E**), 31.7 (**A**), and 32.6 kcal/mol (**H**), indicating a strengthening of the S−N bond upon esterification, *N*-acetylation, or lengthening of the alkyl chain of **C**. To approximate aqueous solvation effects, the self-consistent polarized continuum model was employed to calculate solvation free energies.[22] The resulting solution homolytic dissociation energies for the S−N bond are 29.5 (**C**), 32.2 (**E**), 34.5 (**A**), and 35.8 kcal/mol (**H**), demonstrating that S−N bond homolysis is relatively insensitive to a polar environment. These S−N energies compare well with the published RSNO homolytic dissociation energies of 29−32 kcal/mol obtained from kinetic measurements,[5] and the value of ∼25 kcal/mol deduced from thermochemical data,[6] which provides further support for the model chemistry used here. Given the high calculated and observed homolytic dissociation energies, spontaneous homolysis can be ruled out as an RSNO degradation pathway in vivo.

Table 1. Rate of NO Release (k) and Computed Properties for Cu^I−RSNO Intermediates

k^a	RSNO	$\Delta G_c{}^b$ (PCM)^c	S–N^d	S–N bcp^e ρ_b; $\nabla^2\rho_b$	N–O^d	N–O bcp^e ρ_b; $\nabla^2\rho_b$
3×10^5	**E**		1.87	15.0; −3.3	1.18	54.4; −138
	Cu^I−E	−68.8 (−19.8)	2.10	9.9; 7.6	1.15	59.8; −188
2×10^4	**C**		1.87	14.9;−18.8	1.18	54.7; −141
	Cu^I−C	−63.9 (−22.5)	2.11	9.6; 8.0	1.14	60.0; −190
2×10^1	**H**		1.86	15.2; −3.3	1.18	54.4; −140
	Cu^I−H	−78.1 (−25.3)	2.11	9.6; 7.6	1.14	60.7; −196
0	**A**		1.89	14.5; −0.4	1.18	55.3; −146
	Cu^I−A	−53.9 (−8.3)	2.15	8.8; 8.4	1.14	61.5; −202
	NO				1.16	43.5; −82

[a] Observed rate constants ($M^{-1}\,s^{-1}$) for NO release, assuming rate = k[Cu][RSNO].[2,9] [b] Gas-phase B3LYP/6-311+G(2d,2p)//B3LYP/6-31G* free energies ΔG_c (kcal/mol) of the Cu^I−RSNO complexes with respect to the reactants. [c] Aqueous ΔG_c values using a polarized continuum model (PCM)[22] for the solvent with $\epsilon = 80.2$. [d] Bond lengths (Å) calculated from the B3LYP/6-31G* geometries. [e] Electron densities ($\rho_b \times 10^{-2}$ e/a_0^3) and their Laplacians ($\nabla^2\rho_b \times 10^{-2}$ e/a_0^5) at the S−N and N−O bond critical points (bcp).[23,24] Note that small ρ_b values (∼7×10^{-2} e/a_0^3) are typical of a closed-shell interaction (ionic in this case), whereas large ρ_b values (>30×10^{-2} e/a_0^3) are indicative of covalent interactions.[23]

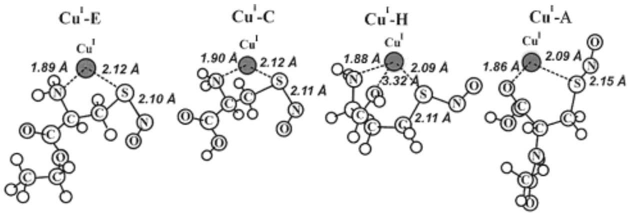

Figure 1. Optimized B3LYP/6-31G* geometries of the thermodynamically most stable **Cu^I−E**, **Cu^I−C**, **Cu^I−H**, and **Cu^I−A** complexes. The dashed lines between Cu^I and the coordinating atoms represent bond critical paths[23,24] predicted by the AIM analysis.

Figure 1 shows the gas-phase Cu^I−RSNO intermediates in Cu^I-catalyzed RSNO decomposition predicted by the present calculations. For **Cu^I−C**, the thermodynamically most stable complex (Figure 1) corresponds to a five-membered ring in which Cu^I binds to the amino N and S atoms. The gas-phase complexation enthalpy calculated for **Cu^I−C** (−73.4 kcal/mol) is comparable to the experimental value measured for Cu^I−cysteine (−81.1 kcal/mol).[20] The gas-phase geometries for **Cu^I−E** (N and S coordination) and **Cu^I−A** (S and COOH carbonyl coordination) are also characterized by bidentate chelation, while **H** chelates Cu^I in a tridentate manner via its S, N, and hydroxyl O atoms (Figure 1). Both **Cu^I−H** and **Cu^I−A** possess six-membered ring structures, while intermediate **Cu^I−E** is a five-membered chelate very similar to **Cu^I−C**. Remarkably, the calculated S−N bonds are 0.23−0.26 Å longer in the Cu^I−RSNOs versus the free RSNOs, and the N−O bond, shorter by 0.02 Å than in free NO (Table 1). The longer S−N bonds in

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the calculated Cu^I−RSNO structures correspond to a ∼5 kcal/mol decrease in the bond energy, which is close to 20% of the homolytic dissociation energy. Thus, Cu^I-complexation promotes S−N bond cleavage from an energetic point of view.

To gain insight into chemical bonding in the intermediates and to elucidate the changes in electronic structure upon complexation, we make use of the atoms-in-molecules (AIM) theory.[23,24] This is based on a topological analysis of the electron density (ρ) and its Laplacian ($\nabla^2\rho$) at bond critical points. Inspection of the electronic densities ρ_b (Table 1) indicates a 34, 35, 37, and 39% depletion of ρ_b at the S−N bond critical point in **Cu^I−E**, **Cu^I−C**, **Cu^I−H**, and **Cu^I−A**, respectively, versus the free ligands. In contrast, ρ_b increases by 10−12% at the N−O bond critical points upon Cu^I-binding to the RSNOs (Table 1). This, combined with large negative $\nabla^2\rho_b$ values (Table 1), indicate that the NO bond has enhanced covalent character in the Cu^I−RSNOs. On the other hand, the positive $\nabla^2\rho_b$ values at the S−N bond critical points (Table 1) reflect a decrease in covalency when Cu^I is present.[21] Given the significant weakening of the S−N bond and strengthening of the N−O bond upon Cu^I chelation predicted by AIM analysis, the Cu^I−RSNO intermediates can clearly be considered as precursors to NO release. We note that, in contrast, Cu^I complexation to the carbonyl O of formamide was shown to weaken the next neighboring C−N bond rather than the stronger, adjacent C−O bond;[25] Cu^I complexation thus appears to weaken the weaker of the adjacent and next-neighboring bonds in the ligand. Use of other approaches to analyze electronic structures, such as natural bond orbital theory[26] and the electron localization function,[27] paint a similar picture yielding intermediates with a weakened S−N bond and an NO moiety closer to that of free NO.[28]

Since the reactivity of RSNOs has been correlated with their abilities to chelate copper,[2,8] we turn our attention next to the free energies of the complexes (ΔG_c). The thermodynamic stabilities calculated for the gas-phase complexes decrease as **Cu^I−H** > **Cu^I−E** > **Cu^I−C** > **Cu^I−A**, and the same trend is followed when solvent is added except that **Cu^I−C** > **Cu^I−E** (ΔG_c, Table 1). The AIM analysis indicates the existence of a bond critical point (bcp) between the hydroxyl O and Cu^I in **Cu^I−H**, and this third ligand (at 3.3 Å from the metal) contributes to the stabilization of the intermediate. Our calculations also revealed the existence of a less stable ($\Delta G_c = -56.7$ kcal/mol) **Cu^I−H** intermediate with pure bidentate Cu^I in an unfavorable seven-membered ring (not shown). Thus, if only bidentate Cu^I-coordination is considered, the gas-phase stabilities (**Cu^I−E** > **Cu^I−C** > **Cu^I−H** > **Cu^I−A**) follow the trend expected from the reported degradation rate constants (Table 1).

The **Cu^I−A** intermediate is considerably less stable than the other **Cu^I**−RSNOs, consistent with the kinetic stability of **A** (Table 1). However, the shorter Cu^I−S and longer S−N bonds reveal stronger Cu^I−S interaction in **Cu^I−A** relative to the other bidentate complexes (Table 1). Thus, rates of Cu^I-catalyzed RSNO breakdown reflect the stabilities of both the Cu^I−S bond and the Cu^I complex. The latter is expected to vary considerably with the coordination of the copper.[29,30] A requirement for bidentate Cu^I chelation in RSNO decomposition[2] must also be questioned since CuZn-superoxide dismutase, an abundant enzyme in cells with Cu-(histidine)$_4$ at its active site, is an efficient catalyst of RSNO decomposition in the presence of thiol donors.[31] Thus, catalysis by protein-bound Cu in vivo is possible since significant amounts are found complexed to CuZn-superoxide dismutase and other proteins,[11] whereas no free aquated Cu^I is expected in cells.[32]

In conclusion, the results of this preliminary computational study support a role for Cu^I in RSNO degradation. Our calculations clearly show that the formation of a Cu^I−RSNO intermediate weakens the S−N bond and strengthens the N−O bond, thereby promoting NO release from RSNO. Further theoretical investigations of Cu^I−L_n species of likely biological significance and of the electron-transfer processes associated with NO release in such species are underway.

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