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Reduction of Nitrous Oxide to Dinitrogen by a Mixed Valent Tricopper-Disulfido Cluster

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Abstract

The greenhouse gas N_2O is converted to N_2 by a μ -sulfido-tetracopper active site in the enzyme nitrous oxide reductase (N_2OR) via a process postulated to involve μ -1,3 coordination of N_2O to two Cu(I) ions. In efforts to develop synthetic models of the site with which to test mechanistic hypotheses, we have prepared a localized mixed valent Cu(II)Cu(I)_2 cluster bridged in μ - η^2 : η^1 : η^1 fashion by disulfide, $[\text{L}_3\text{Cu}_3(\mu_3\text{-S}_2)]\text{X}_2$ ($\text{L} = 1,4,7$ -trimethyl-triazacyclononane, $\text{X} = \text{O}_3\text{SCF}_3^-$ or SbF_6^-). This cluster exhibits spectroscopic features similar to those of the active site in N_2OR and reacts with N_2O to yield N_2 in a reaction that models the function of the enzyme. Computations implicate a transition state structure that features μ -1,1-bridging of N_2O via its O-atom to a $[\text{L}_2\text{Cu}_2(\mu\text{-S}_2)]^+$ fragment and provide chemical precedence for an alternative pathway for N_2O reduction by N_2OR .

Nitrous oxide (N_2O) is an important greenhouse gas and component of the global nitrogen cycle.¹ Its reduction to dinitrogen (N_2) is thermodynamically favorable ($E^\circ = 1.76 \text{ V}$), making it attractive as an environmentally benign oxidant, yet its utility in this regard is limited by high kinetic barriers that limit reaction rates. Transition metals facilitate the reduction of N_2O , although in most heterogeneous catalytic systems high temperatures are required² and homogeneous processes that operate under mild conditions generally use highly reducing low-valent metal complexes.^{3–5} In Nature, conversion of N_2O to N_2 and H_2O is catalyzed under ambient conditions during microbial denitrification by the metalloenzyme nitrous oxide reductase, N_2OR .⁶ X-ray crystallographic,⁷ spectroscopic, and theoretical studies⁸ have identified the active site of N_2OR as a μ -sulfido-tetracopper cluster without precedent in biology or synthetic chemistry, which cycles through tetracopper(I) and mixed-valent states during catalysis.⁹ A provocative mechanism for N_2O reduction has been suggested that involves μ -1,3-coordination and bending of N_2O between two of the copper ions in the fully reduced (all copper(I)) cluster, with the μ -sulfide acting to facilitate electron delocalization during the redox process.⁹ Inspired by a desire to test this mechanistic hypothesis and to better understand the properties of the intrinsically novel active site $[(\text{His})_7\text{Cu}_4\text{S}]$ cluster, multicopper-sulfur complexes supported by N-donor ligands have been targeted for synthesis and characterization.¹⁰ To date, however, none contain reduced copper or react with N_2O . We have resolved these shortcomings, and report the characterization of a unique mixed-valent tricopper cluster bridged by disulfide that converts N_2O to N_2 at low temperature. A novel pathway for the reaction is suggested on the basis of theory that has potential implications for understanding the N_2OR enzyme mechanism.

Reaction of Na_2S_2 with $[\text{LCu}(\text{CH}_3\text{CN})]\text{X}$ ($\text{L} = 1,4,7\text{-trimethyltriazacyclononane}$, $\text{X} = \text{O}_3\text{SCF}_3^-$ or SbF_6^-)¹¹ in THF at room temperature over ca. 90 min resulted in the formation of known complex **2a**^{11b} or new variant **2b** (Figure 1). The structures and spectroscopic properties of these complexes are similar to each other and to those of others with antiferromagnetically coupled ($\mu\text{-}\eta^2\text{:}\eta^2\text{-disulfido}$)dicopper(II) cores.^{11b,12} For example, they are EPR silent and exhibit an intense $\text{S}_2^{2-} \rightarrow \text{Cu(II)}$ charge transfer transition at ~ 395 nm ($\epsilon \sim 15,000 \text{ M}^{-1}\text{cm}^{-1}$, Figure 1c, red line), excitation into which ($\lambda_{\text{ex}} 406.7$ or 457.0 nm) results in resonance enhancement of a peak in the Raman spectrum at $\sim 431 \text{ cm}^{-1}$ ($\Delta^{34}\text{S} = 19 \text{ cm}^{-1}$) attributable to an S-S stretching mode.

Monitoring the reactions by UV-vis spectroscopy revealed the formation and subsequent decay (at room temperature, $t_{1/2} \sim 45$ min) of an intermediate with $\lambda_{\text{max}} = 634$ (**1a**) or 631 (**1b**) nm, respectively, the lifetime of which can be extended significantly by lowering the temperature (Figure 1c). For the case of **1b**, crystals suitable for characterization by X-ray diffraction were obtained, although larger scale solid samples for reactivity studies (see below) were more readily isolated for **1a**. The structure of **1b** (Figure 1b) features two O_3SCF_3^- anions associated with a $[\text{L}_3\text{Cu}_3\text{S}_2]^{2+}$ unit. This unit contains a disulfide (S_2^{2-}) bridging the three copper ions in a manner unique in copper chemistry,¹⁰ albeit preceded for other metal ions¹³ and analogous to a proposed motif for a peroxide intermediate in dioxygen reduction by copper oxidases.¹⁴ A localized mixed valent Cu(II)Cu(I)_2 electronic structure for the $[\text{Cu}_3\text{S}_2]^{2+}$ core is suggested by metal-ligand bond distances¹⁵ and metal ion coordination geometries, with 4-coordinate C_{3v} -distorted tetrahedral Cu1 and Cu2 in the +1 oxidation state and 5-coordinate Cu3 in the +2 state.

In the ESI mass spectrum of **1a** in CH_2Cl_2 (Figure S5) a parent ion envelope with the appropriate isotope pattern for $[\text{L}_3\text{Cu}_3\text{S}_2](\text{SbF}_6)^+$ confirms that the trinuclear cluster is retained in solution (calc. m/z 1003.1463; found 1003.1426). Nonetheless, loss of a $[\text{LCu(I)}](\text{SbF}_6)$ fragment to yield $[\text{L}_2\text{Cu}_2\text{S}_2]^+$ is facile, as indicated by an intense peak envelope for this species (calc. $m/z = 532.1499$; found 532.1521); this observation is relevant to reactivity studies described below. The EPR spectrum of a frozen solution of **1b** (X-band, 3.5K, Figure S4) exhibits an essentially axial signal with slight rhombicity ($g_x = 2.079$, $g_y = 2.024$, $g_z = 2.023$) and a four line hyperfine splitting in g_x due to coupling to a single Cu nucleus ($A_{\text{Cu}} = 138 \times 10^{-4} \text{ cm}^{-1}$). The 631 nm absorption band for **1b** resembles that observed for a mixed-valent form of Cu_Z in N_2OR ($\lambda_{\text{max}} = 640$ nm) that has been attributed to a $\text{S} \rightarrow \text{Cu}$ ligand-to-metal charge transfer (LMCT) transition.^{8b,c} Excitation into the 631 nm band for **1b** leads to resonance enhancement of a peak in the Raman spectrum (Figure 2b) at 453 cm^{-1} ($\Delta^{34}\text{S} = 19 \text{ cm}^{-1}$); similar data were observed for **1a**.¹⁶ These results are consistent with assignment of the 631 nm band as a Cu/S_2^{2-} charge transfer transition and the 453 cm^{-1} peak as a predominantly $\nu(\text{S-S})$ mode.

These assignments were investigated further using Density Functional Theory (DFT) calculations. Geometries of the $[\text{L}_3\text{Cu}_3\text{S}_2]^{2+}$ cluster were optimized in the gas phase and including self-consistent reaction-field THF solvation effects. The two optimized geometries were similar and differ little from the single-crystal X-ray structure, except that the S-S bond is longer ($2.21 \text{ \AA}$) in the computed structures than in the crystal structure ($2.13 \text{ \AA}$). UV-vis spectra calculated at the time-dependent B98 density functional level of theory were insensitive to choice of structure (computed or X-ray) and showed excellent agreement with the experimental spectrum when computed transitions were blue-shifted by 0.2 eV (Figure 2a; the blue shift is consistent with the tendency for most TD-DFT protocols to underestimate the energy of charge-transfer excitations¹⁷). Importantly, and in contrast to the assignments for Cu_Z ^{8b,c} the absorption near 630 nm can be attributed to a metal-to-ligand charge transfer (MLCT) from the Cu(I) centers into a π^* orbital of the S-S bond (SOMO, Figure 2a). Similarly, the peak at around 340 nm originates mainly from a MLCT from the Cu(I) centers into the

σ^* orbital of the S-S bond (LUMO). With respect to the resonance Raman spectrum, theory predicts an S-S stretch at 406 cm^{-1} and Cu(I)-S stretches at 366 cm^{-1} and 347 cm^{-1} . The discrepancy of the S-S stretch with the experimental value is probably due to the elongated S-S bond in the gas-phase optimized structure, which was used for these computations (a frequency of 504 cm^{-1} is predicted when the non-stationary X-ray crystal structure is used). Finally, the calculations show that 71% of the spin density is located on the sulfur atoms and the Cu center coordinated η^2 to the disulfide unit (therefore denoted Cu(II) with all other atoms having less than 5%).

In addition to converting to **2a** upon warming to room temperature, **1a** in CH_2Cl_2 (or generated in situ in THF) under a He atmosphere at $-80\text{ }^\circ\text{C}$ slowly ($t_{1/2} \sim 6\text{ h}$) reacts with excess N_2O to quantitatively (based on **1a**) yield N_2 as determined by GC/MS. In control experiments, identical procedures were followed but with either no complex present, or with the complex present but without injecting N_2O ; in both cases, N_2 production was not observed. UV-vis spectroscopic changes during the reaction of **1a** with N_2O show bleaching of the 631 nm band and concomitant formation of the features due to **2a** ($\sim 60\%$ yield¹⁸), the identity of which was confirmed by resonance Raman spectroscopy.^{11b} In addition, an ESI mass spectrum of the final reaction solution revealed the presence of $\{[\text{L}_2\text{Cu}_2(\text{OH})_2]\text{SbF}_6\}^+$ (calc. m/z 739.1048, found 739.0960).^{19,20} Peaks corresponding to this product are not present in the spectrum of the solution resulting from decay of **1a** in the absence of N_2O . We have not been able to isolate $[\text{L}_2\text{Cu}_2(\text{OH})_2](\text{SbF}_6)_2$ from the product mixture, and suspect that it may not be the primary oxygen-containing copper byproduct of the reduction of N_2O ; further experimental mechanistic evaluation is needed to address this issue. Importantly from a mechanistic perspective, the presence of a ~ 7 -fold excess of $[\text{LCu}(\text{CH}_3\text{CN})](\text{SbF}_6)$ in solutions of **1a** in CH_2Cl_2 inhibited both the decomposition of **1a** in the absence of N_2O ($t_{1/2} \sim$ several days at $-20\text{ }^\circ\text{C}$) and its reaction with N_2O to form N_2 ($40 \pm 10\%$ yield after 10 h). These results, in conjunction with the ESI-MS data indicating the feasibility of $[\text{L}_2\text{Cu}_2\text{S}_2]^+$, are consistent with a pre-equilibrium step (eq. 1) that generates $[\text{L}_2\text{Cu}_2\text{S}_2]^+$ as the active species in the N_2O activation process, with added LCu(I) shifting the equilibrium to the presumably less reactive trinuclear cluster.

In computational efforts to evaluate this hypothesis, we calculated that the disproportionation shown in eq. 1 in THF is endergonic by only 2.7 kcal/mol (with THF bound to $[\text{LCu}(\text{I})]^+$). As a result, exhaustive searches at the M06L DFT level for bound complexes and transition-state (TS) structures with N_2O were performed for both $[\text{L}_3\text{Cu}_3\text{S}_2]^{2+}$ and $[\text{L}_2\text{Cu}_2\text{S}_2]^+$. In addition to a number of TS structures found for both clusters with free energies of activation too high to be experimentally relevant (see supporting information), we discovered a low-energy ($\Delta G^\ddagger = 26.6\text{ kcal mol}^{-1}$) TS structure for $[\text{L}_2\text{Cu}_2\text{S}_2]^+$ that evolves to an oxo-bridged intermediate (not observed experimentally) in an exergonic process ($\Delta G = -12.5\text{ kcal mol}^{-1}$, Figure 3 and Figure S13). The nature of the bridging in the TS structure, involving only the substrate O atom, differs from the μ -1,3 binding of N_2O proposed for N_2OR by Solomon and coworkers,⁹ but the $\Delta G^\ddagger$ we compute is similar to their calculated value for the enzyme model (after entropic effects are added to their reported activation potential energies). We were unable to find similar μ -1,3-bridged species for either binuclear or trinuclear clusters; plausible starting structures inevitably dissociated N_2O or relaxed to monodentate coordination.

The ground state of our computed product is a quartet and this, together with an analysis of its charge and spin density, indicates oxidation of the original Cu(I)/Cu(II) binuclear reactant to a Cu(II)/-O $\bullet$ /Cu(II) product. As has been noted previously,²¹ high-spin (triplet) Cu(II)-O $\bullet$ is preferred over a closed-shell Cu(III)/oxo formalism, and coupling of this fragment with the

remaining Cu(II) is ferromagnetic leading to an $S = 3/2$ ground state. In the TS structure, oxidation of the original Cu(I) atom is moderately advanced, but the doublet $S = 1/2$ state remains the ground state. Thus, spin crossing occurs subsequent to commitment to reaction.

We conclude by noting that although the disulfido (S_2^{2-}) unit in complex **1** differs from the sulfido (S^{2-}) ligand in the active site of N_2OR , key properties of Cu_Z are modeled, such as S-bridging between Cu(I) and Cu(II) sites supported by N-donor ligands and similar UV-vis absorption features. Importantly, the complex exhibits reactivity relevant to that of the enzyme, insofar as it converts N_2O to N_2 . The finding of N_2O reduction by a discrete copper-sulfur complex under mild conditions is significant, as such reactions have only been observed for Cu oxide surfaces²² and Cu-doped zeolites²³ at elevated temperatures and for excited state Cu (I) ions in the gas phase.²⁴ We propose a mechanism involving pre-equilibrium formation of a dicopper complex, which subsequently reduces N_2O via a transition state that features bridging of substrate between the two copper ions through a single O atom. Although previously discounted,⁹ the evidence we have obtained suggests that such a pathway may represent a feasible alternative to the mechanism involving μ -1,3-coordination of N_2O proposed previously for the enzyme.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

a) Synthesis of disulfido complexes. **b)** X-ray structure of cationic portion of **1b**, with all nonhydrogen atoms shown as 50% thermal ellipsoids. Selected bond distances: Cu1-S1, 2.1416(8) Å; Cu2-S2, 2.1367(8) Å; Cu3-S1, 2.2548(8) Å; Cu3-S2 = 2.2751(8) Å; S1-S2, 2.1267(10) Å. **c)** UV/Vis spectra obtained during the reaction of $[LCu(MeCN)]O_3SCF_3$ (3.1 mM, black line) with Na_2S_2 in THF. The green line is the spectrum obtained after 90 min at $-20^\circ C$ (**1b**), which transforms upon warming to room temp to **2b** (red line).

Figure 2.

(left) Experimental absorption spectrum of the crude reaction solution containing **1b** obtained upon slow addition of Na_2S_2 (0.5 eq) to $[\text{LCu}(\text{MeCN})]\text{O}_3\text{SCF}_3$ in THF at -20°C (green line) and TD-B98 calculated spectrum (red line), with drawings of the acceptor orbitals for the respective features shown. (right) Resonance Raman spectrum obtained from the reaction of $[\text{LCu}(\text{MeCN})]\text{O}_3\text{SCF}_3$ with Na_2S_2 in THF (-196°C , $\lambda_{\text{ex}} = 647.1\text{ nm}$; ^{32}S , blue line; ^{34}S , red line).

Figure 3. Transition state structure for the N-O bond cleavage computed at the M06L DFT level with selected interatomic distances (Å). C and H atoms of the Me₃tacn ligands are not shown for clarity. Key: green = Cu, blue = N, yellow = S, and red = O.