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N₂O Reductase Activity of a [Cu₄S] Cluster in the 4Cu^I Redox State Modulated by Hydrogen Bond Donors and Proton Relays in the Secondary Coordination Sphere

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Abstract

The synthetic model complex [Cu₄S(dppa)₄]²⁺ (**1**, dppa = (Ph₂P)₂NH) was found to have N₂O reductase activity in methanol solvent, mediating the 2H⁺/2e[−] reduction of N₂O to N₂ + H₂O in the presence of an exogenous electron donor (CoCp₂). A stoichiometric product featuring two deprotonated dppa ligands was characterized, indicating a key role of second-sphere N–H residues as proton donors during N₂O reduction. The activity of **1** towards N₂O was suppressed in solvents that are unable to provide hydrogen bonding to the second-sphere N–H groups. Structural and computational data indicated that second-sphere hydrogen bonding induces structural distortion of the [Cu₄S] active site, accessing a strained geometry with enhanced reactivity due to localization of electron density along a dicopper edge site. The behavior of **1** mimics several aspects of the Cu₂ catalytic site of nitrous oxide reductase: activity in the 4Cu^I:1S redox state, use of a second-sphere proton donor, and reactivity dependence on both primary and secondary sphere effects.

Graphical Abstract

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N₂O reductase activity of a synthetic model compound relies on both primary and secondary coordination sphere effects.

Keywords

cluster compounds; nitrous oxide; hydrogen bonds; bioinorganic chemistry; second sphere

Anthropogenic nitrous oxide (N₂O) emissions are a leading cause of both global warming and ozone layer depletion,^[1,2] motivating studies related to its atmospheric regulation and synthetic consumption. The uncatalyzed reduction of N₂O is thermodynamically favoured ($\text{N}_2\text{O} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{N}_2 + \text{H}_2\text{O}$, $E^\circ = 1.35$ V at pH 7) but is associated with a large kinetic barrier ($\Delta G^\ddagger = 59$ kcal/mol).^[3] Furthermore, designing catalysts to lower this barrier is challenging due to the weak σ -donating and π -accepting properties of N₂O that make it a poor ligand for most transition metals.^[4,5] In fact, transition metal complexes featuring N₂O ligands have only been crystallographically characterized recently,^[6–9] in part because weakly donating ligands such as N₂ have long been known to bind competitively with N₂O in classic complexes such as $[\text{Ru}(\text{NH}_3)_5\text{L}]^{2+}$ (L = N₂O or N₂).^[10] Thus, fundamental knowledge about how transition metal active sites can be designed to bind and activate N₂O is very valuable.

As part of bacterial denitrification, the $2\text{H}^+/2\text{e}^-$ reduction of N₂O is catalysed by the metalloenzyme, *nitrous oxide reductase* (N₂OR).^[5] Structural characterization of N₂OR reveals that its catalytic site, Cu_Z, has an unusual $[\text{Cu}_4(\mu_4\text{-S})]$ core structure.^[11,12] Given the difficulties of designing synthetic catalysts for N₂O activation, it is intriguing to consider how this unusual 4Cu:1S cluster binds and activates N₂O under physiological conditions.

A large body of enzymological, spectroscopic, and computational work from Solomon, Moura, and co-workers^[13] has led to a recently updated mechanistic proposal for N₂O reduction at Cu_Z.^[14] In this proposal (Figure 1a), the fully-reduced 4Cu^I state of Cu_Z binds N₂O along the Cu_I-Cu_{IV} edge of the cluster with assistance from a second-sphere LysH⁺ residue (Lys397). Bending of the N₂O molecule upon coordination, along with electron donation from Cu_Z into the N₂O π^* levels, induces N–O bond cleavage and loss of N₂. The hydroxo ligand thus produced from N₂O is subsequently converted to H₂O with assistance from Lys397 acting as a proton shuttle during coupled proton/electron transfer. The structural asymmetry of the 4Cu:1S core is thought to be crucial for this mechanism, as the asymmetric distribution of electron density among the four Cu sites is thought to provide a low-energy pathway for electron transfer from Cu_Z to N₂O via Cu_{IV}. It should be noted, however, that this proposal is under some doubt as Einsle and co-workers showed that anoxic preparations of N₂OR feature a $[\text{Cu}_4(\mu_4\text{-S})(\mu_2\text{-S})]$ form of Cu_Z where the additional $\mu_2\text{-S}$ ligand blocks access to the dicopper edge proposed by Solomon et al. to be the N₂O binding site.^[12,15]

Synthetic model compounds can be used to probe various aspects of the Cu_Z mechanism.^[16] Tolman, Torelli, and our group have each reported N₂O reductase activity with copper-sulfur clusters that do not mimic the structural features of Cu_Z in terms of aggregation state or Cu:S stoichiometry.^[17–19] The first $[\text{Cu}_4(\mu_4\text{-S})]$ cluster was reported in 1993 by Yam and co-

workers using bridging dppm ligands (dppm = (Ph₂P)₂CH₂).^[20] This 4Cu^I complex lacks any well-defined redox chemistry or N₂O reactivity but, separately, has shown intriguing photochemical properties.^[21] Our group recently accessed the 2Cu^I:2Cu^{II} and 3Cu^I:1Cu^{II} redox states of a [Cu₄(μ₄-S)] cluster using the bridging formamidinate ligands [(2,4,6-Me₃C₆H₂N)₂CH]⁻,^[22,23] but the 4Cu^I state that would model the active form of Cu_Z was unstable and could not be accessed synthetically.

Our group has also reported new derivatives of the original Yam-type 4Cu^I complex, including [Cu₄(μ₄-S)(μ₂-dppa)₄]²⁺ (**1**, dppa = (Ph₂P)₂NH, see Figure 1b).^[24] A unique feature of **1** is the ability of its second-sphere N–H groups to act as hydrogen bond donors towards anions and solvent molecules. In our previous study, we showed that complex **1** exhibits reactivity that Yam's complex lacks, including activation of triatomic substrates isoelectronic to N₂O (e.g. N₃⁻) and strong binding of I⁻, which is a known inhibitor of N₂OR.^[5] In this report, we disclose conditions under which **1** mediates 2H⁺/2e⁻ reduction of N₂O, with two second-sphere N–H groups acting as the terminal proton donors to produce H₂O. The activity of **1** is modulated by second-sphere hydrogen bonding interactions and can be suppressed in the absence of effective hydrogen bond acceptors. Significant aspects of these findings include: 1) the first report of a synthetic cluster in the physiologically relevant 4Cu^I:S²⁻ redox state activating N₂O, 2) second-sphere amine residues that model the Lys397 residue of N₂OR, and 3) biomimetic interplay of primary and secondary structure on active site function.

In our previous study,^[24] we reported that complex **1** did not react with N₂O under any conditions we examined. Now we have found that bubbling excess N₂O into a MeOH solution of **1**, followed by slow addition of CoCp₂ (2 equiv) as an electron donor, results in a fairly rapid color change, with complete conversion within 1 h as determined by ³¹P NMR spectroscopy. The main Cu-containing product was identified as neutral Cu₂(μ₂-dppa)(μ₂-dppa')₂ (**2**, dppa' = (Ph₂P)₂N⁻), which was produced in 90% yield according to ³¹P NMR and was isolated in 67% recrystallized yield. Adding less than 2 equiv of CoCp₂ resulted in incomplete conversion of **1** according to ³¹P NMR spectroscopy, consistent with a two-electron reduction reaction (more details below). No reaction was observed between CoCp₂ and N₂O in the absence of **1**. Upon scale-up, the resulting [CoCp₂][PF₆] was isolated in pure form after workup (see Supporting Information, page S14). Based on these observations, we hypothesize a balanced reaction as shown in Scheme 1. The yield of N₂ was determined to be 86 ± 5 % by GCMS analysis, and formation of ¹⁵N₂ was observed when using ¹⁵N₂O. Thus, the yield of **2** is representative of the production of N₂ from N₂O. Due to the incompatibility of various reaction components with reagents employed in Karl-Fischer and other chemical H₂O assays, the formation of H₂O was verified qualitatively using an established near-IR assay,^[25] although H₂O quantification proved to be challenging by this method (see Supporting Information, page S23-S25).

Single-crystal X-ray diffraction studies of **2** (Figure 2a) revealed a pseudo-threefold symmetric dicopper(I) lantern structure with a Cu...Cu distance of 2.6718(6) Å. The N–H proton for the single dppa ligand was located in the Fourier difference map, but no evidence for N–H protons was found for the two dppa' ligands. The dppa ligand also has elongated P–N distances (P1–N1, 1.6824(15) Å; P2–N1, 1.680(2) Å) compared to the dppa' ligands (P3–

N2, 1.6312(15) Å; P4–N2, 1.635(2) Å; P5–N3, 1.6340(15) Å; P6–N3, 1.634(2) Å), presumably due to enhanced N/P hyperconjugation in the anionic dppa' ligands involving the π -symmetry N lone pair. The ^1H NMR spectrum of **2** is consistent with this structural assignment: the integral of the N–H resonance indicates the presence of only one remaining N–H proton (Figure S1). The ^{31}P NMR spectrum of **2** features two distinct resonances for the two chemically inequivalent phosphorous ligands, with each displaying complex second-order J_{PP} coupling patterns.

Varying the reactions conditions gave further insight into the factors controlling the ability of **1** to mediate N_2O reduction (Table 1). The conversion to **2** was determined to be 90% by NMR spectroscopy when conducted as described above (Entry 1). Performing the reaction under N_2 atmosphere rather than N_2O resulted in decomposition of **1** to tricopper(I) species **3** (Entry 2), as we have noted before,^[24] with no evidence for formation of **2**. Exposing **1** to N_2O in the absence of CoCp_2 also resulted in **3** (Entry 3), indicating that any meta-stable N_2O adduct of **1** must immediately be trapped by (proton-coupled) reduction in order for the reaction to proceed. Use of 1 equiv rather than 2 equiv of CoCp_2 resulted in only partial conversion to **2** along with decay to **3** (Entry 4), consistent with our proposal of a 2-electron reaction. Performing the reaction in the poor hydrogen bond acceptor solvents THF or toluene gave only unreacted **1** and decomposition product **3** (Entries 5–6), indicating that N_2O activation by **1** requires the second-sphere N–H groups to be engaged in hydrogen-bonding interactions with the solvent medium. Indeed, performing the reaction in the stronger H-bond acceptor solvent, acetone, reestablished the reaction (Entry 7).^[26] Interestingly, replacing MeOH with $\text{MeOH-}d_4$ also suppressed N_2O activation and resulted in unreacted **1** (Entry 8). The ^1H NMR spectrum of **1** in $\text{MeOH-}d_4$ does not have an observable N–H resonance, indicating deuterium exchange with the solvent. The complete suppression of reactivity due to this deuteration is puzzling. One possibility is the presence of a particularly large kinetic isotope effect during one or both O–H(D) bond forming steps. Exposing Yam's $[\text{Cu}_4(\mu_4\text{-S})(\mu_2\text{-dppm})_4]^{2+}$ complex (**1'**)^[20] to the reaction conditions did not result in any conversion (Entry 9). Collectively, considering the observations that replacing MeOH solvent with acetone solvent does not turn off the reaction but replacing NH groups in the ligand backbone with CH_2 groups does, it is likely that the backbone NH groups are acting as the hydrogen atom source to generate H_2O , akin to the Lys397 residue in N_2OR .

To better understand the sensitivity of N_2O reductase activity of **1** to solvent environment, we decided to examine its solvent-dependent structure. Repeated attempts at solving the solid-state structure of **1** using single crystals grown from $\text{MeOH-}d_4$ indicated the presence of hydrogen-bonded solvent molecules in the second sphere. However, Yam's $[\text{Cu}_4(\mu_4\text{-S})(\mu_2\text{-dppm})_4]^{2+}$ complex (**1'**)^[20] can be viewed as a model for the structure of **1** in the absence of any hydrogen-bonding interactions. The $[\text{Cu}_4\text{S}]$ core of **1'** has local C_{2v} symmetry, with a rectangle-based pyramidal core featuring $\text{Cu}\cdots\text{Cu}$ distances of 2.869(2) and 3.128(1) Å, and a $\mu_4\text{-S}$ -atom with a τ_4 parameter^[27] of 0.56. Crystals of **1** obtained from MeOH solution provided a structure with some key differences (Figure 2b). Two of the N–H groups are acting as hydrogen bond donors in the solid-state: the N1–H group to a PF_6^- counterion, and the N4–H group initiating a $\text{NH}\cdots\text{MeOH}\cdots\text{MeOH}\cdots\text{PF}_6^-$ network. The $[\text{Cu}_4\text{S}]$ core of **1** is highly unsymmetrical, with the three Cu sites distal to the N4–H

hydrogen-bonding network clustered close together ($\text{Cu1}\cdots\text{Cu2}$, 2.6969(6) Å; $\text{Cu2}\cdots\text{Cu3}$, 2.6690(6) Å) while the Cu4 site is pulled further away ($\text{Cu4}\cdots\text{Cu3}$, 3.0175(7) Å; $\text{Cu4}\cdots\text{Cu1}$, 3.542(1) Å). The S-atom geometry is also perturbed ($\tau_4' = 0.72$) compared to **1'**. These metrical parameters are almost identical to what we previously reported for **1** interacting with acetone molecules.^[24] Furthermore, these parameters are quite similar to the Cu_Z structure,^[11] which is also highly unsymmetrical with one Cu site distal (3.00–3.33 Å) from the other three and which has a similar S-atom geometry ($\tau_4' = 0.77$). Structural comparisons of the two synthetic $[\text{Cu}_4\text{S}]$ cores are shown in Figure 3a.

To evaluate the electronic impact of these structural changes, we studied **1'** and **1** computationally. When examining calculated NBO atomic charges, a notable difference between the two complexes is the buildup of additional negative charge on the Cu2 site, i.e. the middle site among the more closely clustered Cu centers, in unsymmetrical **1** (Figure 3a). When examining the frontier MOs produced by DFT calculations, another notable difference involves the HOMO-3 level, which is highly delocalized across the entire $[\text{Cu}_4\text{S}]$ core for **1'** but is localized mostly at the Cu1 and Cu2 sites in **1** (Figure 3b) and is also 573 cm^{-1} closer to the HOMO level. A similar phenomenon is observed for the LUMO level, which is localized at the sulfur atom for **1'** but mostly localized at the Cu2 site for **1** (see Supporting Information, page S46). Collectively, these observations indicate that for **1**, hydrogen bond-induced structural distortion creates localization of frontier MO density at the Cu1-Cu2 edge site, both making Cu2 more electrophilic towards N_2O binding and making the Cu1-Cu2 edge better able to π -backbond into the π^* manifold of bound N_2O . In a related discovery, recently Agapie showed that structural distortion of tetrametallic models of the oxygen evolving complex (OEC) of photosystem-II through steric pressure modulates the clusters' reduction potentials.^[28] The additional contribution of our system is the correlation between structure and chemical reactivity with the relevant substrate, N_2O . A similar correlation between substrate activation and localization of frontier MO density has recently emerged to describe the octanuclear FeMo-cofactor of nitrogenase.^[29] In our system, no visual changes are observed unless all three reaction components (**1**, CoCp_2 , and N_2O) are present. Because complex **1** is in the $4\text{Cu}^{\text{I}}:1\text{S}^{2-}$ redox state, it is unlikely that reactivity initiates with reduction of **1** by CoCp_2 . Instead, we favor a sequence where the π -accepting molecule N_2O binds to **1**, likely along the Cu1-Cu2 edge, thus raising the reduction potential such that CoCp_2 can donate to the newly introduced electron holes of **1**- N_2O .

To summarize, the N_2O reductase activity of **1** depends on a subtle interplay of primary and secondary coordination sphere effects. In the presence of appropriate hydrogen bond acceptor molecules (MeOH, acetone) in the reaction medium, second-sphere hydrogen bonding induces structural distortion in the primary coordination sphere of the $[\text{Cu}_4\text{S}]$ active site. The resulting strained geometry enhances the active site's ability to bind and activate N_2O through localization of frontier MO density along one edge site of the cluster. Upon activation, the N_2O substrate is converted to N_2 and H_2O with H^+ donation directly from the second coordination sphere. These behaviors closely mimic phenomena previously hypothesized for the Cu_Z site of N_2OR and, we hope, provide an entryway to future Cu_Z modeling studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) Proposed mechanism for N_2O reduction at Cu_4Z , with key ET and PT pathways highlighted in red; (b) Synthetic model complex 1 featured in this work.

Figure 2.

Solid-state structure of (a) **2** and (b) **1** (from MeOH solution) as 50%-probability ellipsoids (non-C,H atoms) or wireframe (C atoms). C-H hydrogens have been omitted, and the N-H hydrogens shown were located in the Fourier difference map. Selected distances (Å) and angles (°) for **2**: Cu1–Cu2, 2.6718(6); P1–N1, 1.6824(15); P2–N1, 1.680(2); P3–N2, 1.6312(15); P4–N2, 1.635(2); P5–N3, 1.6340(15); P6–N3, 1.634(2); P1–N1–P2, 123.3(1); P3–N2–P4, 117.3(1); P5–N3–P6, 120.0(1). For **1**: Cu1–Cu2, 2.6969(6) Å; Cu2–Cu3, 2.6690(6) Å; Cu3–Cu4, 3.0175(7) Å; Cu1–Cu4, 3.542(1).

Figure 3.

(a) Comparisons of the [Cu₄S] cores of **1'** and **1**, with bond distances shown in black and NBO charges shown in red; (b) comparisons of the HOMO-3 orbitals for **1'** and **1** (B3LYP/6-31++G**).

Table 1.

Variations on N₂O reduction reaction by 1.^[a]

Entry	Variation from standard conditions	Unreacted 1 (%)	Product 2 (%)	Decomposition product 3 (%)
1	None	0	90(67 ^[b])	0
2	N ₂ atmosphere	54	0	25
3	No CoCp ₂	60	0	15
4	1 equiv CoCp ₂	6	38	44
5	THF solvent	38	0	48
6	Toluene solvent	67	0	6
7	Acetone solvent	0	42	0
8	MeOH- <i>d</i> ₄ solvent	77	0	0
9	dppm in place of dppa	>95	0	0

^[a]Yields were determined by ³¹P NMR using tri-*o*-tolylphosphine as the internal standard.

^[b]Isolated yield.