

Published in final edited form as:

Chem Commun (Camb). 2014 February 4; 50(9): 1104–1106. doi:10.1039/c3cc47705c.

Orbital Contributions to CO Oxidation in Mo-Cu Carbon Monoxide Dehydrogenase

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Abstract

A molecular orbital analysis provides new insight into the mechanism of Mo/Cu carbon monoxide dehydrogenase, and reveals electronic structure contributions to reactivity that are remarkably similar to the structurally related molybdenum hydroxylases. A calculated reaction barrier of ~12 kcal/mol is in excellent agreement with experiment.

The carbon monoxide dehydrogenase (CODH) from *Oligotropha carboxidovorans* and *Hydrogenophaga pseudoflava* possess a μ -sulfido bridged heterobinuclear Mo/Cu active site.^{1–5} This unique Mo/Cu CODH is a member of the xanthine oxidase (XO) family of pyranopterin molybdenum enzymes that typically catalyse the oxidative hydroxylation of N-heterocycle and aldehyde substrates^{6, 7}. In marked contrast to XO, CODH catalyses the oxidation of CO to CO₂ according to



with the Mo ion cycling between the Mo(VI) and Mo(IV) oxidation states. The Cu ion remains at the Cu(I) oxidation level throughout the catalytic sequence². A recent kinetic study of CODH² showed that the activation energy for CO oxidation is 11.4 kcal/mol at pH 7. Of particular note is the lack of a CO concentration dependence on the kinetic parameters. This suggests that CO binding to the enzyme is rapid, followed by a slower substrate oxidation step. Support for CO binding directly to the Cu(I) center derives from recent ¹³C and ^{63,65}Cu ENDOR studies of a paramagnetic Mo(V)/Cu(I) intermediate state trapped during reduction of the enzyme by CO.⁸

DFT computations^{9, 10} suggest that the CO carbon present in the Mo(VI)/Cu(I)-CO complex formed after substrate binding is susceptible to nucleophilic attack by a metal activated water (Mo=O/Mo-OH) to yield a cyclic μ_2 - η^2 CO₂ bridged intermediate (**1**) (Figure 1). Intermediate **1** has been proposed to undergo a large geometric rearrangement to form a very stable C-S bonded intermediate (**2**) (Figure 1). The stability of **2** leads to large computed reaction barriers for the formation of the reduced Mo(IV)/Cu(I) cluster and oxidized product⁹. Support for intermediate **2** in the catalytic cycle is based upon x-ray crystallographic studies of an *n*-butylisocyanide inhibited form of CODH³ that also possesses a C-S bond. Interestingly, EPR and ENDOR spectroscopic studies of the related

XO enzyme reveal the presence of an enzyme-substrate C-S bond in an inhibited XO enzyme form (aldehyde inhibited XO) obtained under turnover conditions with certain aldehydes.^{6, 11, 12} In light of the inhibitory nature of C-S bond formation in both XO and CODH, we wondered whether the formation of highly covalent enzyme-substrate C-S bonds is a necessary condition for catalysis in CODH. We thought it possible that the elimination of stable C-S bonded structures such as **2** (Figure 1) would lead to reduced activation barriers for CO oxidation and minimal geometric changes at the active site during catalysis. We begin with an electronic description of the cyclic $\mu_2\text{-}\eta^2$ CO₂ bridged intermediate **1**, which is believed to occur prior to C-S bond formation and is common to all currently proposed mechanisms. This provides a convenient starting point for understanding electronic structure contributions to catalysis in CODH, particularly with respect to small molecule activation and the avoidance of stable intermediates that possess covalent C-S bonds.

The structure of putative intermediate **1** possesses a bent $\mu_2\text{-}\eta^2$ CO₂ molecule that bridges the Mo and Cu ions. This structure is remarkably similar to that proposed for the transmetallated product of Aresta's complex using organozinc reagents¹³, where a bent CO₂ molecule is bound $\mu_2\text{-}\eta^2$ to a heterobinuclear transition metal cluster and activated for nucleophilic attack at carbon. While the CO₂ oxygens are weak Lewis bases, the carbon center is electrophilic. Thus, Cu(I) \rightarrow CO₂ charge donation (back bonding) results in partial occupation of the degenerate CO₂ LUMOs (Figure 2), and this will drive a Renner-Teller¹⁴ (R-T) distortion to a bent (C_{2v}), geometry. Structural changes that result from orbital charge transfer between small molecules and transition metals have recently been shown to be a key component in the activation of these ligands¹⁵. With respect to intermediate **1**, the effect of the R-T distortion is to break the degeneracy of the CO₂ LUMOs leading to the in-plane (ip; a_1) LUMO being stabilized relative to the out-of-plane (b_1) LUMO. This bending also results in a LUMO(a_1) orbital with a directional sp hybridized carbon center. Thus, R-T induced bending of CO₂ in **1** may activate the coordinated CO₂ in **1** for nucleophilic attack at carbon.

Both the HOMO and LUMO of **1** possess CO₂ LUMO(a_1) character, highlighting the importance of the CO₂ LUMO(a_1) in the reactivity of **1** (Figure 3). These highly delocalized orbitals are consistent with the results of EPR and computational studies on CODH models, which show a high degree of Mo-S-Cu covalency.¹⁶ The HOMO of **1** clearly shows the key Cu(I) \rightarrow CO₂ σ -type charge donation that leads to CO₂ bending and activation for nucleophilic attack. The CO₂ LUMO(a_1) character present in the LUMO of **1** indicates that **1** possesses an activated CO₂ molecule that is subject to nucleophilic attack by a water or hydroxide at the active site. The LUMO of **1** also has considerable Mo-S π^* character that demonstrates that the Mo ion is not fully reduced. In fact, the occupied Mo character in the HOMO is only ~30%, compared to ~60% for the computed fully reduced Mo(IV) form, *vide infra*. A natural bond orbital (NBO^{17, 18}) analysis of **1** supports this partially reduced character, and further reveals **1** to be an approximate 1:1 resonance hybrid of Mo(VI) and Mo(IV) structures arising from C-Cu $\sigma \rightarrow$ Mo-S π^* charge donation and Mo-S $\pi \rightarrow$ C-Cu σ^* donation (Figure 4 A-D). The NBO description of the bonding in **1** is remarkably similar to what we previously observed in CODH related XO¹¹ (Figure 4). Specifically, the XO intermediate formed by nucleophilic attack of a metal activated water on the carbonyl carbon of aldehyde substrates possesses an analogous combination of C-H $\sigma \rightarrow$ Mo-S π^* and Mo-S $\pi \rightarrow$ C-H σ^* charge donations. These two donor-acceptor interactions in XO lead to the resonance structures in Figure 4B. The similar donor-acceptor interactions in these XO and CODH intermediates underscore their importance in C-H and CO₂ activation, respectively.

The x-ray structure of CODH shows a water molecule weakly associated with the Cu(I) ion at a Cu(I)-O distance of 2.4\AA^3 . With respect to the potential for Cu(I) to activate a water molecule by lowering its pK_a , we computed the reaction coordinate for nucleophilic attack of hydroxide and water on the activated $\mu_2\text{-}\eta^2$ CO₂ carbon centre of **1-OH** and **1-OH₂**. Hydroxide attack proceeds through a single TS to yield a fully reduced Mo(IV)-product complex with a low activation barrier (ΔG^\ddagger of 12 kcal/mol), and the Mo(IV)-bicarbonate product complex (**1-P**) is stabilized relative to **1-OH** with a ΔG of -2 kcal/mol. Water attack on the $\mu_2\text{-}\eta^2$ CO₂ carbon centre of **1-OH₂** results in the formation of carbonic acid with a ΔG^\ddagger of 22 kcal/mol. Thus, activation of a water molecule may contribute up to 10 kcal/mol toward TS stabilization in this process. The CODH active site contains an active site glutamate residue (Glu 763) that is rigorously conserved in XO. Glu 763 is only 3.7\AA from the Cu(I) site, and may further activate a water molecule associated with Cu(I) for nucleophilic attack on the $\mu_2\text{-}\eta^2$ CO₂ carbon centre to yield the Mo-bound bicarbonate product species. Glu 763 may also play an important role in the coupled electron-proton transfer steps necessary to convert Mo(IV)-OH₂ to Mo(VI)=O in the electron transfer (oxidative) half reaction of the catalytic cycle¹⁹.

Conclusions

A reaction mechanism (Figure 5) may now be proposed for CODH that initially involves nucleophilic attack of a Mo=O oxo on the carbon center of Cu(I)-CO, resulting in a 5-membered cyclic intermediate (**1**) that can bind HO⁻/H₂O to yield **1-OH**. This is followed by a second nucleophilic attack on the activated $\mu_2\text{-}\eta^2$ CO₂ carbon centre of **1-OH** to yield a Mo(IV)-bicarbonate product complex, **1-P**. This second nucleophilic attack is suggested based on our electronic structure description of intermediate **1**, which possesses a bent and activated CO₂ bound to the Mo and Cu ions. The low energy barrier ($\Delta G^\ddagger = 12$ kcal/mol) calculated for hydroxide attack on **1-OH** is in excellent agreement with the experimentally determined value of 11.4 kcal/mol. Previous computational work^{9, 10} based on the x-ray structure an *n*-butylisocyanide inhibited form of the enzyme³ suggested the presence of a very stable intermediate (structure **2** in Figure 1) that possesses a C-S bond. These earlier calculations showed that breakdown of intermediate structure **2** occurs with a larger activation energy, and this is likely due to the inhibitory nature of C-S bond formation in XO family enzymes¹¹.

Although CODH and XO oxidize very different substrates (CO vs. purine heterocycles and aldehydes), we observe a remarkable electronic structure similarity for proposed intermediates that directly contribute to CO₂ and C-H bond activation, respectively. In XO, the combination of C-H $\sigma \rightarrow \text{Mo-S } \pi^*$ and Mo-S $\pi \rightarrow \text{C-H } \sigma^*$ charge donation contributes to lowering the activation barrier for C-H bond cleavage that is coupled to Mo reduction¹¹. In CODH we have shown that strikingly similar donor-acceptor interactions are operative, with C-Cu $\sigma \rightarrow \text{Mo-S } \pi^*$ charge donation and Mo-S $\pi \rightarrow \text{C-Cu } \sigma^*$ donation contributing to CO₂ activation and partial reduction of Mo. We anticipate that this work will stimulate further discussion and provide the impetus for new experimental studies directed toward unravelling the remarkable chemistry associated with this unique CODH heterobimetallic active site.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

M.L.K. acknowledges the National Institutes of Health (GM 057378) for generous support of this work.

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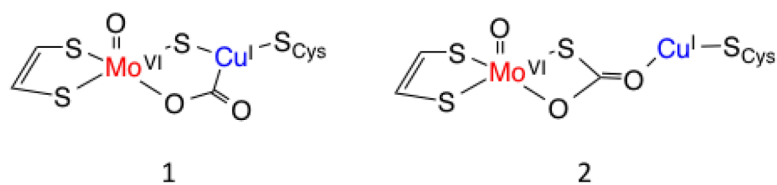


Figure 1.

a) Cyclic $\mu_2\text{-}\eta^2$ CO₂ bridged intermediate, **1**, suggested by computational studies probing the reaction coordinate of CODH. b) Inhibited-type structure with stable C-S bond, **2**.

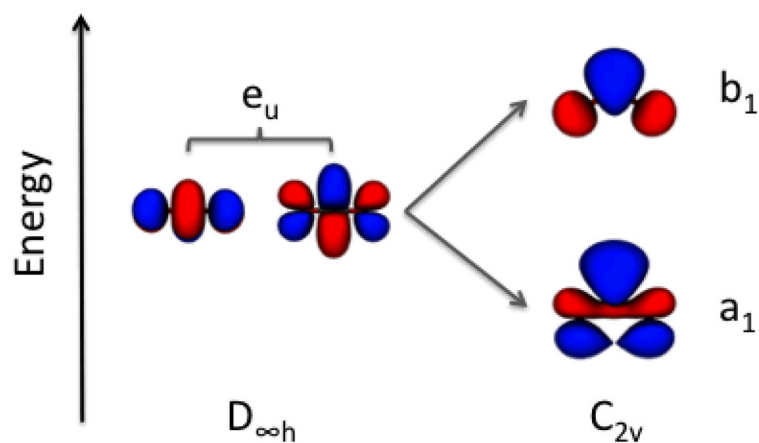
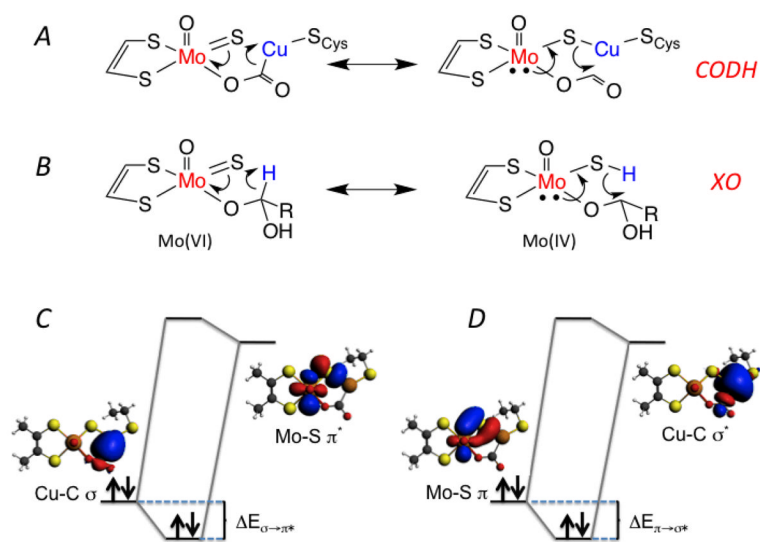


Figure 2. LUMOs of linear (left) and bent (right) CO₂. Note the stabilization of the in-plane a₁ MO upon bending.



Figure 3.
DFT calculated frontier molecular orbitals of **1**. Left: HOMO, right: LUMO. Note the CO₂ LUMO(ip) character in both of these orbitals.

**Figure 4.**

(A) Resonance structures that contribute to the ground state of cyclic intermediate 1. (B) Resonance structures that contribute to the ground state of the corresponding XO intermediate. (C) Principle NBOs involved in Cu-C $\sigma \rightarrow$ Mo-S π^* delocalization. (D) Principle NBOs involved in Mo-S $\pi \rightarrow$ Cu-C σ^* delocalization.

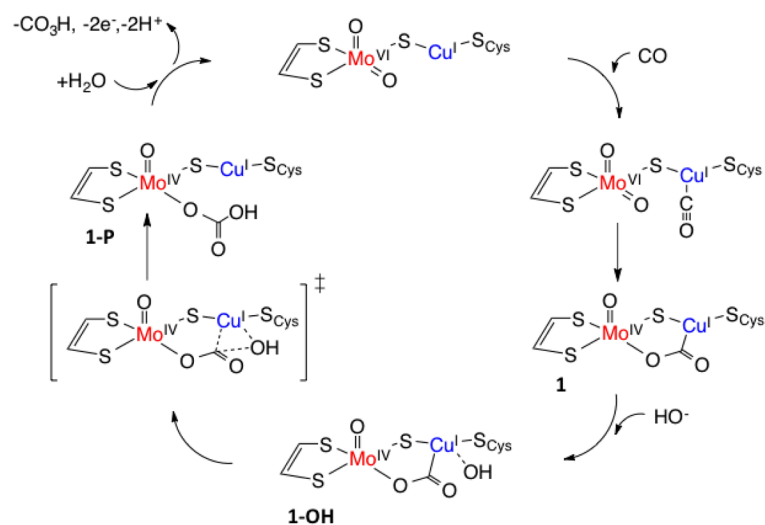


Figure 5. Proposed catalytic cycle for CODH that avoids formation of a stable C-S bonded intermediate.