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ORIGINAL ARTICLE

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The mechanism of Mo-/Cu-dependent CO dehydrogenase

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Abstract Density functional theory computations at the B3LYP/SDDp//B3LYP/Lanl2DZ level were performed on model complexes derived from $[(Me_2C_2S_2)Mo(O)_2\text{-}S\text{-}CuSMe]^{2-}$ or its oxo protonated form to gain insight into the reaction steps involved in substrate oxidation of a Mo-/Cu-dependent CO dehydrogenase. Only the bis-oxo but not the hydroxo oxo complex was found to oxidize CO exothermically. A thiocarbamate complex structurally characterized as the reaction product of the enzyme with the inhibitor *n*-butylisonitrile corresponds to a thermodynamic well on the potential energy surface. For the formation of the analogous thiocarbonate complex from CO oxidation, however, we do not find a significant thermodynamic driving force. In the protein matrix of the enzyme this species should be further destabilized, as it requires the metal centers to move apart considerably from each other.

Keywords Density functional theory · Computational chemistry · Model compound · CO dehydrogenase · Dinuclear molybdenum copper complexes

Introduction

Until recently molybdenum has been known to be biologically relevant in mononuclear hydroxylases and oxo transferases [1–4] and as part of the FeMo cofactor in nitrogenase [5]. The biological role of copper on the other hand is electron transfer and oxygenation by mononuclear enzymes of types I and II, respectively, and oxygen transfer and activation by dinuclear copper enzymes (type III) [6, 7]. A carbon monoxide dehydrogenase (CODH) from the aerobic bacterium *Oligotropha carboxidovorans* has been recognized as the first metalloenzyme containing Mo and Cu in its active site only recently [8, 9]. Originally, the heavy atom in close proximity to the Mo was described as a selenium atom [10]. Other Cu-containing heterodinuclear active sites of metalloenzymes feature Cu/Zn (superoxide dismutase) [11] and Cu/Fe (cytochrome c oxidase) [12], where Cu is redox-active.

As the Mo-/Cu-dependent CODH has an active site of a new metal composition with a distinct role for copper the mechanism of CO oxidation is of particular interest. Other CODHs identified in anaerobes employ Ni as a member of a Fe4S4 cluster as a catalytically active site [13].

In addition to the resting state of the Mo- and Cu-containing enzyme, X-ray crystallographic structures could be determined for the Cu-depleted (inactive) form, the oxidized and the reduced states as well as for a *tert*-butyl isocyanide (*t*-BuNC) inhibited form [9]. The active dinuclear form (Scheme 1, **A**) has Cu(I) bound by a terminal cysteinate (Cys388) and by a sulfide bridging to Mo(VI). The latter is chelated by two sulfur atoms of a metallopterin ligand (mpt), which is also found in all mononuclear molybdenum enzymes. The Mo coordination is completed by one oxo and one hydroxo ligand [9] (or two additional oxo ligands according to extended X-ray absorption fine structure studies [8]). The *t*-BuNC inhibited form has no Mo–S–Cu link but a thiocarbamate coordinated to Mo by O and S and to Cu by N (Scheme 1, **B**).

In analogy to **B** a structure **C** was postulated as an intermediate for the oxidation of CO. This intermediate cannot be formed directly by nucleophilic attack of the equatorial Mo=O at the CO carbon, which supposedly is activated by coordination to copper but requires additional and substantial rearrangement. The reverse rearrangement is needed to reform the Cu–μ–S bond

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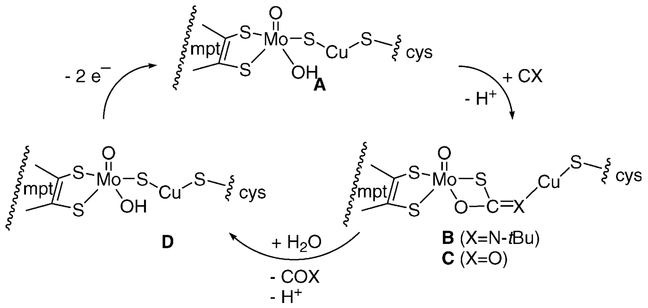

Scheme 1 Proposed catalytic cycle for Mo-/Cu-dependent carbon monoxide dehydrogenase [9]

when CO_2 is split off and substituted by H_2O (**D**). Reoxidation of the Mo(IV) center by an external electron acceptor completes the proposed catalytic cycle (Scheme 1).

We performed density functional theory computations on simple model complexes to investigate the plausibility of the proposed catalytic cycle.

Computational details

Structures were optimized with Gaussian 98 [14] using the B3LYP [15–17] density functional and the Lanl2DZ [18–21] effective core basis set supplemented by d-type polarization functions on S ($\zeta = 0.421$) [22] and characterized by analytical frequency calculations at the same level. The relative energies reported here are derived from additional single-point energy calculations employing the SDD [23–25] basis set augmented by d-type polarization functions on C ($\zeta = 0.600$), O ($\zeta = 1.154$), N ($\zeta = 0.864$) as well as S ($\zeta = 0.421$) [22] and are corrected for zero-point vibrational energies in the case of E_{rel} and for thermal corrections obtained for $p = 1.013$ hPa and $T = 298.15$ K in the case of ΔG^0_{298}. A list with optimized Cartesian coordinates, a table of absolute energies and a display of molecular structure for all structures discussed in the text are provided as supplementary material. Additional self-consistent reaction field single-point computations employed the same method and basis sets and the conductor-like screening model (COSMO) [26] approach as implemented in Gaussian 03 [27], with a dielectric constant and a solvent radius of $\epsilon = 4.0$ and $r = 1.4$ Å, respectively.

Results and discussion

Various computed structures possibly relevant in the oxidation reaction starting with the bisoxo form **1** are shown in Figure 1. Carbon monoxide binds to the copper atom of the bisoxo complex **1** to form adduct **2O** with a binding energy of 7.9 kcal mol^{-1} (Fig. 1). The subsequent oxo attack to form a five-membered cyclic intermediate **3O** is a very easy process: it requires only 3.4 kcal mol^{-1} of activation and is exothermic by 6.9 kcal mol^{-1}.

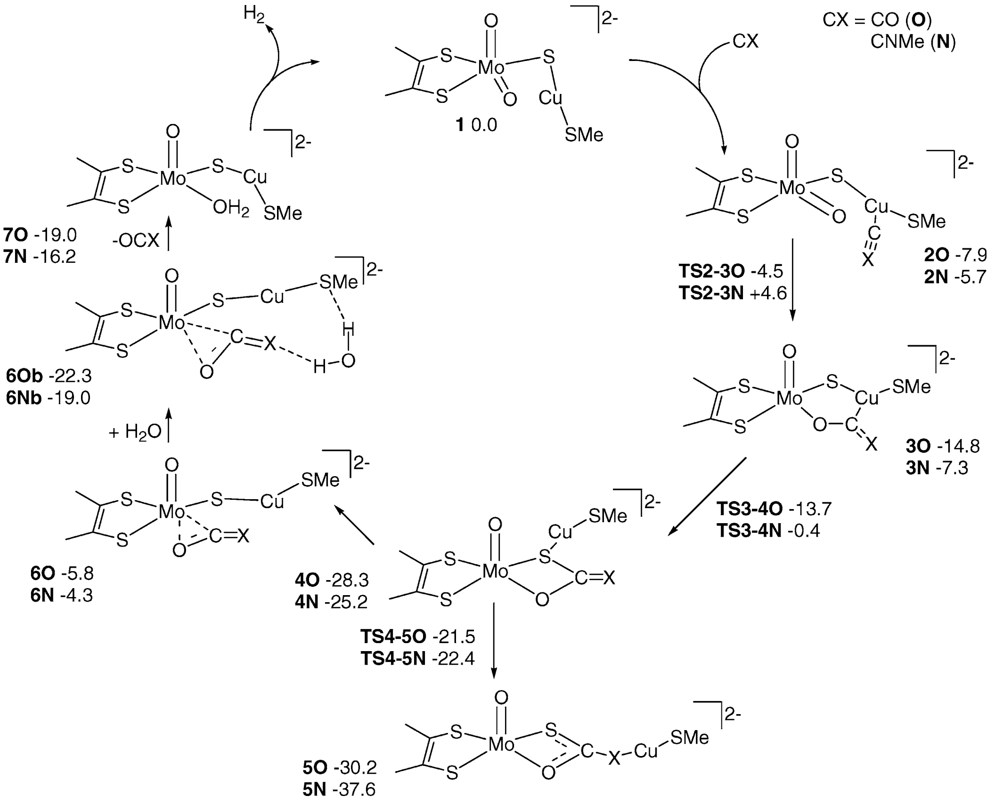

Fig. 1 Reaction cycle for the CO and the MeNC oxidation. Relative energy values ΔE are given in kilocalories per mole

Isomerization of **3O** to the four-membered cyclic intermediate **4O** is very easy (barrier of 1.1 kcal mol^{-1}) and thermodynamically favorable ($\Delta E = -13.5$ kcal mol^{-1}). Further isomerization of **4O** (Cu coordinated at S) to the postulated intermediate **5O** (Cu coordinated at O), however, has only a small thermodynamic driving force ($\Delta E = -1.9$ kcal mol^{-1}) according to our model calculations. Elimination of carbon dioxide from **4O** and addition of a water molecule (**4O** → **7O**) is endothermic by 9.3 kcal mol^{-1}.

Replacing the carbon dioxide moiety of **3O** by a water molecule leads to **7O** more directly. This reaction step is exothermic ($\Delta E = -4.2$ kcal mol^{-1}) and exergonic ($\Delta G^0_{298} = -3.7$ kcal mol^{-1}). The η^2-product complex **6O** could also be the species from which water replaces the oxidation product [28]. We tried to locate minima corresponding to **3O** with an additional water molecule bound to Mo or Cu but could not find such stationary points; neither could a transition state be located connecting **3O** and **6O**. However, we computed a minimum corresponding to **6O** with one additional water molecule bridging the former carbon monoxide oxygen with the copper atom. The reaction step **6O** → **6Ob** is highly exothermic ($\Delta E = -16.5$ kcal mol^{-1}) but less exergonic ($\Delta G^0_{298} = -6.8$ kcal mol^{-1}).

We also computed structures analogous to those just discussed but using MeNC as a reactant to mimic the experimentally investigated inhibitor *t*-BuNC. While both CO and MeNC bind about equally well to the copper center of **1**, the reaction forming the five-membered ring intermediate **3** has a considerably higher barrier of 10.3 kcal mol^{-1} for oxo attack to the carbon atom of MeNC ($E_a = 3.4$ kcal mol^{-1} for CO oxidation). Further significant energetic differences are a much more exothermic formation ($\Delta E = -37.6$ kcal mol^{-1}) of **5N** and a significantly larger driving force for the **4N** → **5N** isomerization ($\Delta E = -12.4$ kcal mol^{-1}) at a reduced barrier involved ($E_a = 2.8$ kcal mol^{-1}). In summary, the formation of **5** is easier and more favorable for an isonitrile substrate in comparison with CO.

As it is not clear which experimentally determined structure, i.e., MOO [8] or MO(OH) [9], is catalytically active, model compounds for oxidations starting with the oxohydroxo complex **8** were computed for comparison (Fig. 2). The carbon monoxide coordinates to the copper atom of **8** to form **9O** slightly more strongly ($\Delta E = -9.8$ kcal mol^{-1}) as compared with the dioxo species **1** ($\Delta E = -7.9$ kcal mol^{-1}). Tautomers to **9** protonated at *X* and having a bisoxo-Mo moiety could not be found. Geometry optimizations of such starting geometries converged to **10** or ring-closed **12**. In the former case, the substrate carbon atom is inserted into the copper–sulfur bond, forming a six-membered ring including an *X*H···O hydrogen bridge.

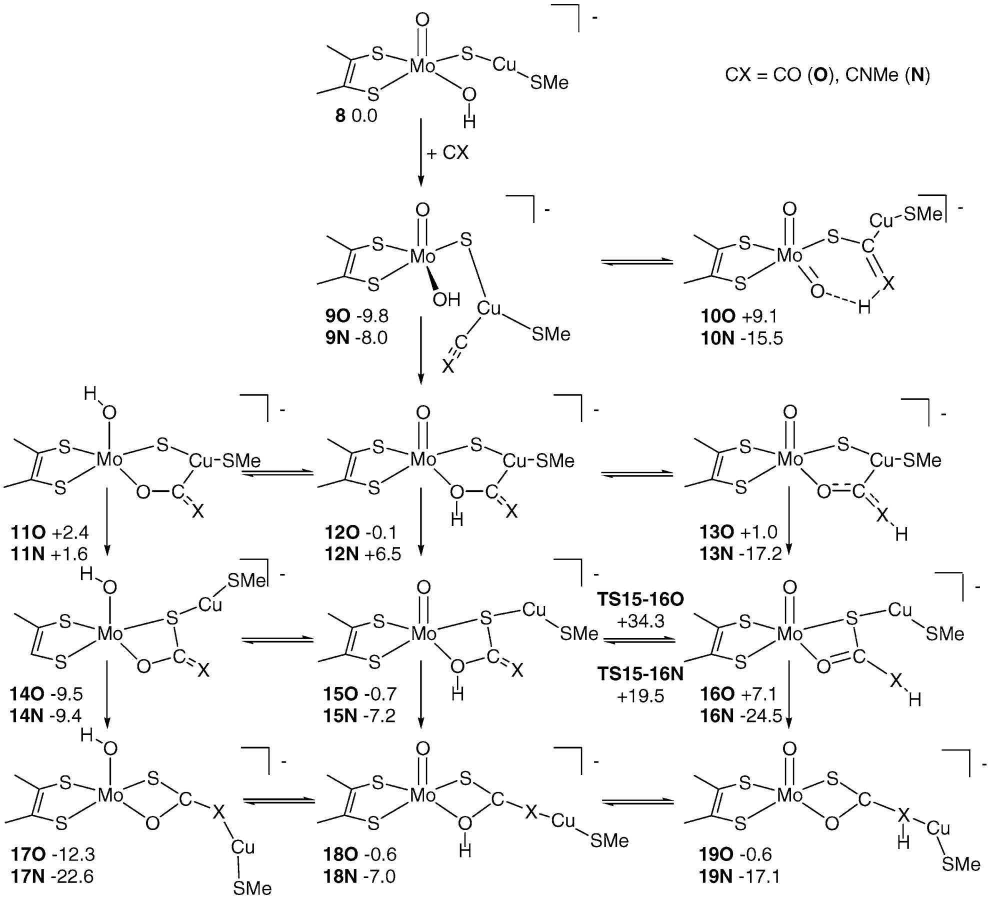

Fig. 2 Possible reaction intermediates for the CO (**O**) or MeNC (**N**) oxidation by the oxo hydroxo model complex **8**. Relative energies are given in kilocalories per mole

The rearrangement of **9O** to **15O** or **18O** with four-membered rings containing molybdenum, sulfur, carbon and the hydroxyl group is endothermic by approximately 9 kcal mol^{-1}. The tautomers **16O** and **19O**, respectively, are not more favorable either. In addition, **16O** is separated from **15O** by a large barrier (35.0 kcal mol^{-1}). Isomers **18O** and **19O**, where copper is bound to the former CO oxygen atom rather than to the sulfur atom, are also less stable than adduct **9O**. Starting geometries constructed by protonating the axial rather than the equatorial oxygen in **1** and **2O** again converged back to conformers with axial oxo and equatorial hydroxo ligands. In summary, the hydroxo oxo pathway starting with **8** seems unlikely as we could not identify any intermediate stabler than the initial CO adduct **9O**. In principle, the working catalytic cycle could consist of some reaction steps from the dianionic reaction sequence (Fig. 1) and some from the monoanionic version, provided suitable acidic and/or basic residues are present to provide and/or accept protons. However, none of the isomerization steps considered in Fig. 2 are energetically as favorable as the analogous steps in Fig. 1.

For the isonitrile oxidation the situation is less clear. From thermodynamic stability considerations of possible intermediates a reaction sequence **9N** → **10N** → **16N** seems competitive with the bisoxo route **2N** → **3N** → **4N**. The final rearrangement to the copper N-bound product complex identified experimentally, however, is only favorable for the deprotonated species, i.e., **4N** → **5N**, while **16N** → **19N** is endothermic by 7.4 kcal mol^{-1}.

We computed ΔG^0_{298} for stationary points found for the oxidation of CO and CNMe by complex **1** both accounting for (COSMO computations) and disregarding medium effects of a surrounding polarizable continuum. The results are displayed in Fig. 3a and for comparison in Fig. 1 of the supporting information, respectively. While the relative ΔG^0_{298} values are somewhat more positive as compared with ΔE, the energy trends are the same. The COSMO results are given in the following. Formation of the oxidation product CO_2 is slightly more exergonic ($\Delta G^0_{298} = -5.1$ kcal mol^{-1}) as compared with that of OCNMe (-1.4 kcal mol^{-1}). The reverse is true for the formation of intermediate **5**

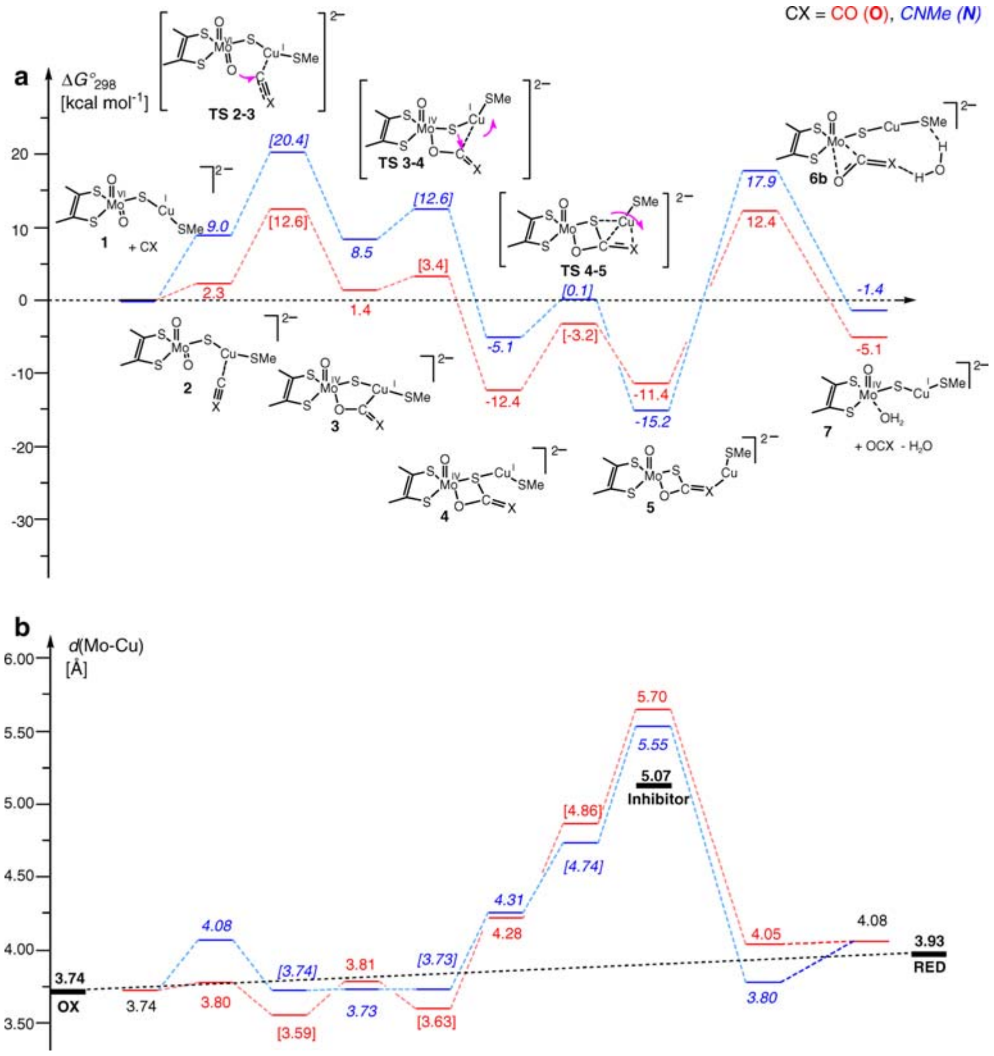

Fig. 3 Energy profiles resulting from computations accounting for medium effects (see "Computational details") for the model oxidation of CO and MeNC (values in *italics*) by the bisoxocomplex **1** in comparison (**a**) and correlation with the Mo–Cu distance in intermediates and transition states (labeled **TS** and included in *brackets*) involved (**b**). Values from enzyme X-ray structures are indicated in *bold*

($\Delta G^0_{298} = -11.4$ and -15.2 kcal mol^{-1} when X is O and NMe, respectively), a model for the inhibited enzyme structure **B**. However, it is more exergonic for both substrates, CO and CNMe, as compared with forming the water complex **7** and the oxidized substrate.

Hence, it remains to be explained why CODH is able to catalytically oxidize CO but is inhibited by isonitriles. We believe that a thiocarbonate intermediate like **5O** is not relevant in the catalytic CO oxidation by CODH. The distances between the metal centers, *d*(Mo–Cu), computed for model complexes **1** (3.74 Å) and **7** (4.08 Å) are in excellent agreement with the X-ray diffraction values of the oxidized (3.74 Å) and reduced (3.93 Å) forms of the enzyme [9], respectively. There is not much change in *d*(Mo–Cu) in going from **1** to **4** (Fig. 3). The isomerization **4** → **5**, however, involves a substantial elongation of the Mo–Cu distance (5.55 and 5.70 Å for *X* is NMe and O, respectively). This requires additional energy in the presence of a protein framework, which does not allow the metal ions to move freely. This is evident from the enzyme inhibitor complex, which is more compressed with respect to the Mo–Cu distance (5.07 Å) than the unrestricted model complexes **5O** and **5N**. The thermodynamical driving force of the copper migration is obviously large enough to overcome this strain in the case of *X* equals NR but not in the case of *X* equals O. The potential energy profile in the presence of a protein framework should be flatter than that shown in Fig. 3, which was computed for an unconstrained model.

Conclusions

Our density functional theory computations suggest the bisoxo form to be the catalytic species of CODH. The main differences of CO versus MeNC oxidation by the $Mo(O)_2SCu$ active center are (1) the initial oxo attack at the substrate carbon atom is easier for CO, and (2) a significant driving force for isomerizing the Cu–S-bound four-membered ring intermediate **4** to the Cu–O-bound or the N–bound intermediate **5** only exists for the isonitrile oxidation.

Hence, a thiocarbonate complex **5**, which was proposed as an intermediate in analogy to a characterized inhibitor complex, is unlikely to be involved in the catalytic cycle of CO oxidation.

Water may replace the oxidation product CO_2 directly from **4**, a process that requires less rearrangement at the active site, especially no Cu–S bond breaking and reforming.

Note added in Proof Recently a similar DFT study on a more extended CODH model was published.[29] The computed energetics reported there agree well with our values. The authors also conclude the bis oxo form of the active center to be the catalytically active one. A direct cycle without the involvement of a type 5 intermediate is also found to be energetically feasible. However, the possibility is raised that such an intermediate nevertheless is part of the cycle. Additional computations indicate that the prohibitively large barrier for Co_2 release is reduced by the involvement of an additional water molecul.

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