Can water act as a nucleophile in CO oxidation catalysed by Mo/Cu CO-dehydrogenase? Answers from theory.

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Abstract

The aerobic CO dehydrogenase from *Oligotropha carboxidovorans* is an environmentally crucial bacterial enzyme for maintenance of subtoxic concentration of CO in the lower atmosphere, as it allows for the oxidation of CO to CO_2 which takes place at its Mo–Cu heterobimetallic active site. Despite extensive experimental and theoretical efforts, significant uncertainties still concern the reaction mechanism for the CO oxidation. In this work, we used the hybrid quantum mechanical/molecular mechanical approach to evaluate whether a water molecule present in the active site might act as a nucleophile upon formation of the new C—O bond, a hypothesis recently suggested in the literature. Our study shows that activation of H_2O can be favoured by the presence of the $Mo=O_{eq}$ group. However, overall our results suggest that mechanisms other than the nucleophilic attack by $Mo=O_{eq}$ to the activated carbon of the CO substrate are not likely to constitute reactive channels for the oxidation of CO by the enzyme.

Introduction

Carbon monoxide dehydrogenase (MoCu-CODH) from the aerobic soil bacterium *Oligotropha carboxidovorans* has an important role in the conversion of an estimated 2 x 10^8 tons of CO from the lower atmosphere and soil per year,[1] following the reaction:

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$$CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^-$$

Analogously to the case of the other known class of CO-dehydrogenases, i.e. the Ni/Fe-dependent enzymes,[2] MoCu-CODH enzymes can also behave as hydrogenases; however, their H_2-oxidizing activity is rather low. MoCu-CODH belongs to the xanthine oxidase enzyme family, molybdenum enzymes that catalyse hydroxylation and oxo-transfer reactions by means of their catalytically labile –OH group in the molybdenum equatorial position.[3] The active site architecture of MoCu-CODH constitutes an exception to the xanthine oxidase family. It is constituted of a unique $MoO_2(\mu S)Cu$ bimetallic centre in the oxidised form of the enzyme. The molybdenum ion shows a distorted square pyramidal geometry, with one axial oxo ligand and four equatorial ligands: a dithiolene ligand from a molybdopterin cytosine dinucleotide (MCD) cofactor, one oxo (O^{2-}) ligand and a μ-sulfido ligand. The latter links the Mo-centre to the Cu-centre, which is also coordinated by the sulphur atom of Cys388 and by a weakly coordinated water molecule.[4−6] Although the protonation state of the Mo(VI)(=O)O unit in the oxidised active site has been subject of debate, due to controversial experimental results obtained by X-ray diffraction (XRD) techniques and by extended X-ray absorption fine structure (EXAFS) spectroscopy,[4,7] a consensus has now been reached on the presence of a MoO_2 core. In fact, recent data in support of this hypothesis has come from electron paramagnetic resonance (EPR) analysis of the Mo(V) analogue and from theoretical calculations.[6,8] The molybdenum ion can also attain a +4 redox state in which the equatorial oxo ligand is found to be di-protonated.[4,6,9] As far as the copper ion is concerned, the +1 oxidation state is maintained throughout the catalytic cycle.[4,7]

The CO molecule binds to the Cu(I) centre[9] and its oxidation takes place at the same site, thanks to the high degree of delocalisation within the Mo(μ-S)Cu unit.[10] Such delocalisation allows for facile electron transfers toward the redox-active Mo ion during catalysis.[10] Notably, a glutamate residue (Glu763), which belongs to the second coordination shell of the metals, is conserved within the xanthine oxidase family of enzymes and occupies a position such that it could facilitate deprotonation events of protic ligands at the equatorial positions of the Mo ion.

The first mechanistic hypothesis for the catalytic cycle of the MoCu-CODH was published in 2002 by Dobbek *et al.*, based on structural evidences obtained from X-ray diffraction experiments.[7] According to their proposal, coordination of CO to the resting state of the enzyme is followed by formation of a thiocarbonate intermediate, obtained through a nucleophilic attack of the equatorial Mo-oxo ligand on the CO carbon atom. The structure of the latter is analogous to the thiocarbamate derivative formed during inhibition of the enzyme by *n*-butylisocyanide. Finally, a water molecule could regenerate the oxygenic molybdenum ligand with the consequent release of CO_2. The completion of the reaction cycle occurs upon reoxidation of Mo(IV) to Mo(VI) via the transfer of two electrons.

Inspired by such proposal, two research groups[11,12] performed quantum mechanical calculations on cluster models of the enzyme active site. Hofmann

et al. noted that the thiocarbonate intermediate corresponds to a very stable side-product, the occurrence of which seems not to be functional for catalysis. However, Siegbahn and co-workers found that the SCO-bond of the thiocarbonate can be broken upon insertion of a water molecule in the first coordination shell of the molybdenum ion but the barriers were estimated to be high.

More recent theoretical studies based on much larger models showed that the thiocarbonate species does not represent a thermodynamic sink on the catalytic energy profile[13,14] but its formation still remains problematic for the CO-oxidation catalysis. In fact, no transition state for CO_2 release could be directly linked to the thiocarbonate intermediate by means of the hybrid quantum mechanics/molecular mechanics (QM/MM) approach .[13]

A different mechanism was suggested by Stein and Kirk,[15] which avoids formation of a stable C–S bonded intermediate based on the hypothesis that a bicarbonate product can be formed after a nucleophilic attack of a water molecule on the μ_2-η^2-CO_2 intermediate. The rate limiting step for such mechanism is lower than the highest barriers occurring in the previously proposed mechanism. However, experimental EPR studies of the partially reduced binuclear centre in complex with bicarbonate showed that bicarbonate coordination to Mo is unlikely to take place during catalysis.[16]

Finally, in a recent theoretical study on the MoCu-CODH hydrogenase activity, as well as in an experimental and theoretical investigation concerning a synthetic model of the enzyme active site, a hypothesis was formulated, according to which the protein active site should be viewed as a Mo^{VI}=O/Cu^I frustrated Lewis pair (FLP), where the Cu^I acts as a Lewis acid and the equatorial oxo ligand of Mo acts as a Lewis base toward the oxidation of both H_2 and CO.[17,18]

The previously proposed mechanisms share the idea that the equatorial oxo-ligand of Mo is responsible of a direct oxygen transfer to CO. However, a consensus on this key issue has not been reached, due to the lack of experimental evidences in support of such scenario. In this regard, Hille and coworkers have recently hypothesised that water might play the role of nucleophile, in place of the Mo-bound oxide.[19] Such a role for water would be favoured by the presence of the Glu763 residue that might facilitate its deprotonation upon CO-oxidation.

Inspired by the latter proposal, in the present theoretical study of MoCu-CODH enzymes we used QM/MM approaches to evaluate the possibility of a direct involvement of water in C-O bond formation on the carbon monoxide substrate, in the context of a revisitation of the overall catalytic mechanism.

Results and Discussion

The present section is composed of two parts. In the first one, we gain deepened insights into plausible poses of H_2O within the active site cavity, functional to the putative nucleophilic attack of water to the metal-bound CO ligand. Then, the second part presents and discusses the energetics computed for the water-dependent catalytic path here investigated that leads to CO_2 evolution.

Unless otherwise stated, all energy differences discussed in the following refer to QM/MM energies refined – as explained in Methods – with the Big-QM technique at the B3LYP-D3(BJ)/def2-TZVPD level of theory of closed-shell systems.

Relevant H_2O poses in the enzyme resting state and in the CO-bound active-site cavity

An obvious premise of an investigation of water as a putative nucleophile in MoCu-CODH catalysis is that water should be able to have access to the active site pocket. Indeed, the substrate channel is known to be extremely flexible, such that rather voluminous molecules (L-cysteine, coenzyme A and glutathione) were experimentally found to be able to reach and bind the bimetallic cofactor.[20]

Moreover, in the crystal structure of the oxidised form of MoCu-CODH (PDB code: 1N5W[7]), one water molecule is found to be placed near the Cu centre (Cu–O(H_2O) distance equal to 2.49 Å), in a position that may suggest its effective activation for the purpose of catalysis. However, despite all efforts, we were unable to localise on the QM/MM PES a minimum energy structure characterized by a Cu–O distance around 2.5 Å. Instead, we obtained two poses for water separated by a relatively low energy gap (ΔE = 4.2 kcal/mol) which place the oxygen atom of the water molecule in front of the Cu ion at a distance of 2.17 and 4.17 Å, respectively (species **A** and **A'**, see Fig. 1). The most stable adduct (**A**) features a tricoordinated Cu centre, with one of the coordination positions occupied by the water molecule. Species **A** shares structural characteristics with an analogous adduct previously described by Rokhsana *et al*, based on a QM-cluster enzyme model.[6]

However, with our all-atom representation of the enzyme, an alternative pose of water was also found, **A'**. In the latter, the solvent molecule is stabilised by an electrostatic interaction with the Cu centre (NBO charges: 0.57 e and -0.97 e for the Cu and O(H_2O) atom, respectively. See Table 1) as well as by other interactions within the active site pocket. In particular, the position of water is modulated by the second-sphere residue Phe390 that forms a H-bond interaction by means of its amide nitrogen (N(HN-Phe390)–H(H_2O) distance: 2.20 Å) and creates steric hindrance thanks to its aromatic ring. The fact that the Cu–H_2O coordinative adduct can be close in energy to a minimum structure in which water establishes intermolecular interactions mainly with the active site pocket indicates that metal coordination and hydrogen bonding to amino acids around the active site are competitive phenomena. This is in line with the hypothesis that the H_2O molecule can occupy slightly different positions within the active site.

The first step of the CO-oxidation catalytic cycle is the substrate binding to the resting state of the enzyme (Mo^{VI} and Cu^I). The hypothesis that water could act as a nucleophile in MoCu-CODH mediated catalysis implies the possibility that CO coordination to Cu occurs with retention of the weak coordination of water on Cu(I); in fact, water coordination could keep the reagent

in the right place to make catalytic the water more acidic, which may favour C–O bond formation with concomitant deprotonation of water. The CO-bound form of the enzyme (**B** in Fig. 2) features a trigonal planar coordination geometry of the Cu ion, as previously reported.[11,12] In this intermediate, the water molecule is involved in a hydrogen bond with the equatorial Mo-bound oxo group, with a O(oxo)–H(H_2O) distance of 2.01 Å, whereas the other H atom of H_2O is in proximity of an amide nitrogen of the protein backbone (N(HN-Phe390)–H(H_2O) distance: 2.20 Å). At the same time, the solvent molecule is found to be decoordinated from the Cu centre (O(H_2O)–Cu distance: 3.50 Å). However, it is placed in a favourable position for the formation of the new C–O bond, with a relatively short distance between the O atom of water and the C atom of the CO ligand (2.86 Å).

Given the features of the second coordination spheres of the metals, a limited number of water poses can be devised that place the O atom of water in proximity of the C atom of the CO ligand. In fact, water may form hydrogen bonds either with the Mo-bound oxo ligand in the proximal position with respect to the CO substrate, or with the sidechain of Glu763. An interesting intermediate was obtained upon geometry optimisation (**Bt** in Fig. 2) in which the water molecule is H-bonded to both Glu763 and the equatorial Mo-oxo ligand, with distances of 1.74 Å and 1.65 Å, respectively. At the same time, H_2O is found to be coordinated to the CO-bound Cu ion (O(H_2O)–Cu distance: 2.12 Å) giving rise to a tetracoordinated Cu(I) adduct. Existence of tetra-coordinated copper(I) complexes has been experimentally confirmed.[21,22] In particular, Olmstead *et al.* were able to synthesize and characterize by means of X-ray crystallography a Cu(1,4-thioxane)$_3$(H_2O)(BF$_4$) compound, which contains a coordinated water molecule (Cu–O 2.234(7)Å).

However, the involvement of **Bt** in the nucleophilic attack of water to the C atom of CO is questionable. In fact, it presents a C(CO)–O(H($_2$)O) distance 0.18 Å longer than the corresponding distance of the **B** intermediate and it is predicted to be 3.9 kcal/mol less stable than the tricoordinated adduct. Moreover, even if a migratory-insertion reaction mechanism on the Cu atom was first hypothesised in the present study, we were unable to localise a reactive channel for C–O bond formation starting from **Bt**.

Given the above results, we assumed that the new C–O bond could be formed starting from intermediate **B** as a result of a nucleophilic addition reaction of the water molecule to the carbon monoxide coordinated to copper. Formation of the new bond may be assisted by the equatorial oxo ligand of molybdenum, which would enhance the water nucleophilicity by formation of a strong H-bond interaction.

Proposed catalytic cycle for CO oxidation mediated by a metal-activated water molecule

On the basis of the previous observations, we investigated a new alternative catalytic cycle for the oxidation of carbon monoxide by MoCu-CODH, based on the properties of its bimetallic active site and the presence of a water molecule,

Table 1: Computed charges (NBO in e) at the B3LYP-D3(BJ)/def2TZVPD level for the MoOOSCu centre, H_2O and CO ligand for each intermediate.

	A'	A	Bt	B	TS1	C	TS2	D
Cu	0.57	0.71	0.83	0.72	0.66	0.66	0.69	0.50
S	-0.88	-0.80	-0.75	-0.83	-0.71	-0.60	-0.59	-1.07
Mo	1.25	1.21	1.18	1.20	1.18	1.04	1.01	0.76
O_{eq}	-0.65	-0.76	-0.78	-0.71	-0.83	-0.94	-1.00	-0.95
O_{ax}	-0.75	-0.74	-0.75	-0.74	-0.72	-0.69	-0.69	-0.74
O (H_2O)	-0.97	-0.10	-1.01	-0.96	-0.77	-0.70	-0.70	-0.47
H_1 (H_2O)	0.48	0.50	0.50	0.48	0,50	0.51	0.51	0.51
H_2 (H_2O)	0.49	0.50	0.49	0.49	0.51	0.49	0.51	0.48
C (CO)			0.39	0.41	0.32	0.40	0.43	1.00
O (CO)			-0.55	-0.51	-0.59	-0.68	-0.67	-0.54

summarised in Fig. 3. Starting from the enzyme resting state **A**, the entrance of the CO substrate would allow the formation of intermediate **B** which is characterised by a tricoordinated Cu-ion and by a weakly coordinated water molecule involved in a hydrogen bond with the Mo=O_{eq} group. The latter would be responsible for the activation of the solvent molecule that enables the formation of a new C–O bond through a nucleophilic attack of the activated water on the C atom of the Cu-bound carbon monoxide. The resulting species (intermediate **C**) is a putative C-bonded Cu-carboxylate, produced by a concerted reaction in which the addition of oxygen to CO occurs concomitantly with the deprotonation of H_2O by the equatorial oxo group of Mo. The same substituent of the molybdenum ion would be responsible for the deprotonation of the HCOO-ligand, allowing the decoordination of the CO_2 product from the copper atom, to generate intermediate **D**. Finally, CO_2 may leave the active site and the $Mo^{VI}(=O)_2Cu^I$ species **A** can be regenerated by the release of two protons and two electrons to the quinone pool of the electron transport chain.[23] The concomitant entrance of a new water molecule in the active site would close the enzymatic catalytic cycle.

The energy profile associated with the CO oxidation steps to give CO_2, as investigated by means of our QM/MM model, is shown in Fig. 4.

As a first step, we focused on the **B** → **C** reaction. The conversion of intermediate **B** to product **C** involves only a small reorganisation of the molybdenum coordination sphere. The latter (Fig. 4b) involves a COOH$^-$ ligand coordinated to the Cu(I) centre at a distance of 1.98 Å. The equatorial oxo ligand of molybdenum is protonated to an hydroxo ligand and it is involved in two hydrogen bonds. It donates one to the Glu763 residue (1.69 Å) and accepts another from the COOH$^-$ molecule (2.71 Å). Intermediate **C** is 12.0 kcal/mol less stable than **B**.

A transition state that links the reagent **B** to the product **C** was easily

located (see Supporting Information Figure S2); **TS1** (Fig. 5) is characterised by a seven-membered ring structure for which we observe that the C–O bond making and the O–H bond breaking occur in a single step. The C(CO)–O(H_2O) distance is 1.61 Å; concomitantly, one of the O–H bond distances of the H_2O molecule elongates to 1.14 Å. This occurs thanks to the presence of the Mo=O_{eq} ligand, acting as a base during the reaction (the O_{eq}–H(H_2O) distance in **TS1** is 1.28 Å). However, the barrier height for this C–O bond formation step was quite high, 29.3 kcal/mol for the closed-shell singlet state. We tested also the open-shell singlet and the triplet states for **B**, **TS1** and **C**, but the closed-shell singlet turned out to be the most favourable (see Supporting Information Section S3).

The next step in the catalytic cycle is the formation of carbon dioxide. This requires deprotonation of the carboxylate group through a second proton transfer to the equatorial Mo-ligand. The passage of the $COOH^-$ proton to the Mo–OH ligand takes place through a transition state (**TS2**, Fig. 5 and Supporting Information Figure S3) whose structure resembles the seven-membered ring observed for **TS1**. While going from intermediate **C** to **TS2**, the O(OH_{eq}-Mo)–H(COOH) distance decreases by 0.71 Å. The energy barrier for the deprotonation reaction is only 0.3 kcal/mol.

The product of the whole CO-oxidation reaction is state **D** (Fig. 4b). It features a reduced $Mo^{IV}Cu^I$ centre in which the molybdenum ion is coordinated by a water molecule in the equatorial position at a 2.18 Å distance from the metal. The water molecule forms a hydrogen bond with the oxygen of Glu763 (1.42 Å), while CO_2 has completely dissociated from the Mo(μS)Cu core (Mo–C = 3.77 Å, S–C = 3.57 Å, Cu–C = 3.43 Å). The reaction product **D** is −27.6 kcal/mol more stable than intermediate **C**.

Despite exact oxidation state for the two metals cannot be unambiguously determined by QM methods, atomic populations from a NBO analysis (Table S1 in the Supporting Information) combined with atomic charges (Table 1) both computed at the B3LYP-D3(BJ)/def2-TZVPD level can give us a general framework of the atomic oxidation number. The oxidation states of the metal ions in intermediate **B** are likely Mo(VI) and Cu(I), as it was observed experimentally for the enzyme resting state (without CO). In intermediate **C** we observe a decrease by 0.17 e in the charge of the Mo atom, which becomes more significant for the final product **D** (0.45 e). The same trend, although less pronounced (by 0.06 and 0.22 e), occurs for the copper atom. At the same time, the carbon atom of the CO molecule undergoes an increase in atomic charge (by 0.61 e), as expected due to the oxidation reaction. Net charges of the CO (**B**), HOCO (**C**) and CO_2 (**D**) groups are –0.16, –0.55 and −0.01 from Table 1, respectively, showing that the electron transfer takes place between **C** and **D**.

Conclusions

In the present study, we tested the hypothesis[19] that water might act as a nucleophile upon C-O bond formation during the CO oxidation reaction catalysed by the MoCu-CODH.

Previous reaction mechanisms have been proposed by Siegbahn and Shestakov[12] and by Xu and Hirao.[13] In both mechanisms, formation of a thiocarbonate intermediate in the course of catalysis is contemplated and it was shown to act as a thermodynamic sink, impeding an easy release of the CO_2 molecule. The alternative mechanism reported in this work naturally avoids formation of the thiocarbonate intermediate, since the proximal oxo ligand of Mo works as a base and not as a nucleophile. Activation of the water molecule is favoured by the presence of the $Mo=O_{eq}$ group. The latter was found to be a better base than Glu763 during catalysis, in line with our previous QM/MM study concerning H_2 oxidation by MoCu-CODH.[17] However, a prohibitively large activation barrier for the formation of the new C–O bond was found (> 29 kcal/mol), suggesting that this mechanism does not constitute a reactive channel for the oxidation of CO by the enzyme. Our theoretical study indicates that the $Mo=O_{eq}$ group represents the only nucleophile able to attack the activated carbon of the CO substrate, corroborating the concept that the Mo ion presents a catalytically labile oxygen in its coordination sphere, similarly to the case of xanthine oxidoreductase and other members of that family of enzymes.

Computational Methods

QM/MM calculations

The hybrid QM/MM model was the same as in our previous work.[14] The system was split into two subsystems: the QM region or System 1, which was treated at the QM level, and System 2, which was represented by an array of partial point charges (electrostatic embedding) and was kept fixed at the crystallographic coordinates. The QM region includes the bimetallic $Mo(O_2)$-μS-Cu unit, a crystallographic H_2O molecule found in proximity of the Cu ion and the truncated MCD cofactor – i.e. the cytidine-triphosphate section was placed in System 2. Moreover, it contains the Cys388 residue, backbones of Arg387 and Phe390 residues and the side-chain of the deprotonated Glu763. The total number of atoms in the QM system (System 1) varied between 64 and 66 atoms (see Supporting Information Figure S1), depending on the presence or absence of the CO molecule, while the total charge of System 1 was -3. Covalent bonds between the QM and MM systems were treated by the hydrogen link-atom approach.[24] The calculations were performed using the ComQum software.[25,26] The QM/MM energy in ComQum is calculated as[25,26]

$$E_{\text{QM/MM}} = E_{\text{QM1+ptch2}}^{\text{HL}} + E_{\text{MM12},q_1=0}^{\text{CL}} - E_{\text{MM1},q_1=0}^{\text{HL}} \qquad (1)$$

where $E_{\text{QM1+ptch2}}^{\text{HL}}$ is the QM energy of System 1 truncated by hydrogen-link (HL) atoms and embedded in the set of point charges modelling Systems 2. $E_{\text{MM1},q_1=0}^{\text{HL}}$ is the MM energy of System 1, still truncated by HL atoms, without any electrostatic interactions. $E_{\text{MM12},q_1=0}^{\text{CL}}$ is the classical energy of the whole system, with carbon-link (CL) atoms and with the charges in System 1 set to zero, in order to avoid double-counting of the electrostatic interactions. Thus,

ComQum uses a subtractive scheme with electrostatic embedding and van der Waals link-atom corrections.[27]

The QM calculations were carried out using TURBOMOLE 7.2 software[28] at the BP86-D3(BJ)/def2-TZVP[29−32] level of theory. The resolution-of-identity technique was used to accelerate the calculations.[33] All states were studied in the closed-shell singlet state (representing Cu(I) and Mo(VI) or Mo(IV)). For each intermediate involved in the CO oxidation reaction, triplet and open-shell singlet states were also considered using the UHF formalism (see Supporting Information Section S3). The MM calculations were carried out by means of the Amber software,[34] using the Amber FF14SB force field for the protein,[35] and the general Amber force field[36] with restrained electrostatic potential (RESP) charges[37] for CO and the MCD cofactor. The two Fe_2S_2 clusters were described with RESP charges and a non-bonded model.[38]

For all the optimised models, atomic charges were computed by means of the natural bond orbital (NBO) approach.[39]

BigQM energy calculation

The BigQM approach was employed to obtain refined energies of each step in the CO-oxidation catalysis. This technique[40,41] has been shown to be a valuable tool in theoretical investigation of this enzyme.[14] It had been demonstrated that models of about 1000 QM atoms are needed to obtain convergence of the energies with respect to the size of the QM system.[42−45] Since QM/MM calculations converge faster than QM cluster calculations, we started from optimised QM/MM geometries to build the large BigQM model of 1033/1035 atoms. We included in the calculations all chemical groups with at least one atom within 6.0 Å of the QM system (System 1) and junctions were moved three amino-acids away from each residue in System 1. In addition, all buried charged groups inside the protein were included, with exception of the two iron–sulfur clusters (see Supporting Information List S2). The BigQM calculations were performed on coordinates from the QM/MM optimisations, with a surrounding point-charge model, at the BP86-D3(BJ)/def2-SV(P) level.[46] The multipole accelerated resolution-of-identity J approach (marij keyword) was employed to accelerate the calculations.[47] The resulting energies were corrected with a QM/MM MM term for the BigQM region:

$$E_{MM} = E_{MM12,q_1=0}^{CL} - E_{MM1,q_1=0}^{HL} \qquad (2)$$

Finally, the energies were also corrected by taking into consideration the B3LYP-D3(BJ)/def2-TZVPD functional and basis set effects, using calculations with the standard QM/MM QM system and a point-charge model of the surroundings:

$$E_{corr} = E_{QM1,ptch2}^{B3LYP/TZVPD} - E_{QM1,ptch2}^{BP86/SV(P)} \qquad (3)$$
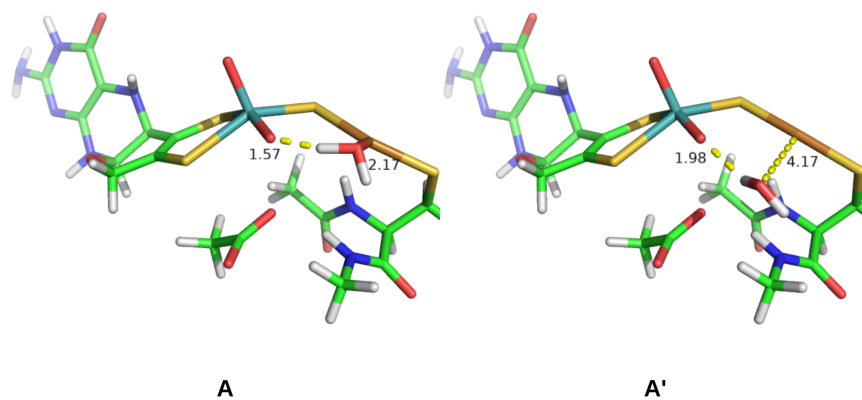
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Acknowledgment

Keywords

Figure 1: Optimised structures of the MoCu-CODH resting state, **A** and **A'**. Colour code: cyan, molybdenum; brown, copper; yellow, sulphur; red, oxygen; blue, nitrogen; green, carbon; white, hydrogen. All distances in Å.
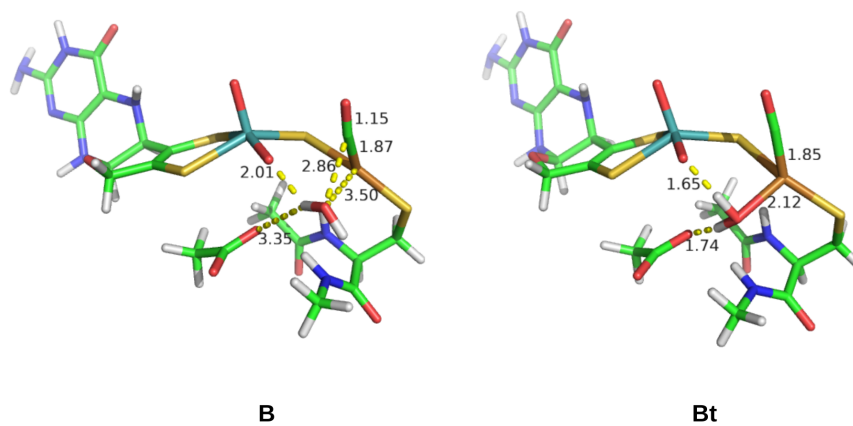

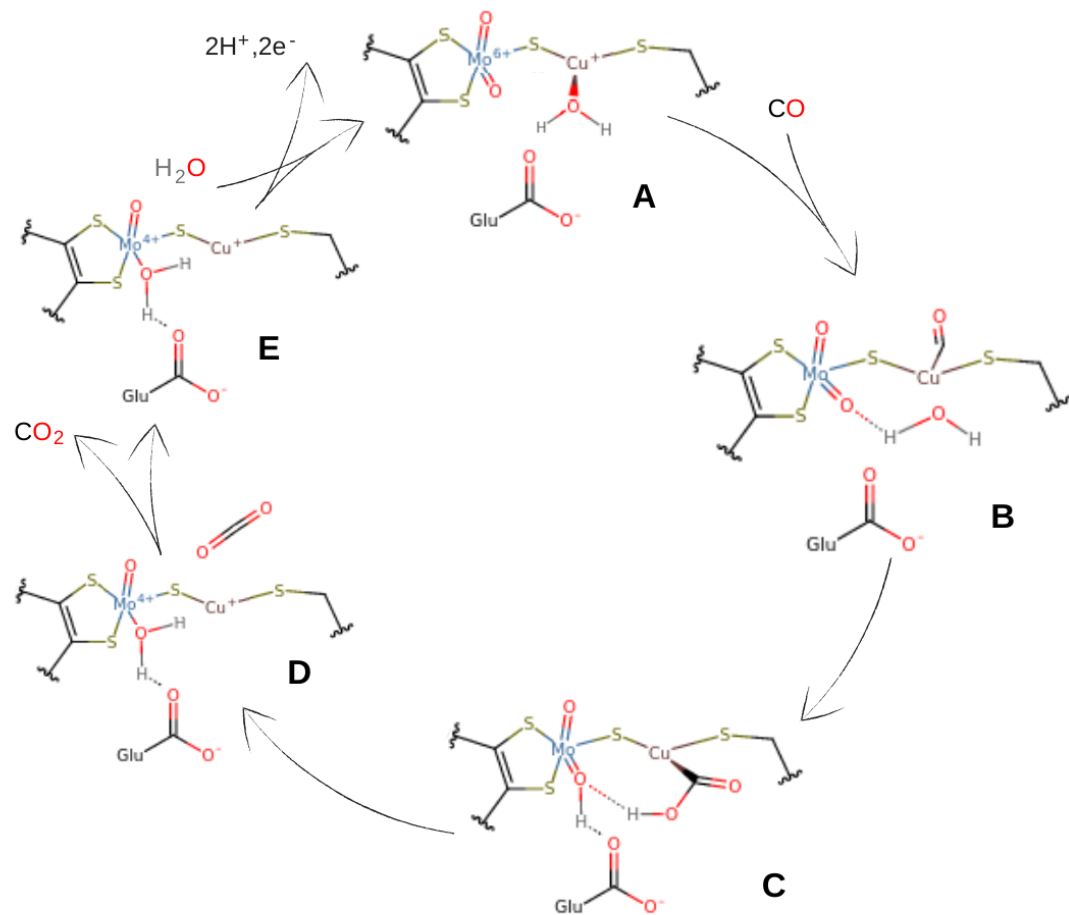Figure 2: Optimised structure of intermediate **B** and **Bt**. All distances in Å.

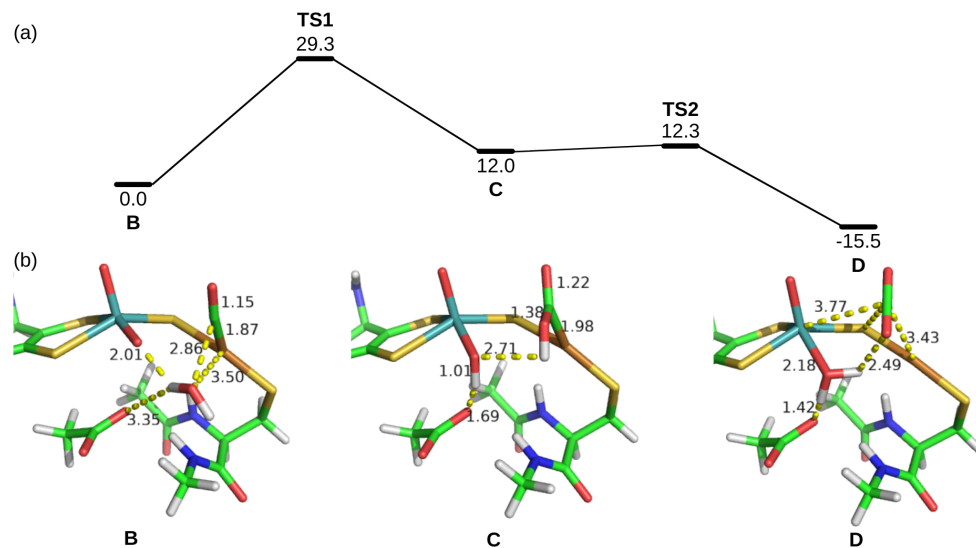Figure 3: Proposed catalytic cycle for CO-oxidation in MoCu-CODH involving a nucleophilic water.

Figure 4: (a) Energy profile (in kcal/mol) for the oxidation of CO in Mo–Cu CODH. (b) Optimised structures of intermediates **B**, **C** and **D**. All distances in Å.
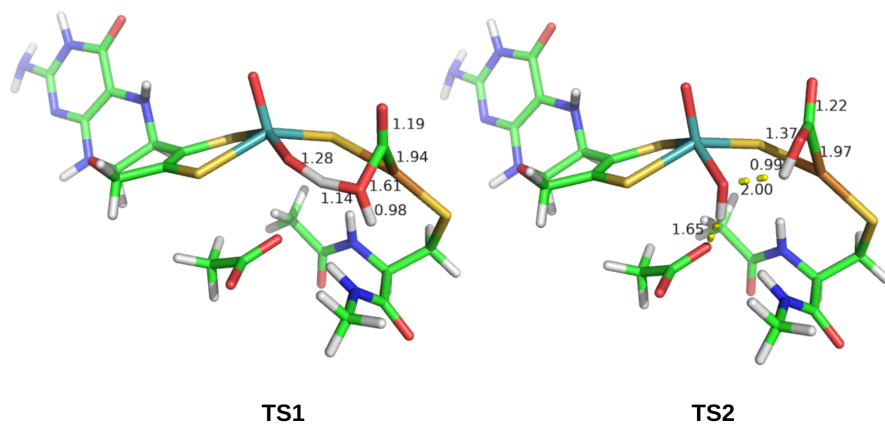

Figure 5: Optimised structures of the transition states **TS1** and **TS2**. All distances in Å.

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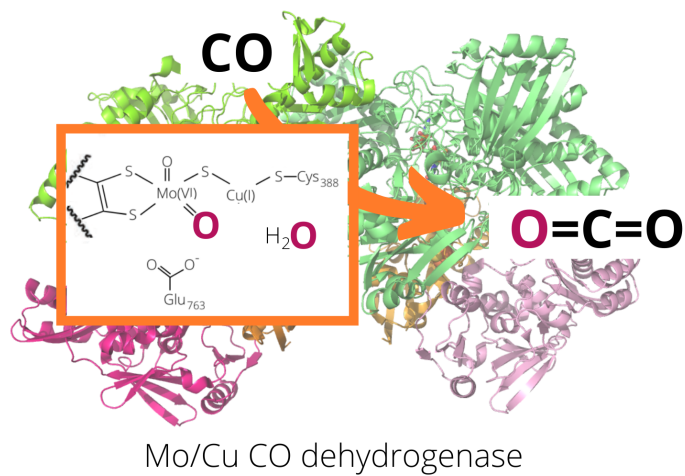**Table of Contents graphical abstract**

Mo/Cu CO dehydrogenase

Figure 6: Can an activated water molecule form the new C-O bond during the CO oxidation reaction catalysed by the aerobic Mo/Cu CO dehydrogenase? Hybrid quantum mechanical/molecular mechanical modelling of the enzyme reveals which oxygen atom makes nucleophilic addition to CO.