

Prediction of Alternative Structures of the Molybdenum Site in the Xanthine Oxidase-Related Aldehyde Oxido Reductase

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Molybdenum oxotransferase enzymes catalyze two-electron redox reactions which involve the transfer of an oxygen atom originating from a water molecule.^{1–3} All enzymes of the xanthine oxidase type possess a structurally similar molybdenum cofactor^{4,5} (Moco) in which a single molybdenum atom is ligated by a dithiolene side chain to a pterin ring. Two oxidation states of Moco are important as they are likely to be involved in the redox reaction: an oxidized state corresponding to Mo(VI) and a reduced state corresponding to Mo(IV) formed after a coupled two-electron + proton transfer from the substrate to the cofactor. On the basis of EXAFS^{6–8} and EPR^{9–11} studies of molybdenum enzymes and their models, “consensus structures”^{12,13} have been proposed both for the oxidized and reduced states of Moco (Figure 1). The X-ray crystal structure of a xanthine oxidase (XO)-related aldehyde oxidase (AOR) at 1.8 Å resolution shows a five-coordinated Mo bound to the molybdopterin.^{14,15} The coordination sphere is distorted square pyramidal with the two dithiolene sulfurs (S_L), an oxo and a hydroxo or water ligand forming the equatorial plane and a sulfido or oxo group (in the desulfo form) located at the apex. The detailed structure of the coordination sphere in the molybdenum cofactor which the crystallographic data at that resolution could not provide is of crucial importance for understanding the catalytic mechanism of xanthine oxidase. The Mo=O and Mo–S bond lengths determined by EXAFS for XO apply most likely also to AOR as suggested by their close similarity in spectroscopic properties and in their protein ligand sphere.¹⁵ In order to clarify the

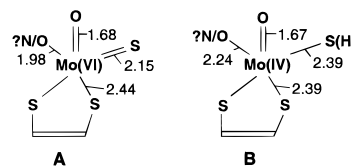


Figure 1. Bond distances (Å) in the “consensus structures” for the Mo site of (A) oxidized and (B) reduced Moco.¹²

structure and energetics of the molybdenum site, we have performed gradient-corrected density functional (DF) calculations; this quantum chemical method is known to provide accurate data for transition metal complexes.¹⁶

In these DF calculations we have found that in addition to the experimental structure, LMoS(OH)₂ and LMoO(SH)(OH), exhibit very similar energies and thus can also represent the oxidized state of Moco. We also predict alternative structures for the reduced Mo site. The calculations show the five-membered ring LMo, dithiolene–molybdenum, to be a very sensitive moiety in Moco. Its geometrical parameters (Mo–S_L distances, S_LMoS_L bond angle, MoSCC dihedral angle δ) may thus be helpful for characterizing the state of the Mo site.

Complexes containing the [MoOS] core are rare as models for xanthine oxidase because of their instability.^{1,2} Therefore, theoretical studies seem highly desirable to elucidate basic aspects of the Moco structure. Recently, DF calculations performed at the local density (LDA) level have been reported for some complexes modeling the active site of xanthine oxidase.¹⁷ However, that study did not consider several essential structures and did not address the energetics of the complexes.

We have performed all-electron nonrelativistic DF calculations at the GGA¹⁸ level using the linear combination of Gaussian-type orbitals method^{19,20} with the following orbital basis sets²¹ contracted in a generalized fashion employing atomic LDA eigenvectors: Mo (19s,14p,9d) → [8s,6p,5d],^{21a} S (12s,9p,1d) → [6s,5p,1d],^{21b,d} O and C (9s,5p,1d) → [5s,4p,1d],^{21c,d} and H (6s,1p) → [4s,1p].^{21c,d} All calculations were performed with full geometry optimization using analytical energy gradients.²⁰

The geometries of the complexes modeling the Moco site are displayed in Figure 2. The ligand L = –S–CR=CR–S– (R = H) represents the dithiolene group of the enzyme pterin moiety. Because LDA geometries¹⁷ of dithiolene–molybdenum systems with R = H and R = CH₃ are very similar, only the simpler ligand was considered in our calculations. The structure of LMoOS (**1**) has C_s symmetry (Figure 2). A significant deviation from planarity is found for the chelated subunit LMo manifested by the dihedral angle δ = 37°. This important structural feature has not been noticed previously.¹⁷ The nonplanarity of this five-membered ring allows two conformers: the C=C fragment and the oxo group can be either on the same side or on opposite sides of the plane S_LMoS_L. The calculated geometrical parameters are very close for both

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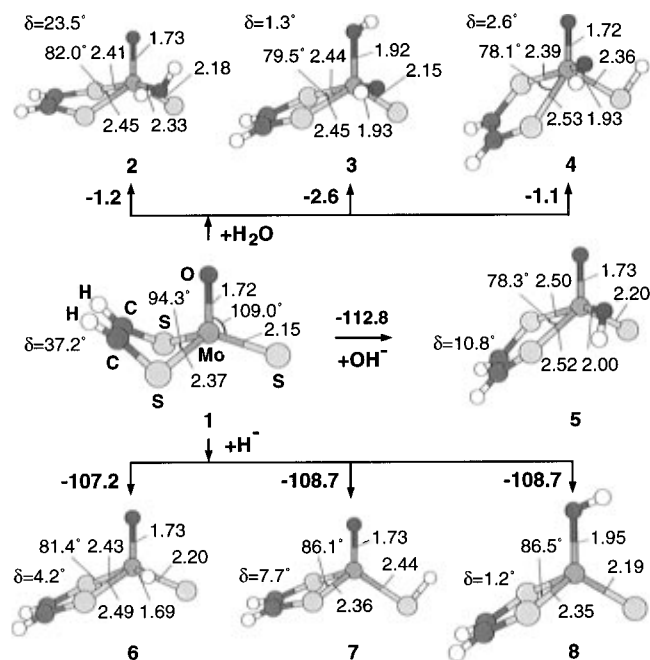


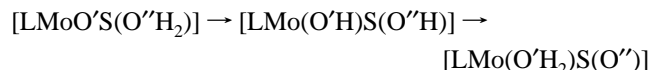
Figure 2. Calculated reaction energies (in kcal/mol, boldface) and selected geometrical parameters (distances in Å, angles in deg) for model complexes of Moco.

conformers, and their total energies differ by only 0.6 kcal/mol. Similar conformations were found for the corresponding dioxo and disulfido derivatives. A coordination number of 4 is not typical for Mo(VI); thus, one expects the coordination sphere of Mo in LMoOS to be easily extended with a species from the environment of the active site (e.g., a carboxylic group of glutamate or a water molecule).¹⁵ The binding energy of a water molecule to Mo in LMoOS is calculated to about 2 kcal/mol. Since Mo essentially lies in the plane defined by the atoms =O, =S, and S_L, one may view the structure of the aqua complex LMoOS(OH₂) (**2**) as a distorted trigonal bipyramid with water and the other S_L occupying opposing axial sites (Figure 2). The calculated Mo–OH₂ distance amounts to 2.33 Å. In contrast to LMoOS where both Mo–S_L bonds are equivalent, those bonds differ in LMoOS(OH₂), 2.41 Å in the axial position and 2.45 Å in the equatorial position of the bipyramidal structure. The five-membered ring LMo of the aqua complex **2** is more planar ($\delta = 24^\circ$) than that in **1** ($\delta = 37^\circ$).

Interestingly, **2** can undergo a rearrangement to LMoS(OH)₂ (**3**) or LMoO(SH)(OH) (**4**) (Figure 2) either through an intramolecular proton transfer or, more likely, by a proton exchange process involving the environment. The total energies of compounds **2–4** are very similar, differing by less than 2 kcal/mol. In **3**, both Mo–OH bond lengths are about 1.92 Å and the Mo–S_L distances are also nearly equal, 2.44 Å. According to EXAFS data,⁸ three thiolate ligands (Mo–SR, 2.45 Å) can be present in the Mo site, and there is also evidence for an oxygen atom at a distance of 2 Å. These experimental structural features seem to be satisfied for moiety **4** (Figure 2). The oxo ligand exhibits a strong *trans* influence resulting in a considerable elongation of the *trans* Mo–S_L bond, 2.53 Å, compared to the *cis* Mo–S_L distance, 2.39 Å (using bipyramidal structure descriptors). Removal of a proton from the coordinated water molecule leads to a considerable shortening of the Mo–O bond (2.33 Å in **2** and 1.93 Å in **3** and **4**). However, protonation of the oxo or sulfido groups induces an elongation of the corresponding bonds by about 0.20 Å. The LMo fragment is almost planar in **3** and **4**; therefore, the neutral compounds **2–4** with a pentacoordinated Mo atom are plausible models for the Mo(VI) center. Despite essential differences in geometries, these three molecules are essentially isoenergetic; H⁺ exchange between Moco and its environment should be almost thermo-

neutral and may thus easily cause a transformation among these three structures. Most likely, a proton transfer will be mediated by the environment (e.g., glutamate) since such a process is known to have small or no activation energies²² while a direct hydrogen rearrange is expected to have a rather high barrier.

A central issue of the reactivity of the Mo site is the source of a labile oxygen. The oxo group¹³ and the water ligand¹⁵ have been discussed as possible donors. However, on the basis of the calculated energies of **2** and **3**, we predict the following exchange to be feasible:



X-ray structure determinations have located a carboxylic group of a glutamate residue in the vicinity of the Mo site.^{14,15} This group can accept a proton from the water ligand in **2** inducing the formation of [LMoOS(OH)][−] (**5**, Figure 2). The calculated structure **5** is in good agreement with the “consensus structure” of the Mo(VI) center (Figure 1 A). From the calculated data for **2–5**, one can expect a substantial alteration in the molybdenum–ligand bond lengths depending on the H⁺ position.

Within the reductive half-cycle of the oxygen transfer reaction, the Mo(VI) center is reduced to Mo(IV) due to a coupled two-electron and proton transfer (formally H[−] transfer). Three possibilities for such a hydride transfer have been discussed: (i) to the sulfido group^{12,15} resulting in the compound [LMoO(SH)][−] (**7**), (ii) to the oxo group yielding [LMoS(OH)][−] (**8**), (iii) to the metal atom forming [LMoOS(H)][−] (**6**) with a pentacoordinated Mo (Figure 2). Quite unexpectedly, structures **6–8** have very similar energies (within 2 kcal/mol) according to our GGA–DF calculations. The geometry changes induced by the hydride transfer to the oxo and sulfido ligands (**7** and **8**, Figure 2) are similar to those found for **2–5**. The hydrido complex [LMoOS(H)][−] (**6**) has a distorted square pyramidal structure where the axial position is occupied by the oxo group; the Mo–H distance is calculated to 1.69 Å. A comparison of the calculated geometries of **1** and **6–8** shows that the Mo–S_L bond becomes slightly shorter by H[−] transfer to the sulfido or oxo ligands, in accord with EXAFS data,⁸ while direct H[−] transfer to Mo leads to an increase in the Mo–S_L distance. The LMo ring is found to be essentially planar in **6–8**.

Note that the structural parameters of LMo (Mo–S_L distances, S₁MoS_L bond angle, MoSCC dihedral angle δ) very sensitively reflect changes in the Mo site. The present DF quantifications should be useful for establishing interrelations between the structure and state of the Moco in oxotransferase enzymes. In summary, for establishing the enzymic mechanism of xanthine oxidase it is necessary to consider more structures than those of complexes **2** and **5**, which have been calculated in agreement with the “consensus structure”. Rather, the alternative structures **3** and **4** which are predicted to exhibit almost the same energy as **2** may also be taken to represent the oxidized state of Moco. For the reduced state, structures **6** and **8** are identified as isoenergetic alternatives to the commonly discussed model **7**.

In closing, we recall that the calculations were performed with isolated Mo complexes. The influence of the protein environment on geometry and energetics of the metal site may vary for the different systems and structures investigated. In particular, the oxo ligand is tightly packed against the protein making its protonation (structures **3** and **8**) unlikely. Indeed, the EXAFS data indicated the presence of a Mo=O group in all oxidation states.

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