The Theoretical Transition State Structure of a Model Complex Bears a Striking Resemblance to the Active Site Structure of DMSO Reductase

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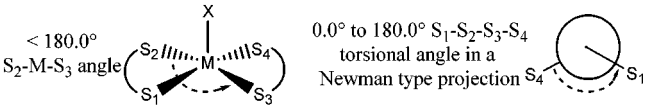
Molybdenum-containing enzymes are ubiquitous in nature and fulfill a variety of biological catalytic functions.[1] Dimethylsulfoxide (DMSO) reductase catalyzes the oxygen atom transfer from the substrate DMSO.[1] Recently, Lim, Sung, and Holm (LSH) have shown that a relatively simple inorganic complex $[Mo[S_2C_2(CH_3)_2]_2OR]^{1-}$ ($R = C_6H_5$) can perform similar chemistry, even though its ligands are severely truncated compared to those of the enzyme.[2] Here, we report density functional calculations on the structures and energies of a model system ($R = CH_3$). Remarkably, the arrangement of the sulfur ligands calculated for the freely optimized transition state of the model, which is quite different from that calculated for the reactant and product, closely resembles the arrangement reported for the crystal structures of the enzyme. The geometric constraint provided by the enzyme not only lowers the reaction barrier but also significantly reduces the exothermicity of the S to Mo oxo transfer.

To describe unambiguously the sulfur ligand orientation in these systems, one must consider two angles: (1) the largest angle with the metal made by two sulfurs from different ligands (e.g., S_2-M-S_3 below) and (2) the torsional angle made by the four sulfurs with the two sulfurs from (1) as the central atoms (e.g., $S_1-S_2-S_3-S_4$ below).[3] For example, a D_{4h} structure would have a $S_1-S_2-S_3-S_4$ torsional angle of 180.0°, and an O_h structure would have one of 90.0°.



X-ray diffraction,[4] EXAFS,[5] and other various spectroscopic techniques[6] have established the general nature of the active site in DMSO reductase. The protein bound, universal molybdenum cofactor (Mo-co) contains a single Mo atom coordinated by four sulfur atoms from two pterins functionalized with a dithiolene-substituted pyran ring (P- and Q-pterin, albeit only weakly coordinated by Q in some structures) and an oxygen atom from a coordinated serine occupying the fifth coordination site. The remaining Mo coordinating ligands remain in disagreement. Rees and co-workers first reported the crystal structure of DMSO reductase isolated from *Rhodobacter sphaeroides* and concluded that the Mo atom was weakly coordinated by one of the two pterin ligands in the Mo^{IV} form, but was a six-coordinate monooxo, seroxy, bispterin structure in the Mo^{VI} form.[4a] Later, Schneider et al. reported a five-coordinate, monopterin, seroxy, dioxo Mo^{VI} form of DMSO reductase isolated from *Rhodobacter capsulatus*.[4b] In contrast, Bailey and co-workers proposed a seven-coordinate, Mo^{VI} dioxo active site based on X-ray crystallography of DMSO reductase from *Rhodobacter capsulatus*.[4c,d] Recently, Schindelin and co-workers[4e] reported a new crystal structure of DMSO reductase isolated from *Rhodobacter sphaeroides*. Their solution of this structure was best resolved by a mixture of a monooxo, bispterin, six-coordinate active site (40%) and a dioxo, five-coordinate active site (60%) with one uncoordinated pterin. These two enzymes isolated from two different species of bacteria have 80% sequence homology, almost superimposable tertiary protein structure, similar UV/VIS absorption spectra for both the Mo^{IV} and Mo^{VI} oxidation states, as well as similarities in the variable-temperature magnetic circular dichroism and EPR spectra of the Mo^{V} form.[1] The orientation of the four sulfurs for these enzyme structures is listed in Table 1.

LSH reported simple molybdenum and tungsten complexes that perform oxygen atom transfer from DMSO, albeit at a slower rate than the in vitro enzyme.[2] The complexes mimic the active site coordination: the linkage to the pterin is simply replaced with a methyl group (a methylated dithiolene) and the serine is modeled with a phenoxy group. Because of the relative stability of the reactants and products, LSH reported the isolation of only the Mo^{IV} reactant and the oxo W^{VI} product.[2,7] The sulfur orientation is tabulated in Table 1. Their kinetic studies offer thermodynamic parameters consistent with an associative mechanism and an enthalpy of activation of 14.8(6) kcal mol^{-1}.

Here, density functional calculations[8] on a simplified LSH model, where the phenoxy group has been replaced by a methoxy group, show predicted structural parameters of the Mo^{IV} reactant that agree with those of the crystal structure of the LSH complex (see Figure 1a). The largest errors are for the Mo−S distances, which on average are calculated to be 0.06 Å too long.

Our results for the model reaction show an associative mechanism for the oxygen atom transfer by the formation of a molybdenum bound oxygen of DMSO. Previous theoretical calculations on a very different model complex with a phosphine substrate also found an associative mechanism and predicted the formation of an intermediate,[9a] which was subsequently found and characterized.[9b] Here, the reaction is assisted by an interaction

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(8) The theoretical calculations have been carried out with the Gaussian 98[10] implementation of B3LYP [Becke three-parameter exchange functional (B3)[11a] and the Lee−Yang−Parr correlation functional (LYP)[11b]] density functional theory.[11c] The basis set for molybdenum is the effective core potentials (ECP) of Hay and Wadt (LANL2DZ)[12a] as modified by Couty and Hall.[12b] The standard LANL2DZ basis set[12a] was used for sulfur supplemented with a *d* polarization function,[12c] the D95* basis set[12d] was used for oxygen, and the D95 basis sets[12d] were used for carbon and hydrogen. All structures were fully optimized without symmetry constraints, and analytical frequency calculations were performed on all structures to ensure a local minimum or 1st order saddle point. All relative energies are reported with corrections for the zero-point energy (ZPE).

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Table 1. Angle Measurements of the Orientation of the Four Sulfurs about the M Atom (alpha numeric codes of the enzyme are those from the PDB[15])

	2DMR[4d]	4DMR[4d]	1EU1(B)[4e]	Mo^{IV} reactant[2]	W^{VI} product[2]	Mo^{IV} (Figure 1a)	Mo-DMSO (Figure 1b)	Mo^{VI} (Figure 1d)	enzyme av	TS (Figure 1c)
S−M−S ∠	147.1	151.7	142.5	140.2	153.5	143.7	137.8	158.7	147.1 (122.4)[a]	146.7 (120.4)[a]
torsional ∠	143.7	146.7	150.7	179.4	110.0	180.0	179.2	102.5	147.0 (57.6)[a]	150.7 (59.6)[a]

[a] The measurements in parentheses are the θ_{fold} and θ_{twist} values of the alternative description of the ligand orientation. See ref 3 for details.

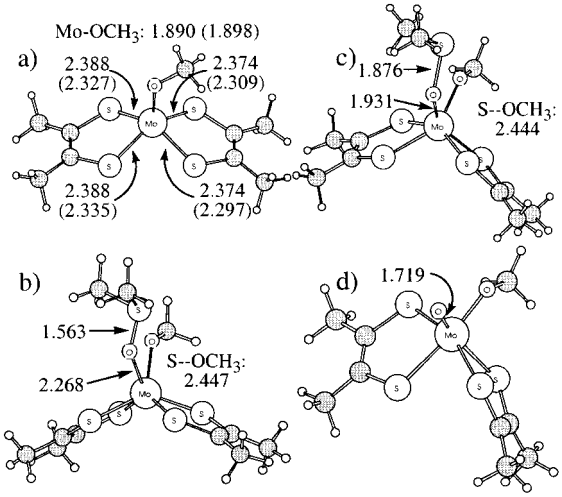

Figure 1. (a) The distances (in Å) for the optimized geometry of the Mo^{IV} model complex (the experimental values[2] are in parentheses); (b) the Mo^{IV} complex with bound DMSO; (c) the transition state for oxygen atom transfer; and (d) the oxo Mo^{VI} product complex.

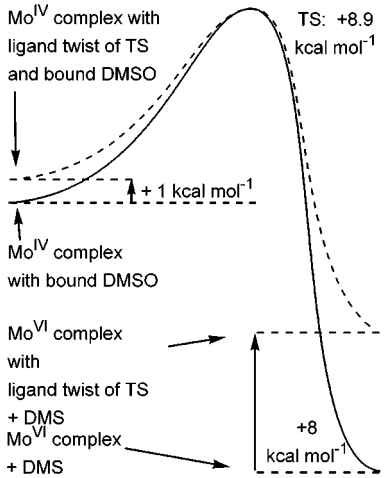

Figure 2. The Mo^{IV} with bound DMSO and the product Mo^{VI} complexes are recalculated with a sulfur ligand twist like that of the transition state. This constraint led to a small decrease of the binding energy (∼1 kcal mol^{-1}) of DMSO with the Mo^{IV} complex, but reduces the exoergicity of the Mo^{VI} product by ∼8 kcal mol^{-1}. The transition state structure has no constraints and is 8.9 kcal mol^{-1} above the DMSO-bound Mo^{IV} complex.

of the DMSO sulfur with the oxygen of the methoxy group. This type of structure has been observed in an experimental protein crystal structure, 4DMR, formed with a reduced form of the enzyme and excess dimethyl sulfide (DMS).[4c] The calculated distance between the sulfur of DMSO and the oxygen of the methoxy group (2.447 Å for the bound DMSO complex and 2.444 Å for the transition state) compares favorably with the distance observed in the crystal structure of 4DMR (2.65 Å). This bound complex then proceeds through a transition state (+8.9 kcal mol^{-1}) that transfers the DMSO oxygen to the molybdenum while breaking the O−S bond of DMSO. The products, an oxo-methoxy Mo^{VI} complex and DMS, are produced with an overall exoergicity of 19.9 kcal mol^{-1} (when comparing separated reactants and separated products).

Besides the DMSO−OR interaction, the most intriguing structural result from these calculations is the arrangement of the dithiolene ligands in the transition state. The calculated transition state angles of 146.7° and 150.7° compare favorably with the average of the enzyme values, which are conspicuously conserved for the Mo^{IV} and Mo^{VI} structures. When the Mo^{IV} and Mo^{VI} complexes are recalculated with the sulfur orientation fixed like that calculated for the TS, the binding energy of DMSO with the Mo^{IV} complex decreases by only ∼1 kcal mol^{-1}, but the exoergicity of the Mo^{VI} product decreases by ∼8 kcal mol^{-1} (see Figure 2).

These calculations demonstrate the entatic principle,[13] which has not been found in previous calculations on Ni−Fe hydrogenase,[14a] blue Cu proteins,[14b] and xanthine oxidase.[14c] By using the rigidity of the pterin ligands (which are embedded in the matrix of the enzyme) and positioning the serine, the enzyme is able to maintain an active site structure that closely resembles the freely optimized transition state for oxygen atom transfer to and from the substrate. Furthermore, this structure reduces the exothermicity of the reaction, decreases the likelihood of producing a thermodynamic sink in the Mo^{VI} oxo product, and diminishes excess energy that would be incompatible with a biological system.

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