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Notes

Computer Modeling of the Oxygen-atom Transfer Reaction between Hydrogen Sulfite and a Molybdenum(VI) Dioxo Complex

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Introduction

The molybdenum-containing oxotransferase enzymes catalyze oxygen atom transfer to/from biological substrates in the nitrogen, sulfur and carbon cycles.[1] The active sites of these enzymes contain a dissociable cofactor known as Moco[2] consisting of a molybdenum atom coordinated to a pterin derivative called molybdopterin. The oxidized forms of the enzymes have been shown to contain *cis*-molybdenum(VI) dioxo units bound to one[3] or two[4] molybdopterins through dithiolene moieties of the pterins. Model studies[5] and ^{18}O labeling[6] experiments indicate that the molybdenum-containing cofactor mediates the oxygen-atom transfer according to eq 1 where X = arbitrary substrate and L = ligand(s). Some hyperthermophilic bacteria contain related tungsten enzymes with similar metal-oxo/pterin cofactors.[7]

$$\mathrm{Mo^{VI}O_2L}_n + \mathrm{X} \rightleftharpoons \mathrm{Mo^{IV}OL}_n + \mathrm{XO} \qquad (1)$$

The sulfite oxidase family consists of sulfite oxidase and assimilatory nitrate reductase.[1] Sulfite oxidase catalyzes the last step in the degradation of sulfur-containing amino acids, the oxidation of sulfite to sulfate. The human,[8] rat,[9] and chicken liver[10] enzymes have been sequenced, and the chicken liver enzyme has been structurally characterized at 1.9 Å resolution.[11] In this structure, the coordination environment of the molybdenum atom is approximately square pyramidal with an oxo ligand in the axial position at an Mo−O distance of 1.75 Å. The equatorial positions are occupied by the two dithiolene sulfurs of one pterin ligand at Mo−S distances of 2.4 Å, one sulfur from a cysteine residue at 2.5 Å from the molybdenum atom and one water/hydroxide ion with an Mo−O distance of 2.2 Å.[11] Extended X-ray absorption fine structure experiments at the Mo K edge of the oxidized enzyme indicate that the molybdenum is coordinated to two oxo ligands at 1.71 Å and three sulfur atoms at 2.41 Å.[12−14] In the reduced form of the enzyme, only one oxo ligand at 1.69 Å from the metal was detected, while the same number of sulfurs were present at a distance of 2.38 Å.[12−14] This suggests that the enzyme is in the reduced form in the crystal structure.

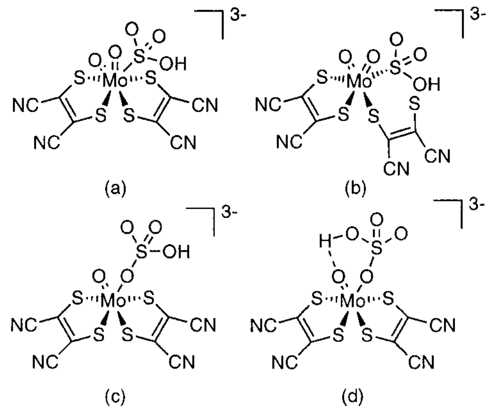

Figure 1. Some possible intermediates for the reaction between $[\mathrm{MoO_2(mnt)_2}]^{2-}$ and $\mathrm{HSO_3}^-$.

Several complexes have been studied as structural and/or functional models of molybdenum-containing oxotransferases. One of these models, $[\mathrm{MoO_2(mnt)_2}]^{2-}$ [mnt^{2-} = 1,2-dicyanoethylenedithiolate], has been found to be able to perform the biologically relevant reaction of oxidizing hydrogen sulfite to $\mathrm{HSO_4}^-$.[15−17] This reaction has been shown to exhibit a Michaelis−Menten type of kinetic behavior in an acetonitrile/water mixture. Different mechanisms have been suggested for the reaction.[15−17] Sarkar and co-workers[15,16] have proposed two possible ways that the sulfur atom of hydrogen sulfite may attack the metal atom to form an intermediate formulated as "$[\mathrm{MoO_2(HSO_3)(mnt)_2}]^{3-}$". The first proposal is an attack in a preequilibrium reaction forming a seven-coordinate intermediate. While the authors do not explicitly state the proposed structure of the seven-coordinate intermediate, we interpret this to be that depicted in Figure 1a. The other proposed pathway[15] involves

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breaking one of the Mo−S bonds (preferably the one trans to an oxo group) prior to the attack of the hydrogen sulfite so that the intermediate contains a hexacoordinate molybdenum atom (Figure 1b). Both pathways involve subsequent oxo-transfer to yield the products $[MoO(mnt)_2]^{2-}$ and HSO_4^-.

On the other hand, we[17] have proposed a direct attack of the sulfur atom on one of the oxo groups to yield a six-coordinate intermediate that leads to the same products, $[MoO(mnt)_2]^{2-}$ and HSO_4^-. The determination of the structure of sulfite oxidase from chicken liver revealed a bound sulfate (or sulfite) molecule close to the active site with the sulfur atom directed toward a water/hydroxo ligand on the molybdenum atom.[11] This coordination suggests an attack of SO_3^{2-} on one of the Mo═O bonds in the active site when the enzyme is in the oxidized form.[1,11,18]

As it has not been possible to detect any of the abovementioned intermediates, we report here our attempts to model the reaction of $[MoO_2(mnt)_2]^{2-}$ and HSO_3^- by computational methods using density functional and extended Hückel calculations. As the oxidation reaction has been carried out in slightly acidic solutions[15,17] where hydrogen sulfite predominates, we have chosen to use hydrogen sulfite as "substrate" although the presence of sulfite cannot be ruled out. Sulfite oxidase functions at physiological pH, where hydrogen sulfite and sulfite are in equilibrium. We have investigated three possible pathways for the reaction; (i) direct attack on the molybdenum atom by the sulfur atom of hydrogen sulfite (Figure 1a and b), (ii) direct attack on one of the oxo groups by the sulfur atom of hydrogen sulfite (Figure 1c), and (iii) attack on one of the oxo groups with accompanying hydrogen bonding of the hydrogen atom of HSO_3^- to the second Mo-oxo ligand (Figure 1d).

Computational Details

Extended Hückel calculations were performed using the CACAO 4.0 program package.[19] The geometry of the HSO_3^- ion was calculated using the Gaussian program package.[20] The density functional calculations were performed with the Amsterdam Density Functional (ADF) program version 2.3.0.[21−23] The implementation of the local density approximation (LDA) uses the standard Slater exchange term[24] and the correlation term due to Vosko, Wilk and Nusair.[25] Geometries were optimized at the LDA level using analytical energy gradients[26,27] within a spin-restricted formalism. Total binding energies were calculated at the LDA geometries using Becke's 1988[28] and Perdew's 1986[29] gradient corrected functionals for exchange and correlation, respectively. Default convergence criteria were employed throughout. Static solvation corrections to the total energies were computed based on the simple Born model as described previously[30] assuming a solvent dielectric constant of 80.

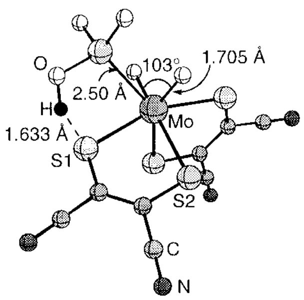

Figure 2. Geometry and labeling used for the modeling of direct attack of hydrogen sulfite on the molybdenum atom by extended Hückel calculations.

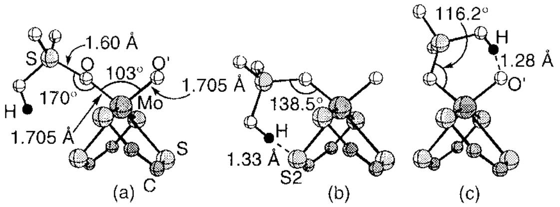

Figure 3. Three geometries used for extended Hückel calculations to model an attack of hydrogen sulfite on an Mo-oxo ligand in $[MoO_2(mnt)_2]^{2-}$. For clarity, the nitrile substituents have been omitted.

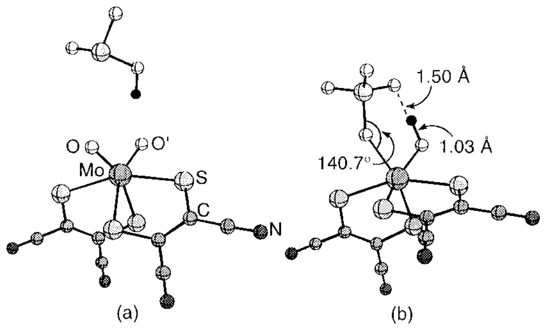

Figure 4. Optimized geometries for the attack of hydrogen sulfite on an Mo-oxo ligand in $[MoO_2(mnt)_2]^{2-}$, before (a) and after (b) formation of a coordinated sulfate.

Results and Discussion

Extended Hückel calculations were used to evaluate orbital differences in possible transition states involving either coordination of hydrogen sulfite to the molybdenum atom, or attack of hydrogen sulfite on one of the oxo moieties. The crystal structure coordinates of the anion of $[NEt_4]_2[MoO_2(mnt)_2]$ [15] were averaged to impose C_2 symmetry on $[MoO_2(mnt)_2]^{2-}$, and the resulting idealized structure was used in the calculations.

To model direct attack on the molybdenum atom, the HSO_3^- molecule was placed as in Figure 2 and the oxo ligands adjusted to accommodate the entering molecule but kept at the same Mo−O distance (1.705 Å) and with the same O−Mo−O angle (103°). The bond between S and Mo consists of the p_z orbital of the attacking sulfur and the d_{yz} orbital of the molybdenum. As the formed Mo−S bond involves the sulfur lone pair, a large rearrangement is necessary for product formation. There are weak interactions between the s and p orbitals of the HSO_3^- oxygen atoms and the oxygens of $[MoO_2(mnt)_2]^{2-}$ and between S1 (cf. Figure 2) and the hydrogen of HSO_3^-.

The direct attack on an Mo-oxo ligand was modeled using the geometry in Figure 3a. An S−O−Mo angle of 170° was found to be lowest in energy. To simulate other possibilites for the intermediate, extended Hückel calculations were also made

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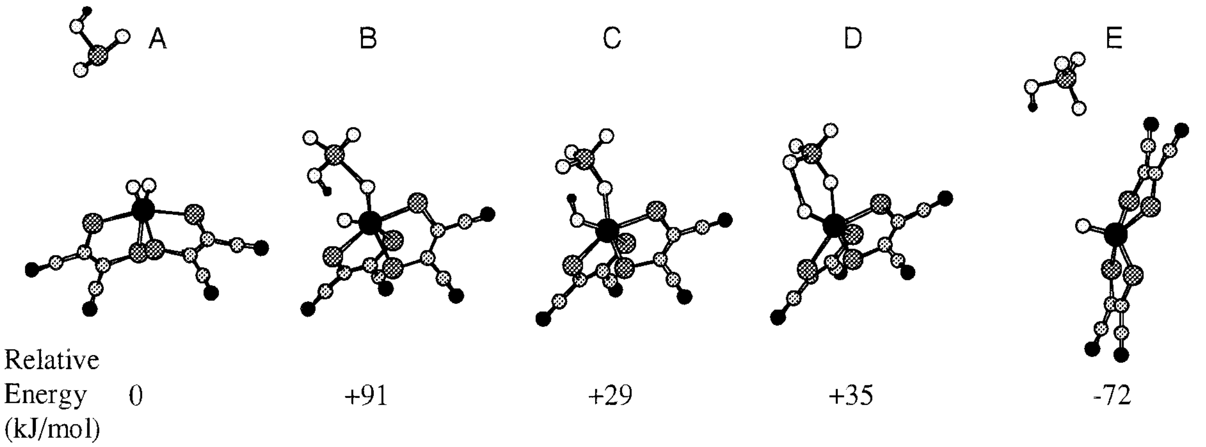
Chart 1. Schematic Depiction of the Calculated Reaction Pathway for the Oxidation of Hydrogen Sulfite by $[MoO_2(mnt)_2]^{2-}$



for the geometries in Figure 3b (the H atom directed toward S2) and Figure 3c (the H atom directed toward the other Mo-oxo ligand). In all three cases, the S—O bond consists of the s and p orbitals of the sulfur atom and the Mo-oxo ligand—this gives a large overlap population which is needed to form the product molecule. The charges of the atoms are in favor of direct attack on the oxygen; both the sulfur atom of HSO_3^- and the Mo atom are positive while there are negative charges on the oxo groups.

Density functional calculations were performed to provide a more quantitative description of the energetics of possible reaction pathways and to optimise the structures of any intermediates in the reaction between hydrogen sulfite and $[MoO_2(mnt)_2]^{2-}$. The solvation-corrected reaction energy is about -70 kJ mol^{-1}, showing that the reaction proceeds “downhill”. To simulate direct attack of hydrogen sulfite on one of the oxo groups, the sulfur atom of the former was positioned above the MoO_2 moiety. The geometry converged to that in Figure 4a with the sulfur lone pair more or less directed at one oxo group (S···O approximately 3.6 Å) while the hydroxide hydrogen makes a hydrogen bond with the other oxo ligand (H···O′ approximately 1.9 Å).

A linear transit calculation was then carried out wherein the S—O distance was decreased from 3.6 Å in several fixed steps while the remaining degrees of freedom were allowed to vary. The solvation corrected energy reaches a maximum at about 2.1 Å representing a forward barrier of about 90 kJ mol^{-1}, before forming an intermediate some 30 kJ mol^{-1} higher in energy than the isolated reactants. During the course of the linear transit, the hydrogen spontaneously transfers to the O′ oxo ligand generating a coordinated sulfate with an S—O bond length of 1.62 Å and an Mo—O bond length of 2.05 Å (Figure 4b). Product dissociation was modeled via a second linear transit, this time lengthening the Mo—O distance of the coordinated sulfate. The energy goes through a shallow maximum with a forward barrier of about 6 kJ mol^{-1} at a distance of 2.3 Å. Moreover, the H atom spontaneously returns to the sulfate oxygen, thus generating the hydrogen sulfate product. The complete reaction pathway is illustrated in Chart 1.

The alternative pathway of a direct attack on the molybdenum atom by the sulfur was also modeled by density functional calculations. Hydrogen sulfite was directed with its lone pair facing the molybdenum atom in the space between the oxo groups and one of the mnt ligands. A complex with an Mo—S distance of about 2.95 Å and with the hydroxide hydrogen oriented toward one of the oxo ligands to make a hydrogen bond was located. The linear transit calculation followed the Mo—S distance which was steadily decreased. The computed energy rose fairly rapidly such that at an Mo—S distance of 2.4 Å, the energy was already some 50 kJ mol^{-1} higher than the highest point on the reaction profile for attack at an oxo group. Direct attack by hydrogen sulfite at the Mo center was therefore already predicted to be unfavorable and further calculations were deemed unnecessary.

In conclusion, our computational modeling of some possible reaction intermediates for the above-mentioned oxidation reaction do not support the formation of a seven-coordinate intermediate. Rather, direct attack of hydrogen sulfite at an oxo ligand, as proposed by us for $[MoO_2(mnt)_2]^{2-}$,[17] and by Hille[1,18b] and Kisker et al.[31] for sulfite oxidase, appears to be a favorable pathway (Chart 1). Our results are consistent with the generally accepted mechanism for oxo transfer by *cis*-dioxo Mo(VI) complexes;[32,33] similar results have been obtained in the computational modeling of the reaction between phosphine and $[MoO_2(Et_2dtc)_2]$ (Et_2dtc = *N*,*N*-diethyldithiocarbamate)[34] and the oxidation of trimethylphosphine by $[MoO_2(NH_3)_2(SH)_2]$.[35] Ab initio studies of hydrocarbon oxidation by $[MoO_2Cl_2]$ [36] have suggested that the second “spectator” oxo ligand achieves partial triple bond character during the course of these reactions and thus plays a critical role in stabilizing intermediates. A similar effect was observed for the oxidation of PMe_3 by $[MoO_2(NH_3)_2(SH)_2]$,[35] where a formal molybdenum—spectator oxo ligand triple bond is formed as a consequence of the direction of the substrate attack. No such effect can be observed in the present model system, but the hydrogen transfer to the “spectator” ligand appears to be important in stabilizing the found intermediate (cf. Chart 1).

The results presented here apply specifically to the oxidation of HSO_3^- by $[MoO_2(mnt)_2]^{2-}$. It is harder to assess the relevance of our calculations to the oxidative half-reaction of the sulfite oxidase enzyme; not only is our model relatively simple when compared to the enzyme, but the direct molybdenum coordination environments in the model and the enzyme (vide supra)

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differ. The hydrogen transfer observed in our modeling may not be applicable to the enzymatic reaction but the crystallographic location of a sulfite molecule in the active site of sulfite oxidase implies that our proposed intermediate and mechanism are likely. Further structural and mechanistic studies of the enzyme system are needed in order to determine its mechanism.

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Supporting Information Available: Tables containing structural parameters and total energies for relevant transition states and intermediates as well as atomic coordinates (in PDB format) for the structures depicted in Chart 1 (7 pages). Ordering information is given on any current masthead page.

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