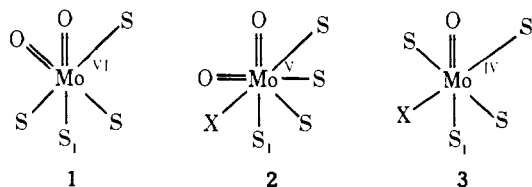lengthened significantly, we arrive at the proposed Mo-site structures **1-3** for the three oxidation levels of the enzyme.

In our EXAFS-derived model, the Mo^{VI} site possesses two cis oxo groups, two sulfurs with relatively short bond lengths trans to each other, one long distance sulfur trans to Mo=O, and possibly a final intermediate distance sulfur, also trans to Mo=O. We assume this final Mo—S distance to be longer than, but unresolvable from, the pair of shorter Mo—S distances. That is, in view of the results for the Mo^V and Mo^{IV} states, we suspect that the 2 S atoms calculated at 2.42 Å for Mo^{VI} in fact represent a weighted average of 2 S at 2.36 Å and 1 S at 2.52 Å, or some similar combination. Upon partial reduction to the Mo^V state, the three medium distance Mo—S bond lengths become more nearly equal. The new scatterer, labeled X (which could be either oxygen or nitrogen), is assigned as the oxygen of bound sulfite or sulfate, although the emergence of a previously obscured protein or cofactor ligand cannot be ruled out without additional data. Finally, reduction to the Mo^{IV} state results in loss of one oxo group.

Clearly, other structural models for the Mo states of sulfite oxidase may be proposed from the calculated values of Table I. Such models would have to include Mo=O and Mo—S units, but the numbers and lengths of the latter bonds could vary somewhat (there is substantial uncertainty in our assignment of the number of Mo—S bonds in the Mo^{VI} state, for example). We expect that chemical and EXAFS studies now in progress will elucidate further the structures of the Mo^{VI}, Mo^V, and Mo^{IV} sites of the enzyme.

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Structural Results Relevant to the Molybdenum Sites in Xanthine Oxidase and Sulfite Oxidase. Crystal Structures of MoO2L, L = (SCH2CH2)2NCH2CH2X with X = SCH3, N(CH3)2[1]

Sir:

X-ray absorption spectroscopy has proven to be useful in elucidating accurate structural details of metal sites in macromolecules. Recent results on xanthine oxidase[2a] and sulfite oxidase[2b] have established that the Mo coordination environments in these two enzymes are similar and that each contains both terminal oxo groups ($Mo-O_t$) and sulfur ligands. This molecular information now makes it possible to establish the relevance of low-molecular-weight model complexes and to compare their structures with those determined for the molybdenum oxidase enzymes by EXAFS. It is such a comparison that we are communicating here, based on the crystal structures of two compounds which were synthesized as a part of an ongoing program to model Mo-containing enzymes.

The two compounds, of the form MoO_2L, contain the tripodal tetradentate ligands $(SCH_2CH_2)_2NCH_2CH_2X$, X = SCH_3 (compound **1**) and X = $N(CH_3)_2$ (compound **2**), and are representative of a new class of complexes containing a polydentate ligand with permutations of O, N, and S coordinating atoms. The syntheses[3] and other physical properties of these compounds will be reported in detail elsewhere.[4] The Mo(VI) complexes of these two ligands have previously been studied by EXAFS and the distances to the atoms coordinating the Mo were reported.[5] We have now completed single-crystal X-ray diffraction studies on both complexes, and the crystallographic results fully confirm the prior EXAFS determinations. These results assume further significance as the Mo environment in these complexes bears resemblance to that found in sulfite oxidase and xanthine oxidase.

The crystal structures of compounds **1** and **2** have been determined using conventional heavy-atom techniques and refined as described previously.[6] The two structures (Figure 1) are distinctly similar in overall geometry and in conformation of the multidentate ligand. Both have distorted octahedral geometry with approximate C_s symmetry. The mirror plane is defined by the MoO_2^{2+} core and contains the central tripodal nitrogen and the other nonthiolate donor atom. The thiolate sulfurs are approximately trans with S(1)–Mo–S(2) angles of 151.2 (**1**) and 154.3° (**2**). The MoO_2^{2+} core has the cis geometry characteristic of all known dioxo Mo(VI) compounds,[8] with O(1)–Mo–O(2) angles of 108.6 (**1**) and 107.9° (**2**). Within each compound, the two Mo=O bonds are equivalent with the distances averaging 1.695 (**1**) and 1.702 Å (**2**). Differences in Mo–N distances and in individual bond angles between the two complexes can be attributed in part to the smaller bite of the nitrogen ligand **2** when compared with the sulfur in **1**. This is reflected in the Mo—N (tripod) bond length

Table I. Summary of Distances in Model Compounds and Proteins

system	method	Mo=O	Mo—N	Mo—S	Mo—S′
MoO$_2$[(SCH$_2$CH$_2$)$_2$NCH$_2$CH$_2$SCH$_3$], **1**	EXAFS[a]	1.693		2.401	2.803
	diffraction 1[b]	1.695	2.418	2.406	2.769
	diffraction 2[b]	1.695	2.394	2.400	2.790
MoO$_2$[(SCH$_2$CH$_2$)$_2$NCH$_2$CH$_2$N(CH$_3$)$_2$], **2**	EXAFS[a]	1.694		2.424	
	diffraction[b]	1.702	2.372	2.415	
			2.510		
Mo(SC$_6$H$_4$NH)$_3$	EXAFS[a]		1.996	2.419	
	diffraction[c]		1.997	2.418	
oxidized xanthine oxidase	EXAFS[d]	1.71		2.54	2.84
oxidized sulfite oxidase	EXAFS[e]	1.71		2.42	2.85

[a] Reference 5. [b] This work, diffractions 1 and 2 refer to the two different crystal forms of **1**. [c] Reference 9. [d] Reference 2a. [e] Reference 2b.
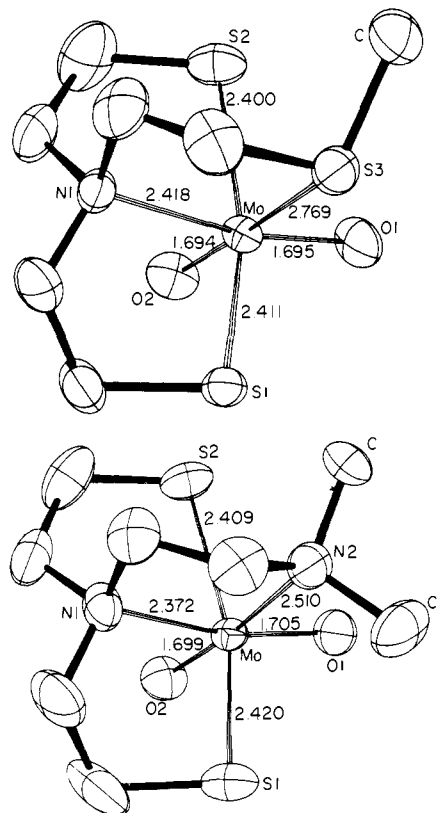

Figure 1. ORTEP views for MoO$_2$[(SCH$_2$CH$_2$)$_2$NCH$_2$CH$_2$SCH$_3$] (compound **1**) (top) and MoO$_2$[(SCH$_2$CH$_2$)$_2$NCH$_2$CH$_2$N(CH$_3$)$_2$] (compound **2**) (bottom). Ligand conformation is clearly similar despite the substitution of an -N(CH$_3$)$_2$ for a -SCH$_3$ ligand. Selected bond angles are given in the text.

with Mo—N distances of 2.418 (**1**) and 2.372 Å (**2**).

EXAFS was used to determine the distances to the oxo groups and sulfur ligands in **1** and **2** prior to the availability of the crystal structures.[5] A comparison with the crystallographic results reported herein (see Table I) reveals the mean difference in distances determined by the two methods to be 0.012 Å. The largest discrepancy was 0.03 Å, found for the Mo—S (thioether) distance[7] in **1**. The crystal structure of a third Mo complex for which Mo—L distances were previously determined by EXAFS has recently been reported by Yamanouchi and Enemark.[9] The Mo—N and Mo—S distances in Mo(SC$_6$H$_4$NH)$_3$[10] determined from the crystal structure[9] were found to be within 0.001 Å of our EXAFS results.[5] All of these results, which are summarized in Table I, together impressively verify the ability of EXAFS to determine distances about metal centers for previously unknown structures.

The EXAFS spectra of compounds **1** and **2** are compared in Figure 2 with the spectra of the oxidized forms of xanthine
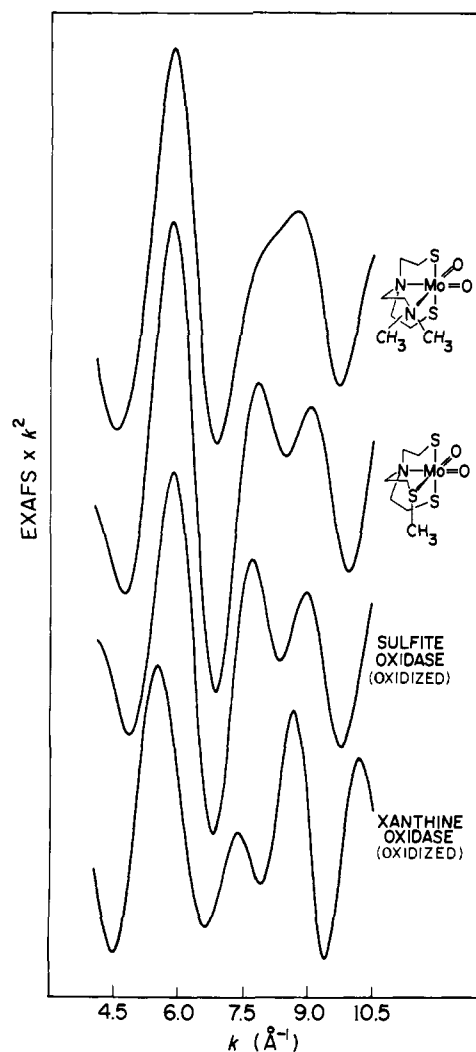

Figure 2. Comparison of the EXAFS spectra for compounds **1** and **2** with those of oxidized xanthine oxidase and sulfite oxidase. The overall similarity in the spectra, especially at high and low k, is striking. The maximum in all four spectra at about k of 5.5 Å^{-1} corresponds to the point where the shorter sulfur and the oxo waves first come in phase, thereby adding to give a strong peak. This maximum is shifted slightly to lower k for xanthine oxidase because of the longer distance to the short sulfur. Between k of 6 and 9 Å^{-1}, the S and O waves begin to go out of phase, giving rise to the "beat" which is characteristic of two different neighboring shells of atoms around the Mo. It is the beat region which is most sensitive to small differences in distances and also to the nature of additional ligands. For example, the appearance of the beat region is altered by changing the longer sulfur in **1** to a nitrogen in **2**. Xanthine oxidase and sulfite oxidase differ in the beat region because of the difference of ~0.1 Å in the Mo-S(short) distance. Around k of 9.5 Å^{-1}, the oxo and short sulfur waves again come in phase and add to give the large negative maximum. Detailed curve-fitting analysis of these spectra has been carried out to determine accurate distances and coordination numbers as described in the text and in ref 2.

oxidase and sulfite oxidase. There are substantial differences between the two model compound spectra in the beat region between k of 6.5 and 8.5 Å⁻¹ despite the basic structural similarity of the compounds. This points out the ability of the EXAFS method to easily distinguish the substitution of one ligand (-SCH₃ vs. -N(CH₃)₂) in the metal's coordination sphere. There is a striking similarity between the EXAFS of **1** and that of the oxidized form of sulfite oxidase.[2] While it cannot be concluded that the Mo environments are identical, there must be many common features.

Curve-fitting analysis of the EXAFS data for xanthine oxidase[2a] and sulfite oxidase[2b] suggests that, in their oxidized forms, both enzymes contain the MoO_2^{2+} unit. The average Mo=O distances, 1.71 Å, are the same for both proteins. Sulfur atoms are also present, at average distances of 2.42 and 2.54 Å for sulfite and xanthine oxidase, respectively. A more distant sulfur is also present at ~2.85 Å in both enzymes. The reason for the similarity of the EXAFS spectra for sulfite oxidase and **1** is clear—the Mo—O and both average Mo—S distances are very similar. The different appearance of the beat region of xanthine oxidase is simply a manifestation of the longer distance to the shorter sulfur ligands.

Comparison of the Mo—S distances in the enzymes with those of compounds **1** and **2** suggests configurational features about the Mo sites in the enzymes. The thiolate sulfurs in **1** and **2** are trans to each other and cis to the oxo groups with the Mo—thiolate distances all between 2.40 and 2.42 Å. The distances are consistent with other known structures containing thiolates cis to Mo=O.[8] Sulfite oxidase has similar Mo—S distances, but in xanthine oxidase a longer distance (2.54 Å) is observed. Since a pronounced trans effect is known to exist for Mo=O bonds, this longer distance may be characteristic of a thiolate sulfur trans to a Mo=O.

For every case in which structural information is available on Mo thiolate complexes (either from X-ray diffraction studies such as those reported herein or from ¹H NMR studies on several other complexes yet to be published[4]) the thiolate sulfurs are trans to each other and cis to the oxo groups. Attempts to synthesize complexes with three or four aliphatic thiolate groups about Mo(VI) have been unsuccessful, leading invariably to internal reduction of Mo(VI) to Mo(IV) or Mo(V). Three thiolate ligands on a MoO_2^{2+} core would require that at least one sulfur be trans to a Mo=O and that at least two thiolates be mutually cis. The strongly σ and π donating thiolate may thereby labilize the trans Mo=O, while the oxidation of two thiolates to a disulfide would be facilitated by their cis orientation on the Mo center. These factors may combine to allow facile reduction of Mo(VI) with concomitant loss of an oxo group. Should oxo activation be involved in enzyme–substrate reactions (either for Mo=O bond cleavage[11] and/or for proton transfer to oxygen),[11,12] the orientation of the thiolate ligands might be crucial for activating the Mo=O bond while mediating the redox potential of the Mo site.

These results further establish the validity of EXAFS analysis in the determination of accurate metal–ligand distances in molecules under noncrystalline conditions. The relevance of model complexes containing the MoO_2^{2+} core to the resting state structures of Mo in oxidized xanthine and sulfite oxidase has been defined by EXAFS. Further synthetic studies and EXAFS data analysis on the proteins in various states should lead to a more thorough understanding of their mechanisms of action on a molecular level.

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(3) The new ligands (HSCH₂CH₂)₂NCH₂CH₂X where X = SCH₃, N(CH₃)₂, were prepared by reaction of NH₂CH₂CH₂X with ethylene sulfide. The complexes were synthesized by the reaction of MoO₂(acac)₂ with the free ligand (1:1) in CH₃OH. Crystals of the yellow X = SCH₃ complex were grown either from a CH₂Cl₂ solution at 4 °C or from a 4:6:3 CH₃CN–CH₂Cl₂–C₆H₆ solution at room temperature. Two different crystal forms were observed. Crystals of the orange X = N(CH₃)₂ complex were grown from a solution of CH₂Cl₂–CH₂ClCH₂Cl at room temperature (acac = acetylacetonate). MoO₂[(SCH₂CH₂)₂NCH₂CH₂SCH₃)] (1): ν(MoO) 910, 880 cm⁻¹. Anal. Calcd: C, 24.92; H, 4.48; N, 4.15. Found: C, 25.38; H, 4.52; N, 4.18. MoO₂[(SCH₂CH₂)₂NCH₂CH₂N(CH₃)₂] (2): ν(MoO) 911, 882 cm⁻¹. Anal. Calcd: C, 28.74; H, 5.43; N, 8.38. Found: C, 28.48, H, 5.47, N, 8.43.
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(6) Crystal data: **1**, a = 7.235 Å, b = 7.717 Å, c = 24.527 Å, β = 119.86°, P2₁/n, Z = 4; **2**, a = 7.188 Å, b = 22.708 Å, c = 7.746 Å, P2₁2₁2₁, Z = 4. Data processing and structural solution methods are described in M. A. Bobrik, K. O. Hodgson, and R. H. Holm, *Inorg. Chem.*, **16**, 1851 (1977). The current unweighted R factors are 3.8% and 2.3% for **1** and **2**, respectively.
(7) Compound 1 was also observed to crystallize in a second crystal form which had two molecules per asymmetric unit. The structure of this crystal form was also determined by X-ray diffraction analysis and the basic structural features were the same as those for the crystal form reported in this communication. There were small differences in distances and angles and it was interesting to observe that the mean Mo—S (thioether) distance was 2.79 Å, a distance only 0.01 Å different from the EXAFS result. The two different unique Mo—S (thioether) distances in the asymmetric unit differed by 0.04 Å. Such a static disorder would result in a low number of atoms being predicted by the EXAFS analysis. Indeed, the number calculated from the EXAFS analysis was 0.5 rather than 1.0.[5] It is uncertain if the EXAFS data were collected on this crystal form, but it is noteworthy that the distances and calculated numbers of atoms are even more consistent with the X-ray result than for the form with only one molecule per asymmetric unit. Regardless of which form was used in the EXAFS analysis, the distinct similarities in the numbers compared with the crystallographic results remain.
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The Molybdenum Site of Xanthine Oxidase. Structural Evidence from X-ray Absorption Spectroscopy

Sir:

We report here structural features of the molybdenum site of milk xanthine oxidase, as determined by analysis of the enzyme's X-ray absorption spectrum. Despite the prodigious amount of study which this metalloprotein has received over several decades,[1,2] this is the first definitive structural char-