(4) Ardon, M.; Pernick, A. *J. Am. Chem. Soc.* **1973**, *95*, 6871.
(5) Ramasami, T.; Taylor, R. S.; Sykes, A. G. *J. Am. Chem. Soc.* **1975**, *97*, 5918.
(6) Ardon, M.; Bino, A.; Yahav, G. *J. Am. Chem. Soc.* **1976**, *98*, 2338.
(7) Chalilpoyil, P.; Anson, F. C. *Inorg. Chem.* **1978**, *17*, 2418.
(8) Cramer, S. P.; Hodgson, K. O. *Prog. Inorg. Chem.*, in press.
(9) Shulman, R. G.; Eisenberger, P.; Kincaid, B. M. *Annu. Rev. Biophys. Bioeng.* **1978**, *7*, 559.
(10) Cramer, S. P.; Hodgson, K. O.; Stiefel, E. I.; Newton, W. E. *J. Am. Chem. Soc.* **1978**, *100*, 2478.
(11) Mo(IV) (0.1 M) in 4 M aqueous HCl solution was prepared by a standard procedure.[4] It was loaded into a 0.5-cm path-length Lucite cell just prior to recording its spectrum at the Stanford Synchrotron Radiation Laboratory. The data presented here were obtained in a period of ~45 min under 3.5-GeV, 50-mA single-electron beam conditions. All data processing utilized previously described programs (Eccles, T. K. Ph.D. Thesis, Stanford University, Stanford, Calif., 1977). The spectra were calibrated assuming the first inflection point for Mo foil to be 20 003.9 eV.
(12) Cramer, S. P.; Hodgson, K. O.; Gillum, W. O.; Mortenson, L. E. *J. Am. Chem. Soc.* **1978**, *100*, 3398.
(13) Cramer, S. P. Ph.D. Thesis, Stanford University, Stanford, Calif., **1977**.
(14) Cramer, S. P.; Gray, H. B.; Rajagopolan, K. V. *J. Am. Chem. Soc.*, following paper in this issue.
(15) Kreale, G. K.; Geddes, A. J.; Sasaki, Y.; Shibahara, T.; Sykes, A. G. *J. Chem. Soc., Chem. Commun.* **1975**, 356.
(16) Cotton, F. A.; Morehouse, S. M. *Inorg. Chem.* **1965**, *4*, 1377.
(17) Knox, J. R.; Prout, C. K. *Acta Crystallogr., Sect. B* **1969**, *25*, 1857.
(18) Brandt, B. G.; Skapski, A. C. *Acta Chem. Scand.* **1967**, *21*, 661.
(19) Delbaere, L. T.; Prout, C. K. *Chem. Commun.* **1971**, 162.
(20) Ricard, L.; Martin, C.; West, R.; Welss, R. *Inorg. Chem.* **1975**, 14, 2300.
(21) Lippard, S. J.; Nozaki, H.; Russ, B. J. *Chem. Commun.* **1967**, 119.
(22) Robinson, P. R.; Schlemper, E. O.; Murmann, R. K. *Inorg. Chem.* **1975**, *14*, 2035.
(23) See paragraph at end of paper regarding supplementary material.
(24) The EXAFS fits were very poor for structures in which two Mo atoms are placed at a given distance from a third. Thus a cyclic trimeric Mo(IV) structure for Mo(IV) (4 M HCl) is unlikely; however, we will not consider this aspect of the structural formulation settled until we have completed EXAFS experiments on the known cyclic Mo(IV) trimers: Bino, A.; Cotton, F. A.; Dori, Z. *J. Am. Chem. Soc.* **1978**, *100*, 5252.

S. P. Cramer, Harry B. Gray*
Contribution No. 5895
Arthur Amos Noyes Laboratory of Chemical Physics
California Institute of Technology
Pasadena, California 91125

Zvi Dori
Department of Chemistry
Technion—The Israel Institute of Technology
Technion City, Haifa, Israel

Avi Bino
Department of Inorganic and Analytical Chemistry
Hebrew University, Jerusalem, Israel
Received October 3, 1978

The Molybdenum Site of Sulfite Oxidase. Structural Information from X-ray Absorption Spectroscopy

Sir:

Very little is known about the structures of the active sites of molybdenum enzymes. Although knowledge of the molybdenum coordination sphere is essential both for rational synthetic modeling and for determination of the catalytic mechanism, no molybdenum protein structure has yet been crystallographically determined. Recent work has shown that X-ray absorption spectroscopy is a valuable tool for the determination of metalloprotein active-site structures,[1-4] and such studies of nitrogenase have suggested that the Mo in this enzyme is present in a cluster that includes Fe and S atoms.[5,6] However, biochemical work has shown that the "iron-molybdenum cofactor" of nitrogenase is quite different from the "molybdenum cofactor" [8,9] common to the remainder of molybdenum enzymes (xanthine oxidase–dehydrogenase, sulfite oxidase, aldehyde oxidase, nitrate reductase, and formate dehydrogenase). We now present X-ray absorption results for sulfite oxidase, which indicate both oxo and sulfur coordination for the Mo in this enzyme.

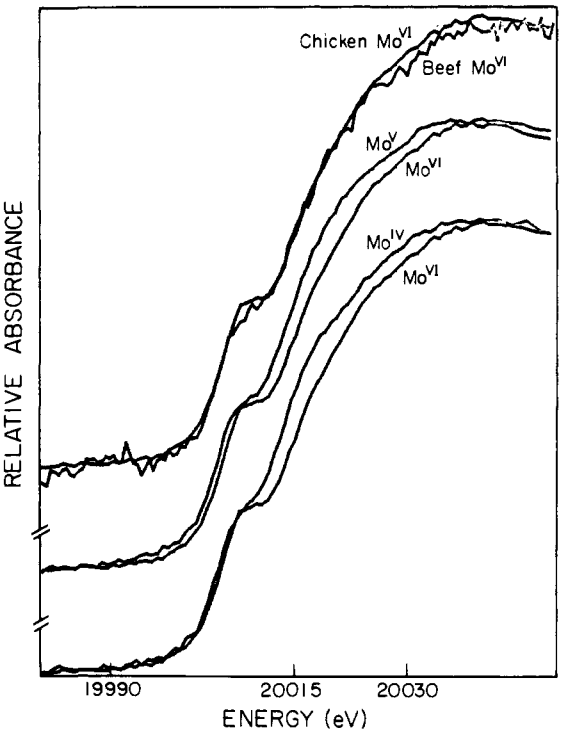


Figure 1. Molybdenum K-absorption edges for sulfite oxidase from different sources and in different states: top curves, oxidized chicken liver sulfite oxidase (CLSO) vs. oxidized beef liver sulfite oxidase; middle curves, oxidized CLSO vs. sulfite-reduced CLSO; bottom curves, oxidized CLSO vs. dithionite-reduced CLSO. All samples were run at ~100 mg/mL in 0.05 M, pH 9.2 Tris-HCl buffer. The reduced samples were prepared with a 50-fold excess of reagent.

Sulfite oxidase catalyzes the oxidation of sulfite to sulfate, using water as the source of oxygen and cytochrome *c* as the physiological electron acceptor.[10] This enzyme has been observed in bacteria, plants, and in mammalian tissues, especially in the liver.[11] Sulfite oxidase contains two *b*-type cytochromes as well as two molybdenums, and it exists as a dimer of roughly 55 000-dalton polypeptide subunits. Several different states of sulfite oxidase have been distinguished by a combination of EPR and optical spectroscopic measurements. The protein as isolated contains Mo^{VI} and a low-spin ferric heme (Soret band at 413 nm).[11] In the sulfite-reduced state there is a low-spin ferrous heme with a Soret band at 423 nm, and a Mo^{V} EPR signal appears at $g = 1.97$.[11] Addition of dithionite causes loss of the Mo EPR, presumably because reduction to diamagnetic Mo^{IV} occurs.[11] As illustrated in Figures 1 and 2, there are significant differences in both the absorption edges and in the EXAFS of all three of these molybdenum oxidation states. By correlating the edge changes with a variety of model compounds, and by curve fitting the EXAFS with previously determined phase shift and amplitude functions,[12] a detailed structural model for the sulfite oxidase molybdenum site may be proposed.

The most prominent feature of the sulfite oxidase Mo absorption edge spectra (Figure 1) is a low-energy bound-state ($1s \rightarrow 4d$) transition that is visible as a shoulder on the main absorption edge. Comparison with model compound spectra[5,13] suggests that the presence of one or two Mo=O groups gives rise to this feature, since Mo complexes without Mo=O generally have a smooth, single inflection point edge,[5] whereas those with three of four oxo groups have a resolved low-energy peak.[13] The major inflection point for the oxidized protein falls at 20 015 eV, which for Mo^{VI} in a protein environment suggests a coordination sphere with a mix of oxygen and sulfur ligands.[14] These results are in direct contrast to previous findings

0002-7863/79/1501-2772$01.00/0

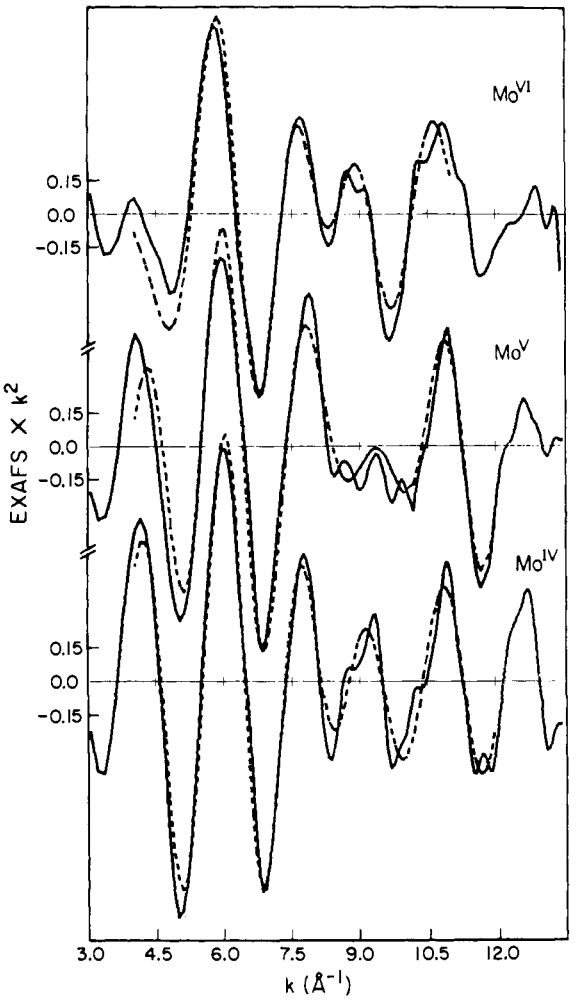


Figure 2. Observed EXAFS (—) and fit (---) for sulfite oxidase in three different states, obtained on same samples used for edges.

for the Mo in nitrogenase,[5,6] where Mo=O groups are absent from the functional enzyme (but appear upon O_2 inactivation), and where the ligands are all or almost all sulfur donors. Upon reduction with sulfite to the Mo^V state, the principal inflection point of the sulfite oxidase Mo edge shifts ~0.5 eV to lower energy, whereas the bound-state transition moves only slightly. Further reduction with dithionite, presumably to the Mo^{IV} state, shifts the principal inflection point to 20 014 eV, whereas the bound-state transition, although not moving appreciably, appears to decrease in intensity.

The EXAFS of sulfite oxidase molybdenum in all three oxidation states (Figure 2) shows a pronounced beat pattern, indicating the presence of at least two strong component waves, and hence two Mo-scatterer distances. From the previous discussion we expect one component to result from Mo=O and a second from Mo—S, and the results of a curve-fitting analysis[12] support this interpretation (Table I). These distances and scatterer numbers were obtained by sequentially adding components to the curve-fitting procedure until an adequate fit to the EXAFS was obtained. For all three oxidation states the major features of the EXAFS may be accounted for by including a short, Mo=O wave and a medium Mo—S wave in the fits. However, a further improvement in the quality of the fits, indicated quantitatively by a reduction in χ^2, is obtained through introduction of a third wave corresponding to a long Mo—S bond. Although the reduction in χ^2 is not dramatic, it is similar to that found upon addition of a long Mo—S component to the EXAFS fit for the complex $MoO_2[(SCH_2CH_2)_2NCH_2CH_2SCH_3]$, whose structure features a Mo—S (thioether) distance of 2.77 Å, as well as two

Table I. Curve-Fitting Results for Sulfite Oxidase[a]

	EXAFS component					
sample	Mo—S	Mo=O	Mo—S′	Mo—O′	Mo—N	χ^2
oxidized sulfite oxidase	2.430[b]					4.364
	(2.3)					
	2.421	1.708				0.752
	(2.1)	(1.7)				
	2.416	1.707	2.840			0.567
	(2.0)	(1.8)	(0.7)			
sulfite-reduced sulfite oxidase	2.375					5.040
	(2.6)					
	2.367	1.710				1.098
	(2.5)	(1.8)				
	2.368	1.711	2.907			0.935
	(2.5)	(1.8)	(0.7)			
	2.362	1.719		2.008		0.764
	(2.9)	(1.8)		(1.0)		
	2.363	1.722			2.008	0.740
	(2.9)	(1.9)			(1.6)	
	2.364	1.723	2.896		2.010	0.561
	(2.9)	(1.9)	(0.7)		(1.6)	
dithionite-reduced sulfite oxidase	2.382					3.204
	(3.0)					
	2.378	1.688				1.336
	(2.9)	(1.2)				
	2.378	1.690	2.839			1.063
	(2.9)	(1.3)	(0.8)			
	2.379	1.682		2.058		0.974
	(3.3)	(1.1)		(1.0)		
	2.380	1.686			2.063	0.937
	(3.2)	(1.0)			(1.6)	
	2.378	1.691	2.849		2.056	0.651
	(3.3)	(1.0)	(0.9)		(1.6)	

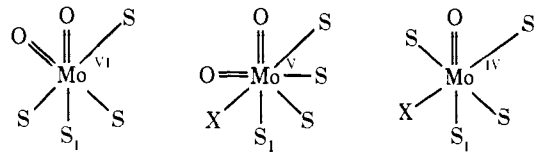
[a] The data were smoothed by convolution with a Gaussian in k space, but *not* Fourier filtered. [b] Ångstroms.

shorter (2.41 Å) Mo—S (thiolate) bonds.[15] For the EXAFS of the two reduced forms, but not the oxidized enzyme, a fourth wave corresponding to either oxygen or nitrogen at ~2.05 Å was also found to improve the fits.

When curve fitting EXAFS data for structure determination, the degree of confidence appropriate for a particular component is proportional to the reduction in χ^2 that results from including that component. Both the short Mo—O and the medium Mo—S components produce dramatic improvements in χ^2 and are essential for a good fit; *thus, we can say beyond doubt that the Mo in sulfite oxidase is bound to a mixture of oxo- and sulfur-donor ligands.* Although the Mo—X (reduced states) and the Mo—S_{long} waves produce lesser improvements in χ^2, the fact that they occur reproducibly for different oxidation levels rules out the possibility of statistical artifacts. Furthermore, the X and S_{long} bonds are necessary to give the molybdenum a reasonable coordination number, and thus we feel only slightly less confident in the reality and accuracy of these components.

Our EXAFS data (Table I) are reasonably accommodated by assuming that the molybdenum site of sulfite oxidase has four sulfur-donor ligands, one or two oxo groups, and the capacity for binding an extra ligand, designated X. By noting further that X-ray crystallographic studies of molybdenum complexes have shown that oxo groups are almost always cis to each other, and that groups trans to Mo=O have their bonds

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



1 **2** **3**

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.

Acknowledgments. We thank Keith Hodgson and Tom Tullius for helpful discussions. Bryan Hainline and Ralph Wiley are acknowledged for their assistance in the protein preparations. S.P.C. is a recipient of a National Institutes of Health Research Fellowship Award GM-06478-01. Data were collected at the Stanford Synchrotron Radiation Laboratory, supported by National Science Foundation Grant DMR-07692-A02 in cooperation with the Stanford Linear Accelerator Center and the Department of Energy. Research at Duke was supported by National Institutes of Health Grant GM00091. Research at Caltech was supported by National Science Foundation Grant CHE77-11389.

References and Notes

(1) Cramer, S. P.; Hodgson, K. O. *Prog. Inorg. Chem.*, in press.
(2) Teo, B. K. *Acc. Chem. Res.*, in press.
(3) Shulman, R. G.; Eisenberg, P.; Kincaid, B. M. *Annu. Rev. Biophys. Bioeng.* **1978**, *7*, 559.
(4) Chan, S.; Gamble, R. *Methods Enzymol.* **1978**, *54E*, 323.
(5) Cramer, S. P.; Hodgson, K. O.; Gillum, W. O.; Mortenson, L. E. *J. Am. Chem. Soc.* **1978**, *100*, 3398.
(6) Cramer, S. P.; Gillum, W. O.; Hodgson, K. O.; Mortenson, L. E.; Stiefel, E. I.; Chisnell, J. R.; Brill, W. J.; Shah, V. K. *J. Am. Chem. Soc.*, **1978**, *100*, 3814.
(7) Shah, V. K.; Brill, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3249.
(8) Pienkos, P. T.; Shah, V. K.; Brill, W. J. *Proc. Nat. Acad. Sci. U.S.A.* **1977**, *74*, 5468.
(9) Johnson, J. L.; Jones, H. P.; Rajagopalan, K. V. *J. Biol. Chem.* **1977**, *252*, 4994.
(10) Cohen, H. J.; Betcher-Lange, S.; Kessler, D.; Rajagopalan, K. V. *J. Biol. Chem.* **1972**, *247*, 7759.
(11) Kessler, D.; Rajagopalan, K. V. *J. Biol. Chem.* **1972**, *247*, 7759.
(12) Cramer, S. P.; Hodgson, K. O.; Stiefel, E. I.; Newton, W. E. *J. Am. Chem. Soc.* **1978**, *100*, 2748.
(13) Cramer, S. P. Ph.D. Thesis, Stanford University, 1977.
(14) Mo^{VI} compounds with all sulfur-donor ligands generally exhibit a major inflection point at about 20 011 eV; the analogous feature for Mo^{VI} with all oxygen-donor ligands falls at ~20 019 eV. See ref 5 and 12 for examples.
(15) Berg, J. M.; Hodgson, K. O.; Cramer, S. P.; Corbin, J. L.; Elsberry, A.; Pariyadath, N.; Stiefel, E. I. *J. Am. Chem. Soc.*, following paper in this issue.
(16) Stiefel, E. I. *Prog. Inorg. Chem.* **1976**, *22*, 1.

Stephen P. Cramer,* Harry B. Gray*
Contribution No. 5894
Arthur Amos Noyes Laboratory of Chemical Physics
California Institute of Technology
Pasadena, California 91125

K. V. Rajagopalan
Department of Biochemistry, Duke University
Durham, North Carolina 27706
Received October 3, 1978

Structural Results Relevant to the Molybdenum Sites in Xanthine Oxidase and Sulfite Oxidase. Crystal Structures of MoO_2L, L = $(SCH_2CH_2)_2NCH_2CH_2X$ with X = SCH_3, $N(CH_3)_2$[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

0002-7863/79/1501-2774$01.00/0