Structural and Spectroscopic Characterization of a Monooxomono(dithiolene)molybdenum(V) Compound and Its Implications for the Low pH Form of Sulfite Oxidase

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Pterin-containing molybdenum enzymes[1] such as sulfite oxidase, xanthine oxidase, and nitrate reductase possess a common molybdenum cofactor that is proposed to coordinate the molybdenum atom via the dithiolene side chain of a 6-substituted pterin.[2] As yet there is no crystal structure available for any pterin-containing molybdenum enzyme, but EXAFS and EPR[3] studies on the Mo(V) state of sulfite oxidase support a mononuclear molybdenum center with a single oxo group and at least two RS ligands bound to the molybdenum center. Tris(dithiolene) complexes of molybdenum have long been known,[4] and recently complexes of the type Cp_2Mo(dithiolene)[5] and $[Mo(dithiolene)_3]^{2-/1-}$,[6,7] in which the dithiolene is appended to a pterin or quinoxaline ring, have been reported. Nakamura and Ueyama[8,9] have prepared $Mo^{VI}O_2(SC_6H_4S)_2$ and $Mo^{IV}O(SC_6H_4S)_2$, which contain oxomolybdenum units and *two* benzenedithiolate ligands. Here we describe the preparation and properties of $LMo^{V}O(SC_6H_4S)$,[10] the first structurally characterized monooxomolybdenum(V) complex that possesses a *single* dithiolene ligand, the *minimum structural feature* for the Mo(V) state of the postulated molybdenum cofactor.

The preparation and characterization[11] of $LMo^{V}O(SC_6H_4S)$ followed previous methods.[12] An X-ray structure determination[13] (Figure 1) revealed distorted six-coordinate stereochemistry about the molybdenum atom, with the oxo group cis to the two sulfur atoms. The two Mo–S distances are slightly shorter than the average Mo–S distance (2.39 Å) in other six-coordinate oxomolybdenum(V) complexes.[12,14,15] The Mo–S distances obtained from the EXAFS studies of the Mo(V) low pH form of sulfite oxidase are in the range of 2.38–2.39 Å.[3]

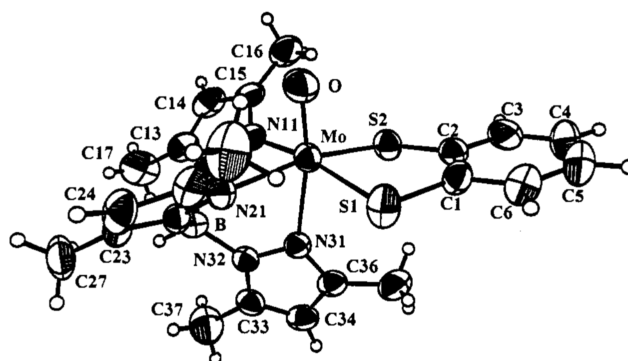

Figure 1. ORTEP drawing of $LMoO(SC_6H_4S)$; the sizes of H atoms have been reduced for clarity. Selected bond lengths (Å) and angles (deg) are as follow: Mo–S1, 2.368(2); Mo-S2, 2.379(2); Mo-O, 1.678(4); Mo-N11, 2.179(5); Mo-N21, 2.178(4); Mo-N31, 2.372(4); S1-Mo-S2, 85.12(6); S1-Mo-O, 101.2(2); S2-Mo–O, 100.7(1).

The X-band frozen EPR spectrum[16] and the simulated[17] spectrum of $LMo^{V}O(SC_6H_4S)$ exhibit a rhombic **g** matrix and an unusual $A(^{95,97}Mo)$ matrix that consists of two large components on the wings of the spectrum and one small component in the center (Table 1 and supplementary material). The similarity of the EPR parameters of $LMo^{V}O(SC_6H_4S)$ and $LMo^{V}O(SCH_2CH_2S)$ shows that sulfur ligation rather than the unsaturated chelate skeleton of the dithiolene ligand is the primary cause of the large g_1 value of these model compounds.[18] In the absence of single crystal EPR data, it is not possible to relate the components of the **g** and $A(^{95,97}Mo)$ matrices to the molecular structure.

The qualitative similarity between the EPR spectrum of $LMo^{V}O(SC_6H_4S)$ and the spectrum of the low pH form of sulfite oxidase in D_2O[19] prompted us to reinvestigate the EPR spectrum of purified chicken liver sulfite oxidase[20] under these conditions.[21] The EPR spectrum observed here agrees[22] with that originally reported, and the calculated g values are consistent with those from other studies.[23] However, simulation of the $A(^{95,97}Mo)$ values (Figure 2) gives quite different results (Table 1) from those originally reported.[19] Experimental determination of $\langle g \rangle$ and $\langle A \rangle$ is not possible due to the slow tumbling rate of sulfite oxidase in solution; therefore, $\langle g \rangle$ and $\langle A \rangle$ were obtained by averaging the respective anisotropic **g** and $A(^{95,97}Mo)$ components.

Comparison of the EPR parameters for $LMo^{V}O(SC_6H_4S)$ and $LMo^{V}O(SCH_2CH_2S)$ to those of the low pH form of sulfite oxidase (Table 1) reveals that these *minimal structural models* for the molybdenum cofactor reproduce g_1 and g_2 of the enzyme, and that the g_3 values of the models are only slightly smaller. The A_1 values for the models are also similar to those for the enzyme.

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(13) The compound forms monoclinic crystals ($P2_1/n$) with a = 10.727(1) Å, b = 14.673(2) Å, c = 15.887(2) Å, β = 100,317(4)°, V = 2460(3) Å^3, and Z = 4. Final refinement using 2708 reflections with $F_o^2 > 2.0\sigma(F_o)^2$ gave R = 0.041 and R_w = 0.051. Structural details are available in the supplementary material.

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(16) EPR spectra were obtained at 77 K with a Bruker ESP 300E spectrometer operating at X-band (ca. 9.1 GHz). Frequencies were measured with an Systron Donner-6530 frequency counter. Samples were prepared as 1 mM solutions in a toluene glass.

(17) Simulation of the frozen solution EPR spectrum was done using a modified version of the program QPOW written by Prof. R. L. Belford and co-workers. The I = $^5/_2$ ($^{95,97}Mo$ isotopes, 25% abundant) and I = 0 ($^{92,94,96,98}Mo$ isotopes, 75% abundant) components were simulated separately and then summed to obtain the complete spectrum.

(18) A low-lying $S(\pi) \rightarrow Mo(d_{xy})$ charge transfer state will give rise to large g values:[12] Carducci, M. D.; Brown, C.; Solomon, E. I.; Enemark, J. H., submitted for publication.

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(21) Initial EPR studies of sulfite oxidase in D_2O at pD 7.0 showed a nearly axial spectrum with distinct $A(^{95,97}Mo)$ hyperfine splittings. The EPR parameters were not simulated but taken directly from the observed spectrum (Figure 12, ref 19) as $g_{\parallel}$ = 2.000, $g_{\perp}$ = 1.968, $A_{\parallel}$ = 58.3 × 10^{-4} cm^{-1}, and $A_{\perp}$ = 43.0 × 10^{-4} cm^{-1}.

(22) Chicken liver sulfite oxidase (SO) was purified by the method of Sullivan *et al*;[20] the enzyme used for the EPR experiment showed a heme to protein absorbance ratio (A_{414}/A_{280}) of 0.81.

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Table 1. EPR Data

	g_1	g_2	g_3	$\langle g \rangle$	A_1[a]	A_2	A_3	$\langle A \rangle$	α_{xy}	α_{xz}	ref
$LMoO(SC_6H_4S)$	2.004	1.972	1.934	1.971	50.0	11.4	49.7	37.0	0	0	this work
$LMoO(SCH_2CH_2S)$	2.018	1.970	1.939	1.975	54.6	3.3	45.5	34.5	0	0	this work
L'MoO(OH)	1.980	1.947	1.944	1.957	64.0[c]	29.0	24.5	39.2	0	26	25
sulfite oxidase (low pH form)	2.003	1.972	1.965	1.980	58.9						23
sulfite oxidase (low pH form)	2.007	1.974	1.968	1.983	56.7	25.0	16.7	32.8	0	18	this work
sulfite oxidase (high pH form)	1.987	1.964	1.953	1.968	51.0						23
desulfoxanthine	1.971	1.965	1.954	1.963	65.4[c]	26.2	27.1	39.6	0	33	25
$[MoO(SPh)_4]^-$	2.017[d]	1.979[e]		1.990	52.6[f]	23.0[g]		31.5	0	0	28

[a] A(^{95,97}Mo, $\times 10^{-4}$ cm.$^{-1}$). [b] α_{xy} is the angle (deg) of rotation about the z axis; α_{xz} is that about the new y axis. [c] A(^{95}Mo, $\times 10^{-4}$ cm^{-1}). [d] $g_\parallel$. [e] $g_\perp$. [f] $A_\parallel$. [g] $A_\perp$.

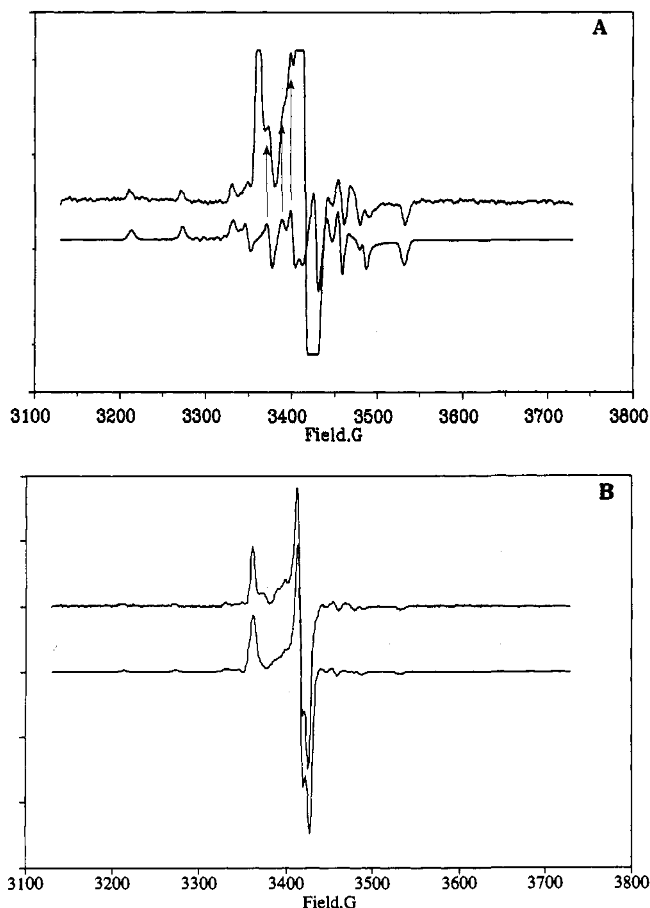

Figure 2. (A) Experimental frozen X-band (top) and simulated ($I = {}^5/_2$ component only, bottom) EPR spectra of sulfite oxidase. The arrows show that the simulated $I = {}^5/_2$ components in the central region match weak spectral details observed in the experimental spectrum (top) that is dominated by the $I = 0$ species. (B) Experimental frozen X-band (top) and composite simulated (bottom) EPR spectra of sulfite oxidase. See refs 16 and 17 for details.

However, the large–small–large pattern of the A(^{95,97}Mo) components in the models is distinctly different from that in the low pH form of sulfite oxidase, whose A(^{95,97}Mo) components are reported here for the first time. The pattern of A components observed for $LMo^VO(SC_6H_4S)$ is also different from the pattern of one large component and two similar moderate components observed for $L'Mo^VO(OH)$,[24,25] a compound that also contains two trans sulfur donor atoms and whose EPR spectrum mimics that of desulfoxanthine oxidase.[25,26]

We reemphasize that $LMo^VO(SC_6H_4S)$ is a minimal structural and spectroscopic benchmark for the proposed oxodithiolene coordination of the molybdenum cofactor. The compound is not a reactivity model because its six-coordinate stereochemistry precludes it from participating in a catalytic cycle of oxygen atom transfer and coupled electron–proton reactions as has been observed for $LMo^{VI}O_2(SPh)$ and related complexes.[27]

In summary, the EPR spectrum of $LMo^VO(SC_6H_4S)$ resembles that shown previously for the low pH form of sulfite oxidase in D_2O,[19] but our reinvestigation and simulation of the EPR spectrum of the enzyme shows that the A_2 and A_3 values for the enzyme are quite different from those of the minimal structural models for the molybdenum cofactor. Indeed, these new values for A_2 and A_3 provide an additional stringent test for spectroscopic mimics of the molybdenum center of sulfite oxidase. In this regard, it is interesting to note that the current values of g_1, A_1, A_2, $\langle g \rangle$, and $\langle A \rangle$ for the low pH form of sulfite oxidase are very similar to these parameters in $[Mo^VO(SPh)_4]^-$.[28] This observation raises the possibility that there may be four sulfur atoms present at the active site of the low pH form rather than two or three as proposed from EXAFS studies.[3] Finally, it is well known from EPR and EXAFS studies that the spectroscopic properties of the Mo(V) center of sulfite oxidase are sensitive to changes in both anions and pH.[3,23,29] This study underscores the need for detailed EPR experiments on catalytically active molybdoenzymes under a variety of conditions and for single crystal EPR studies on low-symmetry mononuclear oxomolybdenum(V) complexes with sulfur donor ligands.

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Supplementary Material Available: Details of the X-ray structure determination (experimental and simulated ($I = 0$ component), EPR spectra for the low pH form of sulfite oxidase and $LMoO(SC_6H_4S)$, and spectroscopic and analytical data for $LMoO(SC_6H_4S)$ (11 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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