# X-ray Absorption Spectroscopy of Dimethyl Sulfoxide Reductase from *Rhodobacter sphaeroides*

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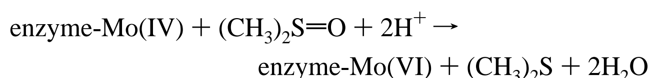
**Abstract:** X-ray absorption spectroscopy at the molybdenum K-edge has been used to probe the molybdenum coordination of *Rhodobacter sphaeroides* dimethyl sulfoxide reductase. The molybdenum site of the oxidized protein possesses a novel Mo(VI) mono-oxo site (Mo═O at 1.68 Å) with additional coordination by approximately four thiolate ligands at 2.44 Å and probably one oxygen or nitrogen at 1.92 Å. The reduced Mo(IV) form of the enzyme is a des-oxomolybdenum with 3−4 thiolates at 2.33 Å and two different Mo−O/N ligands at 2.16 Å and 1.92 Å. Similarly, the stable Mo(V) glycerol-inhibited species is found to be a des-oxomolybdenum with approximately four thiolate ligands at 2.40 Å and (probably) two similarly coordinated oxygen or nitrogen ligands at 1.96 Å.

## Introduction

The molybdenum enzymes comprise a group with quite diverse functionality, catalyzing a variety of two-electron redox reactions coupled to the transfer of an oxygen atom.[1−3] All molybdenum enzymes described to date contain a novel pterin-molybdenum cofactor in which the molybdenum is thought to be bound by a dithiolene side-chain of the pterin ring.[1] Until very recently,[4] the available structural information on molybdenum enzymes has derived almost entirely from spectroscopy of the enzymes and model compound systems.[2,3] The dimethyl sulfoxide (DMSO) reductases from *Rhodobacter sphaeroides*[5] and *Rhodobacter capsulatus*[6] are unique among the molybdenum enzymes reported to date in that they possess the molybdenum cofactor as the sole prosthetic group. DMSO reductases catalyze the reduction of dimethyl sulfoxide to dimethyl sulfide



$$\text{enzyme-Mo(IV)} + (CH_3)_2S{=}O + 2H^+ \rightarrow \text{enzyme-Mo(VI)} + (CH_3)_2S + 2H_2O$$

In *R. sphaeroides*, DMSO reductase is the terminal enzyme in the respiratory chain of the bacterium grown anaerobically in the presence of electron acceptors such as dimethyl sulfoxide or trimethylamine *N*-oxide.[7] The enzyme is a monomeric protein of molecular weight 86 000,[7] and has a molybdopterin cofactor which is subtly different from that of many other proteins,[1,8] with the pterin side chain attached to 5′GMP through a pyrophosphate linkage.[8] The enzyme has been the subject of studies using EPR and optical spectroscopy,[9,10] magnetic circular dichroism,[11] and resonance Raman spectroscopies.[12,13] Recent denaturation experiments suggest that the pterin is in the 5,8-dihydro oxidation level.[13] DMSO reductase is unique among the molybdenum enzymes investigated to date in that it does not contain any distinct redox-active sites other than molybdenum.[8]

We present herein the results of an X-ray absorption spectroscopic (XAS) study of the molybdenum domain of *R. sphaeroides* DMSO reductase. We find that the molybdenum of the oxidized Mo(VI) protein is a novel mono-oxo site, coordinated by approximately four thiolate ligands, and possibly by a single long oxygen or nitrogen. We also investigate two different reduced forms of the protein, which possess a des-oxo Mo(IV) and Mo(V).

## Materials and Methods

**Samples.** *R. sphaeroides* DMSO reductase was purified as previously described.[14] DMSO reductase samples were prepared at approximately 2.4 mM Mo in 25 mM *N*-tris[hydroxymethyl]methylglycine, pH 7.5, and frozen in 10 mm × 10 mm × 3 mm lucite sample cuvettes. Enzyme in the Mo(VI) and Mo(IV) forms was prepared by air-oxidation and by reduction with a two-fold excess (per Mo) of aqueous dithionite, respectively. The Mo(V) glycerol-inhibited form was prepared in the presence of stoichiometric reduced benzyl viologen and an excess (50% v/v) of glycerol as described by Finnegan *et al.*[11] Electron paramagnetic resonance (EPR) spectroscopy and data reduction were performed as described by George *et al.*[15]

**XAS Data Collection.** XAS measurements were carried out at the Stanford Synchrotron Radiation Laboratory with the SPEAR storage ring containing 55−90 mA at 3.0 GeV.

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Molybdenum K-edge data were collected on beamline 7−3 using a Si(220) double crystal monochromator, with an upstream vertical aperture of 1 mm, and a wiggler field of 1.8 T. Harmonic rejection was accomplished by detuning one monochromator crystal to approximately 50% off peak, and no specular optics were present in the beamline. The incident X-ray intensity was monitored using an argon-filled ionization chamber, and X-ray absorption was measured as the X-ray Mo $K_\alpha$ fluorescence excitation spectrum using an array of 13 germanium intrinsic detectors[16] together with a zirconium metal filter (3 absorption-length thickness) and Soller slit assembly. During data collection, samples were maintained at a temperature of approximately 10 K, using an Oxford Instruments liquid helium flow cryostat. For each sample ten 35 min scans were accumulated, and the absorption of a molybdenum metal foil was measured simultaneously by transmittance. The energy was calibrated with reference to the lowest energy inflection point of the molybdenum foil, which was assumed to be 20 003.9 eV.

**Data Analysis.** The extended X-ray absorption fine structure (EXAFS) oscillations $\chi(k)$ were quantitatively analyzed by curve-fitting using the EXAFSPAK suite of computer programs[17] as described by George *et al.*[18]

## Results and Discussion

**Electron Paramagnetic Resonance Spectroscopy.** Finnegan *et al.*[11] have reported a stable Mo(V) complex of DMSO reductase, corresponding to approximately 80% of the total molybdenum, which can be formed by reaction of reduced protein (obtained using reduced benzyl viologen) and 50% v/v glycerol. Enzyme thus treated remains inactive in air, and Finnegan *et al.*[11] have suggested an analogy with the stable Mo(V) desulfo-inhibited species of desulfo xanthine oxidase,[2] which contains glycol covalently bound to the Mo,[19] probably via both oxygens of the diol.[2,19] Bennet *et al.*[9] have investigated the EPR of a similar species (which they called High-g unsplit) obtained using dithionite reduction and ethylene glycol in place of glycerol, although this integrated to only 7% Mo(V) with negligible loss in enzyme activity. We find that preparations using dithionite as a reductant rather than reduced benzyl viologen did not yield appreciable quantities of the DMSO reductase glycerol-inhibited Mo(V) species, while the use of reduced benzyl viologen gave rise to the EPR spectrum shown in Figure 1, which integrated to 85% Mo(V).[21] The reaction thus appears to be sensitive to the nature of the reductant and probably to whether glycerol or glycol is used as well. In agreement with Bennet *et al.*,[9] we find that the signal did not change significantly upon exchange into $D_2O$ buffer and conclude that it lacks resolved hyperfine coupling from exchangeable protons.

**Near-Edge Spectra.** Figure 2 shows the Mo K-edge spectra of the three forms of DMSO reductase investigated. The spectra are broadly similar, with subtle differences that are highlighted by the second derivative plot shown in the inset. The Mo(IV) spectrum is shifted by approximately 1.5 eV to lower energy than the Mo(VI) (see the second derivative plot), consistent with a lower oxidation state for molybdenum, while the Mo(V) spectrum is more subtly shifted (perhaps 0.5 eV), consistent with an intermediate oxidation state.

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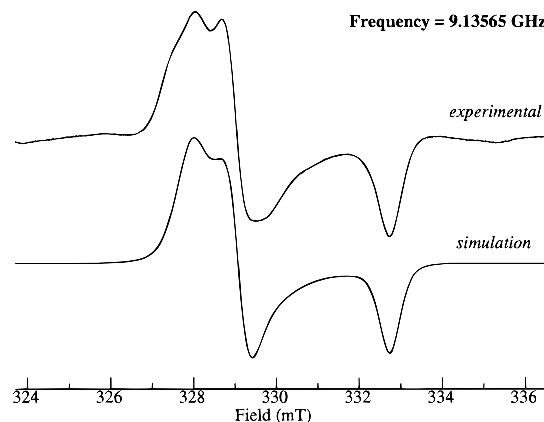


**Figure 1.** Mo(V) EPR spectrum of the glycerol-inhibited enzyme. The upper trace shows the experimental spectrum and the lower trace shows a simulation calculated with the following parameters $g$(x,y,z) = 1.9617, 1.9836, 1.9905, and half-linewidths (lineshape was pseudo-Voigt with a mixing of 0.40) of 0.30, 0.32 and 0.45 mT. The shoulder at approximately 327.6 mT on the high-field side of $g_z$ is probably due to hyperfine structure from the 25 % of $I$=5/2 isotopes ($^{95}$Mo and $^{97}$Mo)[19,20] which are not included in the simulation. Spectra were recorded at a sample temperature of 120 K using 0.16 mT modulation amplitude.

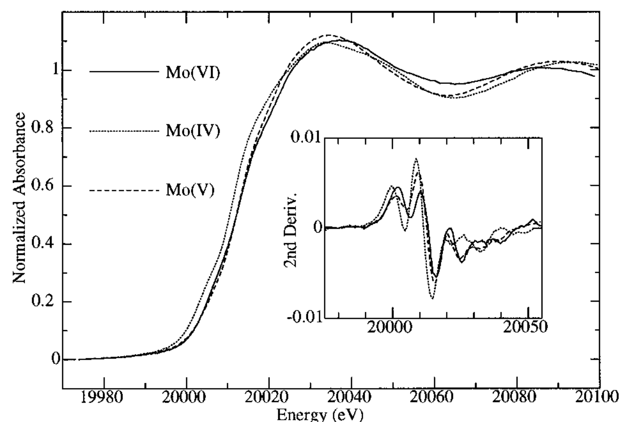


**Figure 2.** Mo K-edge X-ray absorption near edge spectra of DMSO reductase of the native enzyme in the Mo(IV) and Mo(VI) oxidation states, and of the Mo(V) glycerol-inhibited enzyme. The inset shows the respective derivative spectra.

None of the spectra have the pronounced pre-edge feature at about 20 008 eV that is commonly observed in other molybdenum enzymes.[18,22] This so-called oxo-edge feature is characteristic of a species possessing Mo═O groups (or to a lesser extent Mo═S); it arises from formally dipole forbidden 1s → 4d bound-state transitions to antibonding orbitals directed principally along Mo═O bonds.[23] The weak presence of this feature here argues for a low number (*i.e.*, one or zero) of these ligands in the DMSO reductase samples.

**EXAFS Spectra.** Figure 3 shows the EXAFS spectra, the best fits, and the corresponding Fourier transforms of the DMSO reductase in the Mo(VI), Mo(IV) oxidation states and of the glycerol-inhibited Mo(V) species. The results of the curve-fitting analyses are summarized in Table 1.

The curve-fitting results unambiguously indicate that the oxidized Mo(VI) enzyme contains a novel mono-oxo molybdenum site. While mono-oxo Mo(VI) complexes are certainly unusual, they are quite well-known in the extensive coordination

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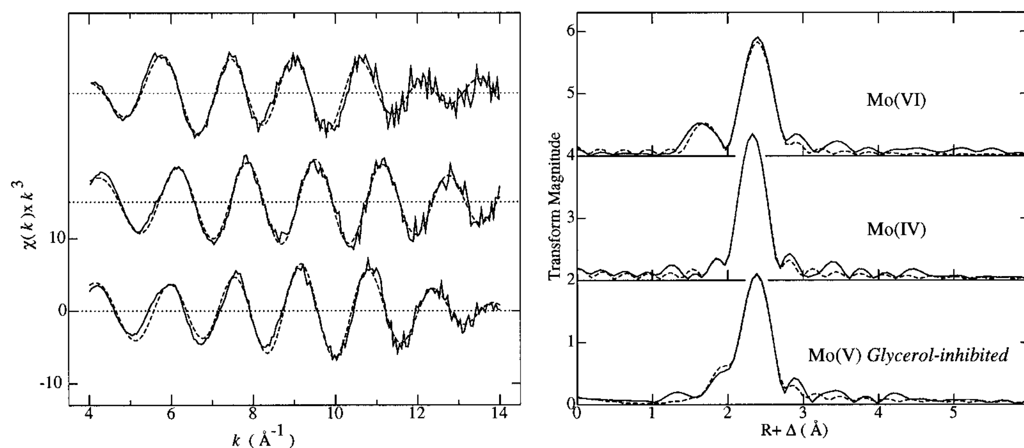


**Figure 3.** EXAFS curve-fitting results. The left panel shows the EXAFS spectra (solid lines) and best-fits (broken lines) of DMSO reductase in the Mo(VI) and Mo(IV) oxidation states, and of the Mo(V) glycerol-inhibited enzyme. The right panel shows the EXAFS Fourier transforms phase-corrected for Mo-S backscattering. The small peak at R+Δ ≈ 1.7 Å in the Fourier transform of the Mo(VI) sample arises from Mo=O EXAFS, while the larger peak at R+Δ ≈ 2.4 Å arises from Mo-S and, to a lesser extent, Mo-O backscattering.

chemistry of molybdenum; Mayer[24] has surveyed bond lengths for a large number of molybdenum oxo species, and his weighted mean of 1.67 Å of 33 Mo(VI) mono oxo complexes compares well with our estimate of 1.68 Å for the Mo=O bond length in oxidized DMSO reductase. The oxidized molybdenum site is also ligated by approximately four thiolate ligands, with Mo−S bond lengths of 2.44 Å, and probably a single Mo−O or Mo−N at 1.92 Å (Table 1). Our estimate of the number of thiolate ligands is larger by a factor of 2 than for xanthine oxidase, consistent with recent chemical analysis of DMSO reductase indicating two molybdopterins per molybdenum,[14] perhaps suggesting a molybdenum bis-pterin dithiolene coordination similar to that observed for tungsten by X-ray crystallography in *Pyrococcus furiosus* aldehyde oxidoreductase.[25]

The reduced Mo(IV) enzyme contains a des-oxo molybdenum, also with three or four thiolate ligands at 2.33 Å, and the absence of Mo=O interactions is evidenced by the lack of a peak at about 1.7 Å in the EXAFS Fourier transform shown in Figure 3. A significant improvement in the fit to the Mo(IV) DMSO reductase EXAFS was achieved by including two different Mo−O/N interactions (Table 1), and it seems probable that one of these originates from a Mo−OH ligation arising from protonation of the Mo=O of the oxidized enzyme. This Mo−OH ligand provides a candidate for the hyperfine coupling to one exchangeable proton that is observed in the so-called High-g split Mo(V) species.[5,9] We note that while three Mo−S ligands do give a marginally better fit than four (Table 1), the improvement in the fit is within the margin of uncertainty of the technique.

The structure of the glycerol-inhibited Mo(V) DMSO reductase, like that of the Mo(IV) enzyme, is a des-oxomolybdenum site. The curve-fitting results indicate four thiolate ligands at 2.40 Å and probably two Mo−O with similar bond lengths of 1.96 Å (Table 1). The lack of any resolved hyperfine interaction to exchangeable protons in the EPR spectrum probably indicates that a Mo−OH group is not present in the signal giving species (although caution must be exercised in forming this conclusion—see George).[26] Additionally, as the inhibition of the glycerol-inhibited Mo(V) species is irreversible (except in the presence of reductant and DMSO), it seems likely that glycerol is covalently bound to molybdenum in the signal giving species, either as a monodentate or as a bidentate ligand. While the hypothesis of a bidentate ligand is highly plausible it is noteworthy that glycerol somewhat resembles dimethyl sulfoxide in shape, and a monodentate coordination to molybdenum via the central −OH is also possible. Such ligation has been suggested by Bennet *et al.*,[9] but due to the presence of two very similar Mo−O bond lengths we prefer the bidentate ligation shown in Figure 4.

Figure 4 shows possible structures for the three forms of DMSO reductase investigated. While the EXAFS data provides no direct information about ligand geometry, in the oxidized Mo(VI) enzyme (Figure 4A), we do not expect the Mo−O to be *trans* to the Mo=O as the bond length of 1.92 Å is rather shorter than would normally be expected from *trans* effects from Mo=O. Similarly, for Mo−S bonds *trans* to the Mo=O long bond lengths of the order of 2.6 Å would be expected.[27] No such interactions were detected; however, we note that such long bonds may not be readily detectable by EXAFS due to large Debye−Waller factors,[28] and given the tentative nature of the longer of the two proposed O/N ligands (see Table 1) and the uncertainty in absolute coordination numbers derived from EXAFS (*ca.* 20%), one or more ligands may differ from the structures given in Figure 4. If the Mo(VI) site does indeed contain four 2.44 Å sulfur ligands, as the EXAFS curve-fitting seems to indicate, plus one Mo−O which is too short to be trans to the Mo=O, then it seems possible that the active site might resemble the distorted trigonal prismatic site proposed for tungsten in the related *P. furiosus* aldehyde oxidoreductase.[25]

Comparison of the EXAFS curve-fitting results shows a systematic decrease in Mo−S bond length on reduction of the active site (Table 1). A significant decrease in Mo−S bond lengths between the Mo(VI) and Mo(V) forms is expected because of the loss of the short Mo=O bond of the Mo(VI) species; however, if we assume a similar coordination for the Mo(V) and Mo(IV) sites, then the observed trend of even shorter bonds in the Mo(IV) form is contrary to expectations. Model compound work [*e.g.*, ref 29] indicates that slightly longer bond lengths are in fact anticipated for the Mo(IV) species, and it thus seems possible that the fully reduced species undergoes a

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**Table 1.** EXAFS Curve Citting Results[a]

| sample | Mo=O | | | Mo−S | | | Mo−O/N[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $N$ | $R$ | $\sigma^2$ | $N$ | $R$ | $\sigma^2$ | $N$ | $R$ | $\sigma^2$ | error[c] |
| Mo(VI) | 1 | 1.677(6) | 0.0035(6) | 3 | 2.434(1) | 0.0026(1) | | | | 0.359 |
| | 1 | 1.676(6) | 0.0039(7) | 4 | 2.435(1) | 0.0041(1) | | | | 0.348 |
| | 1 | 1.676(7) | 0.0041(7) | 5 | 2.437(2) | 0.0054(2) | | | | 0.369 |
| | **1** | **1.683(6)** | **0.0033(6)** | **4** | **2.435(1)** | **0.0041(1)** | **1** | **1.920(15)** | **0.0067(17)** | **0.323**[d] |
| | 1 | 1.680(4) | 0.0018[e] | 4 | 2.434(1) | 0.0040(1) | 1 | 1.898(15) | 0.0073(19) | 0.331 |
| | 2 | 1.690(4) | 0.0018[e] | 4 | 2.433(2) | 0.0039(2) | 1 | 1.866(9) | 0.0015(7) | 0.457 |
| Mo(IV) | | | | 3 | 2.332(1) | 0.0017(1) | 1 | 1.903(8) | 0.0054(9) | 0.239 |
| | | | | 4 | 2.333(1) | 0.0032(1) | 1 | 1.910(5) | 0.0033(6) | 0.237 |
| | | | | 5 | 2.333(1) | 0.0045(1) | 1 | 1.915(5) | 0.0020(5) | 0.279 |
| | | | | **3** | **2.333(2)** | **0.0022(2)** | **1** | **2.160(17)** | **0.0037(15)** | **0.221**[d,f] |
| | | | | | | | **1** | **1.921(8)** | **0.0040(9)** | |
| | | | | 4 | 2.329(21) | 0.0029(10) | 1 | 2.303(101) | 0.0052(31) | 0.226 |
| | | | | | | | 1 | 1.913(8) | 0.0038(7) | |
| Mo(V) | | | | 3 | 2.399(1) | 0.0020(1) | 1 | 1.948(6) | 0.0025[e] | 0.275 |
| | | | | 3 | 2.399(1) | 0.0020(1) | 2[g] | 1.951(3) | 0.0030(3) | 0.278 |
| | | | | 4 | 2.400(1) | 0.0035(1) | 1 | 1.953(6) | 0.0025[e] | 0.277 |
| | | | | **4** | **2.399(1)** | **0.0035(1)** | **2**[g] | **1.955(3)** | **0.0029(3)** | **0.255**[d] |
| | | | | 5 | 2.399(1) | 0.0048(1) | 2 | 1.957(3) | 0.0027(3) | 0.280 |
| | | | | 4 | 2.399(1) | 0.0034(1) | 3 | 1.954(4) | 0.0056(4) | 0.300 |

[a] Coordination number $N$, interatomic distance $R$ (Å), and (thermal and static) mean-square deviation in $R$ (the Debye−Waller factor) $\sigma^2$ (Å$^2$). The values in parentheses are the estimated standard deviations (precisions) obtained from the diagonal elements of the covariance matrix. We note that the accuracies will always be somewhat larger than the precisions, typically ±0.02 Å for R and ±20% for $N$ and $\sigma^2$. [b] Note that EXAFS cannot readily distinguish between scatterers of similar atomic number, such as chlorine and sulfur or nitrogen and oxygen. [c] The fit-error is defined as $\sum k^6(\chi_{\mathrm{exptl}} - \chi_{\mathrm{calcd}})^2/\sum k^6\chi_{\mathrm{exptl}}{}^2$. [d] Fits shown in bold type-face represent the best fit obtained for the sample. [e] The value of $\sigma^2$ was restricted to a chemically reasonable value (0.0018 Å$^2$ for a Mo=O or 0.0025 Å$^2$ for a Mo−O). [f] The improvement in the fit error upon introduction of a second Mo−O interaction is rather subtle, and we note the detection of the presence of this scatterer is correspondingly tentative. We also note that the number of parameters that can be determined is less than the number of independent points which in the present case we conservatively estimate to be ≥8 (using the well-known Nyquist sampling theorum). [g] Fits using two different Mo−O shells converged to the same values of $N$, $R$, and $\sigma^2$.

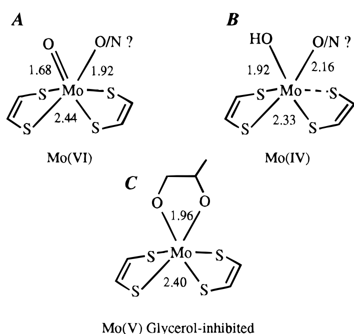


**Figure 4.** Possible structures of DMSO reductase in Mo(IV) and Mo(VI) oxidation states, and of the Mo(V) glycerol-inhibited enzyme. We note that EXAFS provides no direct information about geometry, and the strucrtures postulated are thus tentative. The Mo(VI) from ***A*** might have a trigonal prismatic geometry similar to that proposed for the tungsten site of the related *P. furiosus* aldehyde oxidoreductase by X-ray crystallography.[25] The Mo(IV) form ***B*** may have a decreased Mo-S coordination (see discussion) suggested by a decrease in the average Mo-S bond relative to the Mo(V) form, and the broken line in the figure is meant to suggest a longer Mo···S not detected by EXAFS. Additionally, the assignment of the proton to the shorter (1.92 Å) Mo-O/N ligand in ***B*** is arbitrary, and it might equally well reside on the longer of the two non−sulfur ligands (again as −OH).

significant change in coordination upon reduction. Indeed, as noted above, three Mo−S ligands to the Mo(IV) site do give a marginally better fit than four (although the improvement of the fit is within the uncertainty of the technique), and a reduced Mo−S coordination might provide a possible explanation for this discrepancy in bond lengths. Bond-valence-sum calculations[30,31−33] provide additional support for this hypothesis. For the Mo(VI) and Mo(V) species, the best-fit coordination numbers and bond lengths from Table 1 yield bond-valence-sum values $V$ which are in good agreement with the formal metal oxidation states; $V = 6.0$ and 5.0 for the Mo(VI), and Mo(V) species, respectively. For the Mo(IV) species, if we assume four Mo−S ligands, then we obtain $V = 5.1$, alternatively with three Mo−S ligands we find $V = 4.1$, in good agreement with the metal oxidation state. The observed bond length trends thus suggest a significant change in coordination environment for the fully reduced Mo(IV) species.

We note in passing that the EXAFS of DMSO reductase show some similarity to those reported for *Escherichia coli* nitrate reductase by George *et al.*,[34] which contains a mono-oxo Mo-(VI) site and a des-oxo Mo(IV), with significant thiolate ligation. This enzyme differs from DMSO reductase in exhibiting significant anion effects[9,34,35] which are reflected in the EXAFS.[34]

Our results have implications with respect to EPR studies of DMSO reductase. In an elegant study, Bray and co-workers[9] have shown that *R. capsulatus* DMSO reductase is capable of producing four main types of EPR signal which they called Low-g type-1, Low-g type-2, High-g split, and High-g unsplit. The High-g unsplit signals are similar to the glycerol-inhibited signal of Figure 1 and are probably all inhibited states. The High-g split signal is that studied earlier by Bastian *et al.*[11] and

(30) This empirical treatment assumes that the valence $V$ of an atom can be expressed as a sum of individual bond valences; $V = \sum_j s_j$. In order to the evaluate the $s_j$ we have used the more commonly adopted empirical relationship $s_j = \exp[(R_{0j} - R_j)/b]^{31}$ in which $R_{0j}$ is the bond valence parameter, and $b$ is a "universal" constant with a value of 0.37 Å. In our calculations we have used the values of $R_{0j}$ tabulated by Brese and O'Keeffe[32] with corrections for metal oxidation state where appropriate as described by Brown and Altermat[31], and assuming that the non-sulfur ligands are oxygens (rather than nitrogen).

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(34) George, G. N.; Turner, N. A.; Bray, R. C.; Morpeth, F. F.; Boxer, D. H.; Cramer, S. P. *Biochem. J.* **1989**, *259*, 693−700. Note that an earlier study on enzyme prepared using a different purification method gave quite different EXAFS results [Cramer, S. P.; Solmonson, L. P.; Adams, M. W. W.; Mortenson, L. E. *J. Am. Chem. Soc.* **1984**, *106*, 1467−1471]. The earlier study reported long-distance interactions to an unknown scatterer at about 2.7 Å, and it seems likely that the active site can adopt at least two different structures.

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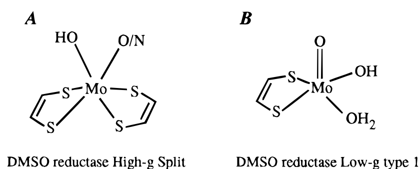


**Figure 5.** Possible structures of the EPR signal-giving species for the High-g split (***A***) and the Low-g type-1 EPR signals (***B***). The Mo-OH ligand on both ***A*** and ***B*** bears the strongly coupled exchangeable protons observed in both EPR signals, while the Mo-$OH_2$ (which might also be Mo-OH) bears the second set of weakly coupled exchangeable protons observed for the Slow signal.[37]

by Benson *et al.*[10] Spectroscopically, it closely resembles the low-pH signals of *E. coli* nitrate reductase,[35] although lacking the pH and anion dependency of the latter signals, having very similar *g*-values and isotropic hyperfine coupling to a single exchangeable proton. The Low-g type-1 EPR signal bears a striking resemblance to the slow EPR signals of reduced desulfo xanthine oxidase, with almost identical *g*-values and proton hyperfine coupling, strongly arguing for a similar coordination around Mo(V). This result is particularly noteworthy given the wide range of spin Hamiltonian parameters accommodated by Mo(V) sites in the various forms of the enzymes.[2] The Low-g type-2 is unique to DMSO reductase, has a large *g*-anisotropy, coupling to a single proton, and will not be discussed further here. Bennet *et al.*[9] interpreted their Mo(V) EPR in terms of exchange of a cysteine ligand of the High-g split species with a lighter atom (oxygen or nitrogen) to yield the Low-g species. They also suggest that such changes might possibly be induced by reduction of pterin from dihydro to tetrahydro.

Our work indicates that the High g-split species is almost certainly a des-oxo species as shown in Figure 5A, resembling the structure of the Mo(IV) form of the native enzyme (Table 1, Figure 4). The Low-g type-1 species, on the other hand, must structurally resemble the slow EPR signal-giving species of desulfo xanthine oxidase. The slow EPR signal is also seen in the desulfo form of the aldehyde oxidoreductase from *Desulfovibrio gigas*,[36] the crystal structure of which has recently been solved.[4] In that enzyme the oxidized molybdenum is coordinated to a *single* pterin dithiolene and to three other terminal ligands (Mo═O or Mo−$OH_2$) which are not well resolved by the crystallography.[4] A combination of the crystallographic,[4] EPR [*e.g.*, ref 2 and references therein], and EXAFS information[2,3,22] allows us to postulate the structure shown in Figure 5B for the slow signal giving species. The presence of two sets of exchangeable coupled protons in the slow EPR signal[37] are consistent with the postulated Mo−OH and Mo−$OH_2$ ligands. Thus, it seems very likely that the Low-g type-1 (and probably also the type-2) lacks one of the two pterin-dithiolene ligands (see above) and has gained an oxo ligand. Such major changes in molybdenum ligation indicate a remarkable structural flexibility at the active site of DMSO reductase and suggests that one pterin may be labile. The unusually flexible coordination properties of molybdenum in DMSO reductase thus appear to be in marked contrast with those previously reported for other molybdenum enzymes.[2,3,4,22]

Oxo transfer has been postulated as a mechanism for a range of molybdenum enzymes, and a variety of functional low-molecular weight model systems have been investigated,[38−40] all of which involve redox chemistry between *cis*-dioxo Mo(VI) and mono-oxo Mo(IV) coordination. Much of this work has been focused on determining the catalytic mechanisms of *cis*-dioxo Mo(VI) enzymes such as sulfite oxidase and of the Mo(VI) oxo-sulfido molybdenum hydroxylases such as xanthine oxidase.[41,42] Irrespective of the catalytic mechanisms of other molybdenum enzymes,[43] we believe that DMSO reductase is likely to function by oxo-transfer from substrate to molybdenum. The formation of the very strong Mo═O bond of the mono-oxo Mo(VI) site should provide a considerable thermodynamic driving force for components of the catalyic cycle. The possible lability of one pterin dithiolene ligand (discussed above) may also have an important role in catalysis. The results of on-going crystallographic studies on DMSO reductase (Douglas Rees, personal communication) should provide a solid basis for extending our knowledge of this novel enzyme system.

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(43) While molybdenum *cis* di-oxo enzymes such as sulfite oxidase may indeed function by oxo transfer, there is some doubt about the molybdenum hydroxylases such as xanthine oxidase which contain molybdenum oxo-thiolato active sites. For these enzymes such a mechanism would involve breaking of the strong Mo═O bond, and destabilization of this by Mo═S (as opposed to another Mo═O) is likely to be subtle at best. While concrete evidence does exist for catalytic oxygen exchange with a solvent-exchangeable water in xanthine oxidase [*see*: Hille, R.; Sprecher, H. *J. Biol. Chem.* **1987**, *262*, 10914−10917]; however, direct evidence for a Mo═O transfer mechanism in the molybdenum hydroxylases is lacking.