Structural and Functional Bis(dithiolene)-Molybdenum/Tungsten Active Site Analogues of the Dimethylsulfoxide Reductase Enzyme Family

Booyong S. Lim, Kie-Moon Sung, and R. H. Holm*

Department of Chemistry and Chemical Biology Harvard University, Cambridge, Massachusetts 02138

Received April 6, 2000

Determination of structures of molybdenum oxotransferase/hydroxylase enzymes[1] by the combined results of crystallography and X-ray absorption spectroscopy enables the conception of synthetic analogues designed to disclose the intrinsic structural, electronic, and reactivity properties of the catalytic sites. Members of the dimethylsulfoxide reductase (DMSOR) enzyme family[1a] include, inter alia, DMSOR itself and trimethylamine *N*-oxide reductase (TMAOR) and contain a cofactor with two pterin dithiolene (S_2pd) ligands bound to molybdenum. The DMSOR of *Rhodobacter sphaeroides* (*Rs*) has been shown to function by a direct oxo transfer pathway from isotope labeling[2] and resonance Raman spectroscopy;[3] TMAOR is likely to behave similarly. Redetermination of the X-ray structure of oxidized *Rs* DMSOR by Schindelin and co-workers[4] at higher (1.3 Å) resolution than the initial structure (2.2 Å)[5] reveals two distinct molybdenum environments, the catalytically competent monooxo site $[Mo^{VI}O(O{\cdot}Ser)(S_2pd)_2]$ with symmetric dithiolene binding, and five-coordinate $[Mo^{VI}O_2(O{\cdot}Ser)(S_2pd)]$. Crystallographic[5] and EXAFS[6] results are consistent with the minimal reduced site formulation $[Mo^{IV}(O{\cdot}Ser)(S_2pd)_2]$. The DMSOR of *Rhodobacter capsulatus* (*Rc*) is reported to contain seven-coordinate dioxo and six-coordinate sites in its oxidized (1.82 Å) and reduced (2.8 Å) forms, respectively.[7] A related seven-coordinate site may exist in the TMAOR of *Shewanella massilia* (2.5 Å).[8] Although the issue currently remains open, it is possible that the same or a similar type of site disorder occurs in these enzymes as in *Rs* DMSOR. In pursuing oxo transfer reactivity (Figure 1), we adopt the minimal reaction paradigm $M^{IV} + XO \leftrightarrow M^{VI}O + X$, utilize complexes whose bis(dimethyl)dithiolene ligand structure closely resembles that of the pterin dithiolene,[9,10] and include systems with M = Mo and W in view of the existence of TMAOR isoenzymes.[11] Our first generation of desoxo M(IV) and monooxo M(VI) structural/reactivity analogues utilized the aromatic dithiolene benzene-1,2-dithiolate (bdt) with M = Mo[12] and W.[13]

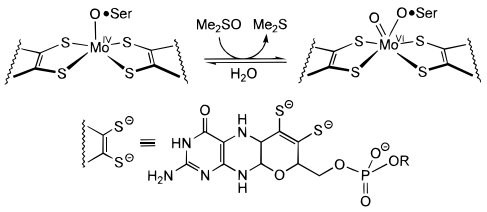


Figure 1. Minimal structures of the desoxo Mo^{IV} and monooxo $Mo^{VI}O$ centers in *Rs* DMSOR, together with the pterin dithiolene cofactor ligand (S_2pd).

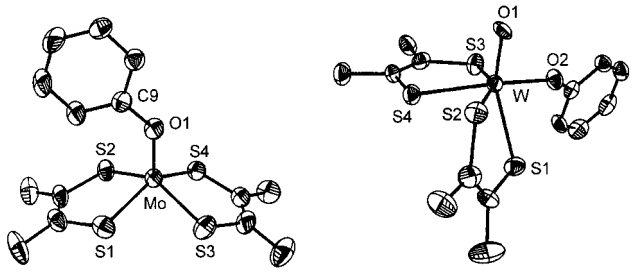


Figure 2. Structures of complexes **1** (left) and **4** (right) with 50% thermal ellipsoids. Selected interatomic distances (Å) and angles (deg): for **1**, Mo−O1 1.895(5), mean Mo−S 2.317(3), mean C−C (ring) 1.33(1), mean C−S 1.768(6), Mo−O1−C9 135.0(4), mean O1−Mo−S 110.1(2); for **4**, W−O1 1.728(3), W−O2 1.994(4), W−S1 2.492(1), mean W−S(2−4) 2.42(1), mean C−C (ring) 1.34(1), mean C−S 1.73(2), O1−W−O2 93.3(2), O1−W−S1 146.0(1), O2−W−S4 154.4(1), S2−W−S3 153.49(5).

Reaction of $[M(CO)_2(S_2C_2Me_2)_2]$[9,14] with 1 equiv each of NaOPh and Et_4NCl in acetonitrile followed by standard workup affords the desoxo complexes $[M^{IV}(OPh)(S_2C_2Me_2)_2]^{1-}$, M = Mo (**1**, brown, 47%) and W (**2**, green-brown, 66%),[10] as Et_4N^+ salts.[15] The complexes are isostructural and possess the square-pyramidal stereochemistry shown for **1**[16] (Figure 2). Related complexes of both metals with isopropoxide and 2-adamantyloxide have been prepared by similar reactions and shown to have square-pyramidal structures; anionic oxygen ligands are intended to simulate serinate binding. In reactions similar to those of bdt complexes,[12,13] treatment of acetonitrile solutions of **1** and **2** with 1−1.5 equiv of Me_3NO yields the monooxo complexes $[M^{VI}O(OPh)(S_2C_2Me_2)_2]^{1-}$, M = Mo (**3,** green) and W (**4**, red-violet, 76%). All attempts to isolate unstable **3** have led to the recovery in high yield of $[Mo^{V}O(S_2C_2Me_2)_2]^{1-}$ (**5**), identified by comparison of absorption and EPR spectra[15] with those of an authentic sample.[9] Complex **3** has been securely established by comparative spectroscopic properties with **4**, including a feature-by-feature red-

(1) (a) Hille, R. *Chem. Rev.* **1996**, *96*, 2757−2816. (b) Romão, M. J.; Knäblein, J.; Huber, R.; Moura, J. J. G. *Prog. Biophys. Mol. Biol.* **1997**, *68*, 121−144. (c) Kisker, C.; Schindelin, H.; Rees, D. C. *Annu. Rev. Biochem.* **1997**, *66*, 233−267.

(2) Schultz, B. E.; Holm, R. H.; Hille, R. *J. Am. Chem. Soc.* **1995**, *117*, 827−828.

(3) Garton, S. D.; Hilton, J.; Oku, H.; Crouse, B. R.; Rajagopalan, K. V.; Johnson, M. K. *J. Am. Chem. Soc.* **1997**, *119*, 12906−12917.

(4) Li, H.-K.; Temple, C.; Rajagopalan, K. V.; Schindelin, H. *J. Am. Chem. Soc.* **2000**, *122*, in press.

(5) Schindelin, H.; Kisker, C.; Hilton, J.; Rajagopalan, K. V.; Rees, D. C. *Science* **1996**, *272*, 1615−1621.

(6) (a) George, G. N.; Hilton, J.; Rajagopalan, K. V. *J. Am. Chem. Soc.* **1996**, *118*, 1113−1117. (b) George, G. N.; Hilton, J.; Temple, C.; Prince, R. C.; Rajagopalan, K. V. *J. Am. Chem. Soc.* **1999**, *121*, 1256−1266.

(7) (a) McAlpine, A. S.; McEwan, A. G.; Shaw, A. L.; Bailey, S. *J. Biol. Inorg. Chem.* **1997**, *2*, 690−701. (b) McAlpine, A. S.; McEwan, A. G.; Bailey, S. *J. Mol. Biol.* **1998**, *275*, 613−623.

(8) Czjzek, M.; Dos Santos, J.-P.; Pommier, J.; Giordano, G.; Méjean, V.; Haser, R. *J. Mol. Biol.* **1998**, *284*, 435−447.

(9) Lim, B. S.; Donahue, J. P.; Holm, R. H. *Inorg. Chem.* **2000**, *39*, 263−273.

(10) Sung, K.-M.; Holm, R. H. *Inorg. Chem.* **2000**, *39*, 1275−1281.

(11) Buc, J.; Santini, C.-L.; Giordani, R.; Czjzek, M.; Wu, L.-F.; Giordano, G. *Mol. Microbiol.* **1999**, *32*, 159−168.

(12) Donahue, J. P.; Goldsmith, C. R.; Nadiminti, U.; Holm, R. H. *J. Am. Chem. Soc.* **1998**, *120*, 12869−12881.

(13) Lorber, C.; Donahue, J. P.; Goddard, C. A.; Nordlander, E.; Holm, R. H. *J. Am. Chem. Soc.* **1998**, *120*, 8102−8112.

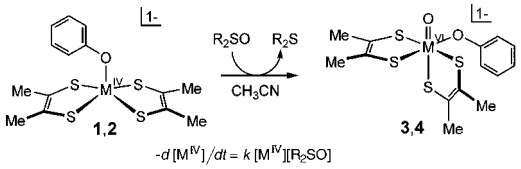
(14) Goddard, C. A.; Holm, R. H. *Inorg. Chem.* **1999**, *38*, 5389−5398.

(15) All reactions and measurements were conducted under anaerobic conditions. Reactions were performed in acetonitrile solutions at ambient temperature. Absorption spectra (acetonitrile): λ_{max} **3**/**4** 395(sh)/337, 475 (sh)/408(sh), 610/514, 795/637 nm. Kinetics parameters for the conversions **1** → **5**, **2** → **4**, and **3** → **5** were obtained from reactions monitored by spectrophotometry. ^{1}H NMR (CD_3CN): δ 2.54 (**1**), 2.61 (**2**), 2.26 (**3**), 2.19 (**4**). Complexes **3** and **4** are fluxional at ambient temperature. IR (KBr): ν_{MO} 917 (^{16}O), 874 (^{18}O) cm^{-1} (**5**); 895 (^{16}O), 848 cm^{-1} (^{18}O) (**4**); EPR (acetonitrile, 298 K): $\langle g \rangle$ = 1.996, $\langle a_{Mo} \rangle$ = 28.8 G (**5**). All (isolated) compounds gave satisfactory elemental analyses.

(16) (a) (Et_4N)[**1**]: monoclinic ($P2_1/c$), a = 17.56(2) Å, b = 8.263(8) Å, c = 18.70(2) Å, β = 102.01(1)°, Z = 4, $R_1(wR_2)$ = 7.00(16.66)%. (b) (Et_4N)[**4**]: monoclinic ($P2_1/c$), a = 10.0883(4) Å, b = 15.8029(6) Å, c = 16.8607(6) Å, β = 98.602(1)°, Z = 4, $R_1(wR_2)$ = 3.61(6.61)%. Data were collected at 213 K with Mo Kα radiation, and structures were solved with direct methods and refined by standard procedures; absorption corrections (SADABS) were applied.

10.1021/ja001197y CCC: $19.00
Published on Web 07/15/2000

Table 1. Kinetics Data for Oxo Transfer Reactions of Mo/W Bis(dithiolene) Complexes with Sulfoxides



M	R_2SO	$k^{298\ K}$ ($M^{-1}\ s^{-1}$)	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta S^{\ddagger}$ (eu)
Mo	$(CH_3)_2SO$	1.3×10^{-6} [a]	14.8(5)	−36(1)
	$(CH_2)_4SO$	$1.5(2) \times 10^{-4}$	10.1(4)	−39(1)
W	$(CH_3)_2SO$	$3.9(4) \times 10^{-5}$	14.4(2)	−30(1)
	$(CH_2)_4SO$	$9.0(3) \times 10^{-4}$	11.6(4)	−33(1)

[a] Obtained from the Eyring plot.

shifted absorption spectrum.[15] In contrast to **3**, (Et_4N)[**4**] is readily isolated as a stable black crystalline solid by addition of ether to the reaction mixture. Acetonitrile solutions of **4** undergo very slow decomposition, yielding $[W^{V}O(S_2C_2Me_2)_2]^{1-}$ (**6**).[14] Complex **4** has a distorted octahedral structure[16] (Figure 2), similar to those of $[MO(OSiPh_2Bu^t)(bdt)_2]^{1-}$ (M = Mo,[12] W[13]), in which the oxo ligand exerts a trans influence on the W−S1 bond length (2.492(1) Å) relative to the other W−S distances (mean 2.42(1) Å). As in the six-coordinate site of *Rs* DMSOR, the two oxygen ligands are cis, but the enzyme site is described as distorted trigonal prismatic with no sulfur atom trans to the oxo ligand.[4] Chelate ring C−C and C−S bond distances point to an enedithiolate ligand description, as is the case for all five-coordinate bis(dithiolene)Mo/W complexes thus far examined.[9,10,14]

Complexes **1** and **2** support oxo transfer reactions of highly variable rates with a variety of molecules XO, including the biological substrates Me_3NO and Me_2SO. Direct oxo transfer was demonstrated in rapid reactions with $Ph_2Se^{18}O$ (62% enriched), which after product isolation gave **5** and **4**, respectively, detected by appropriately shifted ν_{MO} absorptions in their infrared spectra.[15] Reaction of both complexes with 1 equiv of Me_3NO is immediate, thereby allowing identification of **3** in situ[15] before any appreciable decomposition. However, reactions with sulfoxides are relatively slow, even when neat $(CH_2)_4SO$ (**7**) or Me_2SO (**8**) are used as solvents. The clean tungsten conversion $\mathbf{2} + R_2SO \rightarrow \mathbf{4} + R_2S$ was observed in acetonitrile at 290−333 K with both sulfoxides in systems showing a tight isosbestic point at 337 nm. The final spectrum is that of isolated **4** measured separately. Spectrophotometric analysis of reactions conducted under pseudo-first-order conditions affords the kinetics parameters in Table 1. Reactions are second-order; the large negative entropies of activation are consistent with an associative transition state in which, presumably, the phenolate ligand and sulfoxide occupy cis positions in a structure not far removed from that of **4**.

Systems in acetonitrile based on molybdenum complex **1** behave similarly, but react more slowly and with the difference that the final product is **5** rather than **3**. For example, the system **1**/440 equiv of $(CH_2)_4SO$ at 323 K for 8 h formed 92−93% **5** based on absorption spectra and integrated EPR signal intensities. Tetrahydrothiophene was detected by GC. To prove direct oxo transfer to Mo(IV) from a sulfoxide, complex **1** in acetonitrile was treated with 25 equiv of 2-thiaindane *S*-oxide[17] (82% ^{18}O-enriched, from 2-thiaindane *S*-dibromide and $H_2{}^{18}O$) for 2 d. The isolated product was shown to be **5** (30% ^{18}O enrichment) by IR spectroscopy[15] and mass spectrometry. Formation of **5** from **1** in an oxo transfer system is accompanied by the formation of ca. 1 equiv of phenol (^1H NMR). We consider it probable that **3** undergoes an internal redox reaction with the formation of **5** and a phenoxyl radical, which abstracts a hydrogen atom from solvent to form phenol. Under pseudo-first-order conditions at 289−340 K, the slow oxo transfer conversion $\mathbf{1} + R_2SO \rightarrow \mathbf{3} + R_2S$ proceeds followed by the rapid decomposition reaction $\mathbf{3} \rightarrow \mathbf{5} +$ PhOH ($k/[\mathbf{1}] = 0.12(1)\ M^{-1}\ s^{-1}$ at room temperature). The overall transformation $\mathbf{1} \rightarrow \mathbf{5}$ is very clean, with a tight isosbestic point observed at 552 nm; under these conditions, **3** does not accumulate. Kinetics parameters (Table 1) again include $\Delta S^{\ddagger}$ values consistent with an associative pathway. The relative rates $k_7/k_8 \approx 115$ (Mo) and 23 (W) arise because of the sterically less hindered ring structure of $(CH_2)_4SO$ in the transition state.

These results demonstrate that bis(dialkyldithiolene)Mo(IV)/W(IV) complexes with a dithiolene ligand structure and axial ligand closely related to those present in *Rs* DMSOR, and possibly other members of the DMSOR family, sustain direct oxo transfer with biological substrates. The relative rates $k_W/k_{Mo} = 6.0$ ($(CH_2)_4$SO) and ∼30 (Me_2SO) refer to processes in which the metal is oxidized; $k_W > k_{Mo}$ is anticipated from the result that $k_{Mo} \gg k_W$ for the second-order reactions $[M^{VI}O_2(S_2C_2(CN)_2)_2]^{2-} + (RO)_{3-n}R'_nP \rightarrow [M^{IV}O(S_2C_2(CN)_2)_2]^{2-} + (RO)_{3-n}R'_nPO$, in which the metal is reduced.[18] Further, $k_W/k_{Mo} \approx 25$ for the oxidation of $[M^{IV}O(bdt)_2]^{2-}$ by Me_3NO.[19] Rates of reduction of Me_2SO by *Escherichia coli* (*Ec*) DMSOR ($k_{cat}/K_M = 1.9 \times 10^6\ M^{-1}\ s^{-1}$)[20] are much faster than in analogue systems and may arise in part from a protein-induced substrate-bound transition state closer to the distorted trigonal prismatic oxidized enzyme site than is **3**/**4**. The second-order rate constant for reoxidation of Me_2S-reduced *Rc* DMSOR with Me_2SO is $4.3 \times 10^2\ M^{-1}\ s^{-1}$.[21] These analogue systems retain three features of the *Ec* TMAOR isoenzymes: substrate discrimination $k_7/k_8 = 40$ by the tungstoenzyme, $k_W > k_{Mo}$ (ratio 2.3) for reduction of Me_3NO, and a much slower (possibly nil) rate of reduction of $(CH_2)_4SO$ and Me_2SO by the molybdoenzyme.[11] These features suggest active sites similar to the structures of **1**/**2** but subject to modulation by protein structure and environment. Clearly, bis(dithiolene) complexes **1** and **2** are intrinsically reactive to both *N*-oxides and *S*-oxides. Future reports will describe additional structural and reactivity features of bis(dithiolene)Mo/W analogue systems.

Acknowledgment. This research was supported by NSF Grant CHE 98-76457. We thank Prof. H. Schindelin for access to ref 4 prior to publication.

Supporting Information Available: Crystallographic data for the compounds (Et_4N)[**1**] and (Et_4N)[**4**] (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA001197Y

(17) Holland, H. L.; Turner, C. D.; Andreana,. P. R.; Nguyen, D. *Can. J. Chem.* **1999**, *77*, 463−471.
(18) Tucci, G. C.; Donahue, J. P.; Holm, R. H. *Inorg. Chem.* **1998**, *37*, 1602−1608.
(19) Ueyama, N.; Oku, H.; Nakamura, A. *J. Am. Chem. Soc.* **1992**, *114*, 7310−7311.
(20) Simala-Grant, J. L.; Weiner, J. H. *Eur. J. Biochem.* **1998**, *251*, 510−515.
(21) Adams, B.; Smith, A. T.; Bailey, S.; McEwan, A. G.; Bray, R. C. *Biochemistry* **1999**, *38*, 8501−8511.