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Molybdenum and Tungsten Structural Analogues of the Active Sites of the Mo^{IV} + [O] → $Mo^{VI}O$ Oxygen Atom Transfer Couple of DMSO Reductases

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Under the Hille classification scheme for molybdenum oxotransferases,[1] oxidized enzymes with two pterin dithiolene cofactors bound to a $Mo^{VI}X$ unit (X = O, S, Se) are placed in the dimethyl sulfoxide (DMSO) reductase family, named for its prototypic member. Recent crystallographic and EXAFS analyses of oxidized (Mo^{VI}) and reduced (Mo^{IV}) forms of two enzymes of this family, *Rhodobacter sphaeroides*[2,3] (*Rs*) and *Rhodobacter capsulatus*[4,5] (*Rc*) DMSO reductases, together provide structural bases for mechanistic analysis. X-ray and EXAFS results for the *Rs* enzyme agree with one $Mo^{VI}{=}O$ group in the oxidized form, the absence of this group in the reduced form, and serinate ligation in both forms, but are in apparent nonconformity in other structural aspects. In the $[Mo^{VI}O(S_2{\cdot}pterin)_2(O{\cdot}Ser)]$ coordination unit of the *Rs* enzyme, X-ray results indicate one symmetrical (Mo−S 2.4 Å) and one unsymmetrical (Mo−S 2.4, 3.1 Å) dithiolene chelate ring forged with the two pterin cofactors[6] (Figure 1). The symmetrical ligand is retained in the reduced form, but the other dithiolene very weakly interacts with the metal (Mo···S 2.9, 3.7 Å). The EXAFS results show two tightly bound dithiolene ligands (Mo−S 2.44 Å) in the oxidized form and coordination of 3−4 sulfur atoms (Mo−S 2.33 Å) in the reduced form together with two different Mo−O/N interactions. Finally, resonance Raman spectra for this enzyme are consistent with tight binding of both cofactor ligands in the oxidized, reduced, and inhibited forms.[7] The apparent nonuniformity of dithiolene binding in these enzymes renders desirable the synthesis of well-defined active site analogues.

The majority of these results, together with the earlier demonstration using ^{18}O-labeling that an oxygen atom is transferred from substrate to the reduced molybdenum center,[8] lead to the *minimal* oxo transfer reaction couple $[Mo^{IV}(S_2{\cdot}pterin)_2(O{\cdot}Ser)] + Me_2SO \rightarrow [Mo^{VI}O(S_2{\cdot}pterin)_2(O{\cdot}Ser)] + Me_2S$ (Figure 1). The desoxo Mo^{IV} and monooxo Mo^{VI} centers were unanticipated in all earlier oxo transfer systems developed as models for enzymatic reactions.[9] Indeed, synthetic species of these types are far less recurrent than $Mo^{IV}O$ and $Mo^{VI}O_2$ complexes on which the oxo transfer systems are based, including bis(dithiolene)Mo complexes prepared as structural and reactivity models.[10] While it catalyzes a different reaction, we note that an apparently typical member of the principal family of tungsten enzymes,[11] *Pyrococcus furiosus* aldehyde oxidoreductase (AOR), possesses the minimal crystallographic coordination unit $[W(S_2{\cdot}pterin)_2]$, in which there may be an additional one or two oxygen ligands bound.[12] The few known bis(dithiolene)tungsten complexes contain the $W^{VI}O_2$ and $W^{V,IV}O$ units.[13] Here, we disclose synthetic routes to and structures of bis(dithiolene) desoxo M^{IV} and $M^{VI}O$ complexes related to the metal centers in these enzymes.

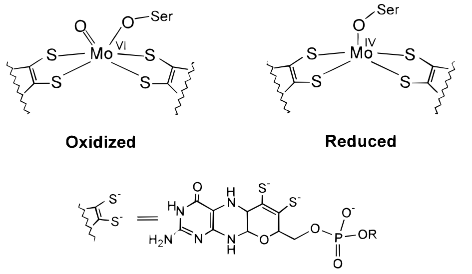

Figure 1. Probable minimal structures of the desoxo Mo^{IV} and monooxo $Mo^{VI}O$ centers in *Rs* DMSO reductase and the pterin dithiolene cofactor (R absent or a nucleotide). Symmetrical chelate rings are depicted; unsymmetrical binding has been detected in the enzyme by crystallography.[2]

Reaction of $[Mo^{IV}OCl(MeNC)_4](PF_6)$[14] with 2 equiv of $Li_2(bdt)$ and excess Et_4NCl in acetonitrile followed by standard workup and recrystallization (MeCN/MeOBu^t) afforded bright orange $(Et_4N)_2[Mo^{IV}O(bdt)_2]$[15a] ($(Et_4N)_2$[**1**], 44%, bdt = benzene-1,2-dithiolate), previously reported[10a] but prepared conveniently by this procedure. Treatment of a slurry of $(Et_4N)_2$[**1**] in acetonitrile with 1 equiv of Ph_2Bu^tSiCl for 1 h resulted in silylation of the oxo ligand; recrystallization of the crude product (MeCN/MeOBu^t) produced green-black diamagnetic $(Et_4N)[Mo^{IV}(OSiPh_2Bu^t)(bdt)_2]$ ((Et_4N)[**2**], 83%).[15b] The corresponding tungsten complex has also been prepared. Reaction of $WOCl_3(THF)_2$[16] with 2 equiv

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(15) All reactions were performed under anaerobic conditions at ambient temperature using dry solvents. All compounds gave satisfactory elemental analyses. IR spectra were determined in KBr, and UV/vis and ^1H NMR spectra were in acetonitrile. (a) $(Et_4N)_2$[**1**]: ν_{MoO} 903 cm^{-1}. (b) (Et_4N)[**2**]: ν_{SiO} 947 cm^{-1}; λ_{max} (ϵ_M) 301 (10 500), 352 (22 200), 388 (sh), 461 (sh), 570 (265) nm. (c) (Et_4N)[**3**]: ν_{WO} 949 cm^{-1}. (d) $(Et_4N)_2$[**4**]: ν_{WO} 905 cm^{-1}. (e) (Et_4N)[**5**]: ν_{SiO} 963 cm^{-1}; λ_{max} (ϵ_M) 307 (21 100), 345 (6740), 420 (420), 553 (85) nm. (f) (Et_4N)[**6**]: ^1H NMR (253 K) δ 7.25 (dd), 6.96 (dd) (bdt); 1.02 (s, Bu^t); FAB-MS *m*/*z* 649 (M^-). (g) (Et_4N)[**7**]: ν_{WO} 888 cm^{-1}, ν_{SiO} 933 cm^{-1}; ^1H NMR δ 7.23 (dd), 6.87 (dd) (bdt); 1.06 (s, Bu^t); FAB-MS *m*/*z* 735 (M^-); λ_{max} (ϵ_M) 296 (14 300), 325 (sh), 340 (sh), 375 (sh), 456 (3720), 600 (1610) nm.

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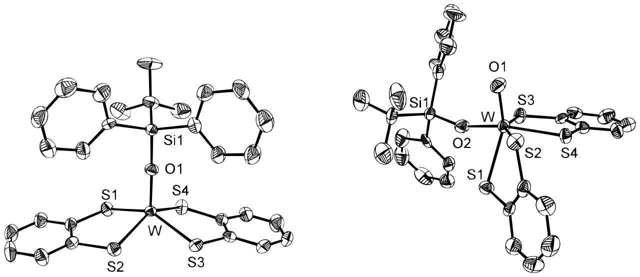

Figure 2. The structures of $[W(OSiPh_2Bu^t)(bdt)_2]^{1-}$ (**5**, left) and $[WO(OSiPh_2Bu^t)(bdt)_2]^{1-}$ (**7**, right). Bond distances (Å) and angles (deg): for **5**, W−O 1.844(6), W−S 2.333(3) (mean), Si−O 1.632(6), O−W−S 107.9(6) (mean), Si−O−W 176.0(6); for **7**, W−O1 1.723(5), W−O2 1.907(5), W−S1 2.489(2), $W-S(2-4)_{av}$ 2.42(1), O1−W−O2 95.0(3), O1−W−S1 145.6(2), O1−W−S2 81.1(2), O1−W−S3 104.6(2), O1−W−S4 103.8(2), S2−W−S3 153.86(8).

of H_2bdt and 4 equiv of Et_3N in acetonitrile followed by precipitation of the product with $(Et_4N)I$ in methanol yielded $(Et_4N)[W^VO(bdt)_2]$[15c] ((Et_4N)[**3**], 79%). Reaction of this compound with sodium acenaphthylenide in THF/MeCN and addition of $(Et_4N)I$ in methanol gave ochre $(Et_4N)_2[W^{IV}O(bdt)_2]$[15d] ($(Et_4N)_2$[**4**], 66%). Complexes **3** and **4** are described elsewhere;[13a] **4** is more efficiently obtained by this procedure. $(Et_4N)_2$[**4**] was silylated by the method for **2**, leading to red $(Et_4N)[W^{IV}(OSiPh_2Bu^t)(bdt)_2]$[15e] ($(Et_4N)$[**5**], 83%). The compounds (Et_4N)[**2/5**] are isomorphous,[17] and their anions are isostructural and nearly isometric. From the structure of **5** (Figure 2, left), it is immediately apparent that it and **2** are desoxo M^{IV} complexes. As in precursor complexes **1** and **4**, the coordination units are square pyramidal, with an axial silyloxide ligand; metric parameters are unexceptional. The two chelate rings of **5** form a dihedral angle of 150.0°, placing the tungsten atom 0.72 Å above the S_4 mean plane; the corresponding displacement in **2** is 0.75 Å.

Monooxo M^{VI} complexes were sought using **2**, **5**, and $M^{VI}O_2$ complexes as precursors. Treatment of $(Et_4N)_2$[**2**] with a 3-fold excess of Me_3NO in acetonitrile proceeded sluggishly to afford partial formation of $[Mo^{VI}O(OSiPh_2Bu^t)(bdt)_2]^{1-}$ (**6**). The preferred method is direct silylation of $[Mo^{VI}O_2(bdt)_2]^{2-}$. Reaction of $(Et_4N)_2[MoO_2(bdt)_2]$[10c] with 1 equiv of Ph_2Bu^tSiCl in acetonitrile resulted in complete conversion to brown (Et_4N)[**6**],[15f] which, however, is intensely hydrolytically sensitive; a crystalline sample has not yet been obtained. The corresponding tungsten system is entirely tractable. Thus, (Et_4N)[**5**] and 2 equiv of Me_3NO in acetonitrile for 1 h followed by isolation and recrystallization ($MeCN/THF/Et_2O$) gave orange-brown $(Et_4N)[W^{VI}O(OSiPh_2Bu^t)(bdt)_2]$[15g] ($(Et_4N)$[**7**], 76%). Alternatively, silylation of $(Et_4N)_2[WO_2(bdt)_2]$ with 1 equiv of Ph_2Bu^tSiCl in acetonitrile for 1 h followed by the same workup afforded the same product in 85% yield. The X-ray structure of **7** (Figure 2, right) reveals a severely distorted octahedral arrangement and confirms the $W^{VI}O$ formulation. Atoms O2 and S(2−4) are cis to the oxo ligand whose bond length (1.723(5) Å) contrasts with the W−O2 single bond of 1.907(5) Å. Atoms O1 and S1 are trans at an angle O1−W−S1 of 145.6(2)°. The trans influence of the oxo ligand is revealed by the W−S1 distance (2.489(2) Å), which is ~0.06 Å longer than any other W−S bond length. The relation between **6** and **7** follows from the near-identity of their ^1H NMR spectra. Consequently, we conclude that **6** is also a monooxo complex and that its structure and metric[18] properties are meaningfully represented by those of congeneric **7**.

Complexes **2** and **6**/**7** provide, in the limit of tight dithiolene chelation, representations of many of the structural aspects of the sites in the $Mo^{IV} + [O] \rightarrow Mo^{VI}O$ oxo transfer couple of *Rs* DMSO reductase. The bdt ligand simulates dithiolene binding, such that the Mo−S bond lengths in the desoxo (2.33 Å) and oxidized (2.44 Å) forms from EXAFS[3] are essentially the same as that of **2** (2.338(2) Å and the mean value of **7** (2.44(3) Å),[18] respectively. In **2**, the silyloxide ligand at a bond distance of 1.840(2) Å simulates serinate binding to Mo^{IV} in reduced DMSO reductase at the crystallographic distance of 1.8 Å.[2] The shorter of the two Mo−O/N EXAFS distances (1.94 Å) in the reduced form may correspond to serinate coordination. The longer distance (2.16 Å) likely involves a neutral ligand, perhaps water. The W═O bond length in **7** (1.723(5) Å) is tolerably close to the Mo═O EXAFS distance (1.68 Å) in the oxidized form of the enzyme. Furthermore, the Mo−O/N distance in this form (1.92 Å)[3] should correspond to the serinate binding established crystallographically.[2] In **7**, this is represented by silyloxide coordination (W−O 1.907(5) Å). EXAFS results described after the X-ray structure report of *P. furiosus* AOR indicate the presence of one W═O (1.75 Å), four to five W−S (2.40 Å), and possibly one W−N/O (1.97 Å) interaction(s) in the dithionite-reduced form.[11] Although apparently more oxidized than these samples, **7** contains the majority of the structural elements of the enzyme site. Because there is no protein ligand, silyloxide might simulate the binding of an anionic ligand such as hydroxide.

In addition to the preparative reactions M^{IV} (**2**,**5**) + Me_3NO → $M^{VI}O$ (**6**,**7**), we have observed additional oxo transfer reactivity. Thus, in systems monitored by ^1H NMR or spectrophotometry, tungsten complex **6** in acetonitrile is converted cleanly and quantitatively to **7** in the presence of 2−10 equiv of the strong oxidants PhIO, *N*-methylmorpholine *N*-oxide, and Ph_2SeO. It reacts very sluggishly with Me_2SO (≤8% conversion to **7** with 60 equiv at 60−80 °C for 3 d), but somewhat more readily with $(CH_2)_4SO$ (≤45% conversion under the same conditions). With molybdenum complex **2**, we have not as yet observed reactions with considerably weaker oxo donors, including sulfoxides. The system **2**/1000 equiv Me_2SO in acetonitrile for 3 d at room temperature evidenced no significant reaction; this system at elevated temperatures resulted in hydrolysis of the silyloxide ligand. On our oxo transfer reaction scale,[19] Me_2SO is a thermodynamically less effective donor than Me_3NO by 25 kcal/mol. In ongoing work, we are examining reactions of sulfoxides, sulfides and other potential substrates with **2** and **5**−**7**. In this way, we hope to learn whether the current level of fidelity of the molybdenum complexes to the sites in the oxo transfer reaction of *Rs* DMSO reductase translates to oxo transfer with biological substrates, and, if not, what requisite structural and/or environmental elements are missing.

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Supporting Information Available: Crystallographic data for the compounds in footnote 17, including intensity collections, positional and thermal parameters, interatomic distances and angles, and calculated hydrogen atom positions (18 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(17) (a) (Et_4N)[**2**] (213 K): monoclinic (*Pn*), $a = 10.6776(1)$ Å, $b = 9.9780(1)$ Å, $c = 17.8337(3)$ Å, $\beta = 96.339(1)°$, $Z = 2$, GOF(F^2) = 1.060, $R_1(wR_2) = 2.29(5.46)\%$. (b) (Et_4N)[**5**] (223 K): monoclinic (*Pn*), $a = 10.616(2)$ Å, $b = 9.968(2)$ Å, $c = 17.804(4)$ Å, $\beta = 96.02(3)°$, Z = 2, GOF(F^2) = 1.000, $R_1(wR_2) = 2.79(6.35)\%$. (Et_4N)[**7**] (223 K): orthorhombic (*Pbca*), $a = 10.662(2)$ Å, $b = 22.730(5)$ Å, $c = 31.414(6)$ Å, Z = 8, GOF(F^2) = 1.226, $R_1(wR_2) = 3.67(7.31)\%$. Data were collected with Mo Kα radiation, and structures were solved by direct methods and refined by standard procedures; absorption corrections were applied to all data sets.

(18) This statement is readily confirmed by comparison of corresponding bond lengths in bis(dithiolene) $M^{IV}O$ and $M^{VI}O_2$ complexes,[10,13] for which the differences are ~0.04 Å.

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