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Geometric Control of Reduction Potential in Oxomolybdenum Centers: Implications to the Serine Coordination in DMSO Reductase

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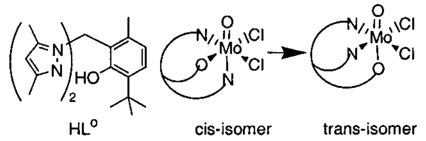
Dimethyl sulfoxide reductase (DMSOR) is a pyranopterin-containing molybdoenzyme that catalyzes the reduction of dimethyl sulfoxide (DMSO) to dimethyl sulfide (DMS).[1−4] Several crystal structures of DMSOR from both *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* have been reported in the last five years.[5] These structural reports reveal a close similarity in the folding of the protein, the presence of two pyranopterin cofactors, and a single coordinated serine per molybdenum atom; however, significant differences in the detailed description of the active site exist. These differences include the number of coordinated pyranopterin ene-1,2-dithiolate donors bound to molybdenum**,** the mode of their coordination, and the number of terminally coordinated oxo groups ($\mathrm{O_t}$). Detailed spectroscopic studies on the *R. sphaeroides* enzyme suggest a monooxo−molybdenum(VI) center coordinated by four sulfur donors.[6] Recently, a high-resolution (1.3 Å) crystal structure of the *R. sphaeroides* enzyme has been reported, which shows that the DMSOR active site is disordered and exists as a mixture of a hexa- and pentacoordinated Mo.[7] It has been suggested that such structural disorder may be a result of different crystallization and/or handling conditions, and this is supported in a detailed biochemical study.[8] We have been interested in understanding the chemical significance of this structural diversity, particularly with respect to the geometric relationship between the terminal oxo ($\mathrm{O_t}$) and serinato oxygen ($\mathrm{O_{ser}}$) donors. A careful inspection of the DMSOR crystallographic data and that of a related enzyme, trimethylamine *N*-oxide reductase (TMAOR), reveals a wide variation in the reported $\mathrm{O_t{-}Mo{-}O_{ser}}$ angle, which ranges from 90° to 146°.[5,7,9] To the best of our knowledge, the implications of this structural parameter on various properties of the active site including the electronic structure and Mo reduction potential have not been previously highlighted.[10] Using well-defined, discrete molecules, we report here the influence of the $\mathrm{O_t{-}Mo{-}O_p}$ angle on the redox and spectroscopic properties of these complexes. Furthermore, we hypothesize that the variability found in the $\mathrm{O_t{-}Mo{-}O_{ser}}$ angle may play a critical role in modulating the reduction potential of the native enzyme.

Scheme 1



We have isolated *cis*-[(L1O)$\mathrm{Mo^VOCl_2}$] and *trans*-[(L1O)$\mathrm{Mo^VOCl_2}$] complexes of the heteroscorpionato ligand, (3-*tert*-butyl-2-hydroxy-5-methylphenyl)bis(3,5-dimethylpyrazolyl)methane (L1OH),[11] where the cis and trans notation refers the position of the phenolato oxygen ($\mathrm{O_p}$) relative to that of the terminal oxo ($\mathrm{O_t}$) ligand. Here, the phenolato oxygen models the coordinated serinato oxygen found bound to the active site Mo, and $\mathrm{O_p} \rightarrow$ Mo LMCT transitions allow us to probe the effect of geometric variation of the phenolato oxygen relative to the metal center on the electronic structure of these complexes. These results are discussed relative to the mode of serine binding to the active site in DMSOR and TMAOR.

[(L1O)$\mathrm{MoOCl_2}$] was synthesized by reacting $\mathrm{(L1O)^-}$ with a slurry of $\mathrm{MoCl_5}$ in THF following deprotonation of the ligand with NaH. The crude product was isolated as a red-brown solid in good yields (50−70%). The two isomers were separated by chromatography on silica gel using dichloromethane as the eluant. A pink band elutes first followed by a yellow one. The molecular composition of the two compounds was confirmed by mass spectrometry. Slow evaporation of dichloromethane or acetonitrile solutions of the pink compound afforded X-ray quality single crystals. The molecular structure of [(L1O)$\mathrm{MoOCl_2}$] was determined by X-ray crystallography, and the complex is found to adopt a six-coordinate distorted octahedral geometry (Supporting Information).[12] The Mo atom is coordinated by a phenolato oxygen ($\mathrm{O_p}$) from the tridentate ligand and two nitrogen donors from pyrazole rings, and the two chloride donors and the terminal oxo ligand occupy the remaining three coordination sites (Scheme 1). Unfortunately, a compositional disorder due to the presence

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(12) Crystal data for [(L1O)$\mathrm{MoOCl_2}$]·2.66$\mathrm{H_xO}$, crystal dimensions, 0.8, 0.4, 0.1 mm; crystal system, rhombohedral; space group, *R*3*m*; $a = 27.982(4)$ Å, $b = 27.982(4)$ Å, and $c = 9.424(2)$ Å; $\alpha = \beta = 90°$, $\gamma = 120°$; $Z = 18$, $V = 6390(2)$ Å^3, Mo Kα ($\lambda = 0.710\ 73$ Å); calculated density 1.34 g/cm^3; abs coeff, μ_{calc} (mm^{-1}) 0.679; total reflections 1855, independent reflections 1009; number of parameters 172, data to parameter ratio 5.86; full matrix refinement; $R(F) = 0.0687$, $R_w(F^2) = 0.2016$, goodness of fit =1.156; largest difference peak and hole 1.046 and −0.69 (e/Å^3).

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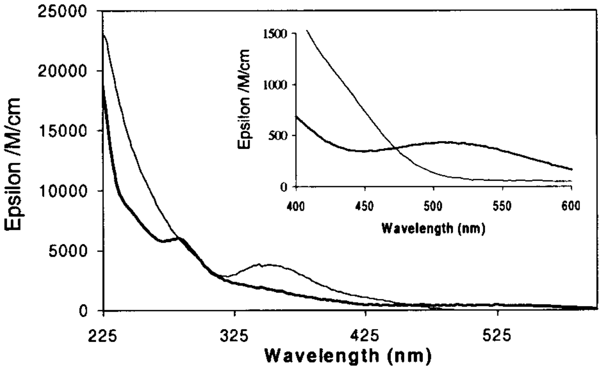

Figure 1. Electronic absorption spectra of the cis (bold line) and the trans isomer (thin line). In the inset the low-energy charge transfer band can be seen for the cis isomer and no such transition was observed for the trans isomer.

of both cis and trans isomers within the asymmetric unit has prevented the positions of the chloride and the terminal oxo (O_t) ligands from being uniquely defined crystallographically.[13]

Several lines of spectroscopic evidence demonstrate that the pink compound is the cis isomer while the yellow compound adopts a trans $O_t{-}Mo{-}O_p$ geometry. The infrared spectrum of the pink compound exhibits a strong Mo═O stretch at 961 cm^{-1} which is shifted 14 cm^{-1} to lower frequency in the yellow compound. The reduction in $\nu_{Mo=O}$ of the yellow isomer is consistent with a trans effect manifested by the phenolato oxygen (O_p). The stronger π-donor ability of the O_p donor competes more favorably than a pyrazolyl nitrogen for the antibonding Mo═O $d_{xz,yz}$ orbitals, thereby weakening the Mo═O bond. More definitive evidence is provided by electronic absorption spectroscopy. Phenolate → Mo d_{xy} LMCT transitions[14] occur at ~20 000 cm^{-1} (526 nm) in oxo−molybdenum(V) complexes possessing equatorial (cis) phenolate donors, and the spectrum of the pink compound exhibits a low-energy (19 340 cm^{-1}, 517 nm, $\epsilon = 430\ M^{-1}\ cm^{-1}$) $O_p^v \rightarrow$ Mo d_{xy} charge transfer transition in this region (Figure 1).[22] It is important to note that charge transfer transitions to the Mo $d_{xz,yz}$ orbitals occur at higher energies (13000−16000 cm^{-1}) than those to the d_{xy} orbital due to their strong π destabilization which results from the terminal oxo ligation (Figure S5, Supporting Information). Thus, the yellow compound is anticipated to be the trans isomer and, as expected, does not exhibit a $O_p^v \rightarrow$ Mo d_{xy} LMCT transition since there is no $O_p^v{-}d_{xy}$ orbital overlap (Figure 2). As a result, the weak $d_{xy} \rightarrow d_{xz,yz}$ LF band can be observed at ~16 500 cm^{-1} (606 nm) in both the absorption and MCD of this complex.[15] Additional support for these assignments derives from the fact that the electronic spectrum of the yellow isomer is virtually identical to that of $LMoOCl_2$ (L^- = 3,5-dimethyl hydrotrispyrazolyl borate) below ~27 000 cm^{-1} (370 nm) (Supporting Information).[16] This is expected since both trans $(L1O)MoOCl_2$ and $LMoOCl_2$ possess N_2Cl_2 equatorial coordination spheres and should have virtually identical low energy Cl^- → Mo d_{xy} LMCT transitions. Taken together, the spectroscopic data is consistent with the formulation of the pink compound as the cis isomer and the yellow compound as the trans isomer.

The redox chemistry of the two isomers was investigated by cyclic voltammetry, and both compounds exhibit well-defined one-electron reductive couples (Mo(V) → Mo(IV)) at −940 mV and at −1160 mV (vs Fc^+/Fc) for the trans and the cis isomers, respectively.[17] Thus the trans isomer is easier to reduce than the cis isomer by ~200 mV.[18] As was previously mentioned, it has been demonstrated that the terminal oxo dominates the Mo ligand field resulting in an energetically isolated d_{xy} redox orbital.[19,20] Therefore, the reduction potentials of monooxo−molybdenum centers are intimately related to the valence ionization energy of this orbital.[21] The low-energy $O_p^v \rightarrow$ Mo d_{xy} LMCT transition in the cis isomer results from π antibonding interactions between the phenolato oxygen and the d_{xy} (redox) orbital which should raise the energy of the redox orbital and make the cis isomer more difficult to reduce. Since the $O_p^v{-}d_{xy}$ interaction is not present in the trans isomer, the redox orbital is not energetically destabilized and the complex is easier to reduce relative to the cis isomer. This is an important observation, demonstrating that the position of an oxygen donor relative to the terminal oxo functions to modulate the reduction potential of the metal center. With respect to the DMSO reductases, this leads to the provocative suggestion that the position of the serinato oxygen relative to the terminal oxo may play a critical role in gating the electron transfer process in the regeneration step.

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Supporting Information Available: An X-ray crystallographic file in CIF format. An ORTEP drawing of $(L1O)MoOCl_2$, electronic spectra for isomeric conversion, electronic and MCD spectra of the trans isomer, electronic spectra of $LMoOCl_2$ and *trans*-$(L1O)MoOCl_2$, and a simplified ligand field diagram (Figures S1−S5). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) The disorder resulted in Mo(1)−O(1)(Cl) bonds being distinctly longer (Mo(1)−O(1) = 2.11(2) Å) than expected for a molybdenum−O^t bond while having the effect of shortening the Mo(1)−Cl(1) and Mo(1)−Cl(2) bonds (2.254(6) Å). The Mo−O_p bond, Mo(1)−O(1), is 1.96(2) Å, and the molybdenum−nitrogen bonds, Mo−N, average 2.23(2) Å. These distances are comparable to those reported in the literature for other oxo−Mo complexes.[37] The average N−Mo(1)−O(2) bond angle is 84.1(6)°, and the N(1)−Mo(1)−N(1a) bond angle is 81.3(8)°.

(14) There are two types of phenolate → Mo LMCT transitions, $O_p^v \rightarrow$ Mo and $O_p^\sigma \rightarrow$ Mo. The superscript v refers to the O_p p orbital oriented perpendicular (vertical) to the O_p−Mo bond, while σ refers to the O_p p orbital oriented along the O_p−Mo bond. The latter orbital is typically found at deeper binding energies due to the σ-bonding interaction; thus the lowest energy $O_p \rightarrow$ Mo CT transitions are $O_p^v \rightarrow$ Mo d_{xy} in oxo−molybdenum complexes.

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