The Oxo-Gate Hypothesis and DMSO Reductase: Implications for a Psuedo-σ Bonding Interaction Involved in Enzymatic Electron Transfer

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The periplasmic dimethylsulfoxide reductases (DMSOR) from *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* are pyranopterin Mo enzymes that contain the Mo active site as their only redox-active center and function as the terminal electron acceptor during anaerobic growth in the presence of the substrate DMSO.[1] The enzyme cycles between Mo(IV) and Mo(VI), with the Mo(V) oxidation state being an obligatory catalytic intermediate in the course of electron transfer regeneration following formal O atom transfer (OAT) between substrate and the des-oxo Mo(IV) site.[1] Considerable debate exists concerning the coordination geometry of the active site and the catalytic mechanism despite numerous structural and spectroscopic studies.[1−10] The results of three separate protein crystallographic studies[2−4] have confirmed the presence of terminal oxo, serinate O, and pyranopterin ene-1,2-dithiolate S donors to Mo, but differ considerably with respect to the coordination geometry and the exact number of oxo and S donors coordinated to Mo. This has provided the impetus for a myriad of proposed mechanistic sequences that involve di-, mono-, and des-oxo Mo coordination, as well as Mo−S bond breaking/weakening steps[3,9] during the course of enzymatic catalysis. However, a recent XAS spectroscopic study of the *R. sphaeroides* enzyme[10] provides strong evidence for the presence of mono-oxo and des-oxo Mo sites for oxidized ($DMSOR_{ox}$) and reduced ($DMSOR_{red}$) enzyme, respectively, with all four pyranopterin dithiolate S donors remaining strongly bound to the metal throughout the course of catalysis. Therefore, the DMSOR active site appears to be structurally similar to the W aldehyde ferredoxin oxidoreductase enzyme from *P. furiosus*.[11] The absence of additional redox-active centers in DMSOR has made it possible to directly probe the electronic structure of the Mo active site by a variety of optical techniques.[5−8] The low-energy ligand-to-metal charge transfer (LMCT) bands observed at ∼720 nm ($\epsilon \sim 2000$ M^{-1} cm^{-1}) and ∼550 nm ($\epsilon \sim 1800$ M^{-1} cm^{-1}) in the electronic absorption spectra of $DMSOR_{ox}$ are uniquely characteristic of this family of pyranopterin Mo enzymes. This is an important observation given that the intensity of a LMCT transition is a function of metal−ligand bond covalency.[12] Thus, S→Mo CT transitions can provide insight into the role of the pyranopterin ene-1,2-dithiolate S donors in catalysis. For example, the intense low-energy S→Cu CT band observed in blue copper proteins has been shown to result from a highly covalent $Cu-S_{Cys}$ bonding scheme, allowing the Cu center to effectively couple into protein-mediated superexchange pathways for long-range electron transfer (ET).[13] Likewise, the intensity of the LMCT bands in $DMSOR_{ox}$ implies considerable covalency in the Mo−S bonds. This work details the assignment of the low-energy LMCT bands in $(PPh_4)[MoO(bdt)_2]$,[14,15] a small molecule paramagnetic analogue of the $DMSOR_{ox}$ active site, and provides detailed insight into the relationship between electronic structure and mechanism in DMSOR.

A similar electronic structure description for $[MoO(bdt)_2]^-$ and $DMSOR_{ox}$ is suggested by a comparison of their electronic absorption spectra (Figure 1) with respect to the number of observed low-energy bands and their relative energies and intensities.[16] Mono-oxo Mo(V) bisdithiolate model compounds possess a distinctive broad low-energy absorption feature at 729−842 nm and a higher energy feature at 446−575 nm.[15,17−20] The two lowest energy absorption bands for $[MoO(bdt)_2]^-$ occur at ∼730 (band 1) and ∼500 nm (band 2). We have used a combination of electronic absorption, MCD, and rR spectroscopies to understand the electronic origin of these low-energy transitions. The MCD spectrum of $[MoO(bdt)_2]^-$ displays C-term features at 735 and 515 nm (Figure S1, Supporting Information) that are consistent with electronic transitions involving one-electron promotions from a dithiolate molecular orbital to the nondegenerate d_{xy} acceptor orbital localized on Mo. Three Mo−S vibrational modes (344, 358, 377 cm^{-1}) are enhanced in the rR spectrum of $[MoO(bdt)_2]^-$ with virtually no enhancement of the Mo≡O stretch (Figure S2, Supporting Information). This is consistent with the assignment of bands 1 and 2 as LMCT transitions to the in-plane (orthogonal to the Mo≡O bond) Mo d_{xy} acceptor orbital, which is nonbonding with respect to the oxo ligand. These spectroscopic results have been utilized to evaluate the results of DFT calculations[21] on the related electronic structure model $[MoO(edt)_2]$[14] in the Mo(V) and Mo(VI) oxidation states.

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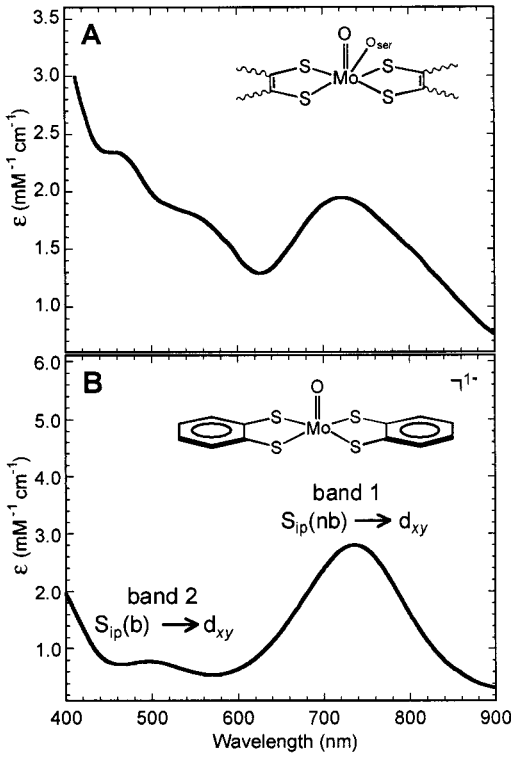

Figure 1. Electronic absorption spectra of (A) $DMSOR_{ox}$ from *R. sphaeroides* (adapted from ref 5) and (B) $(PPh_4)[MoO(bdt)_2]$ in CH_2Cl_2. The consensus structure of the Mo active site of $DMSOR_{ox}$ is shown with only the 1,2-dithiolate linkage of the pyranopterin.

The calculations accurately reproduce the Mo d orbital manifold resulting from terminal oxo ligation,[22,23] and indicate that in-plane dithiolate orbitals (S_{ip}) are more energetically stabilized than out-of-plane dithiolate orbitals (S_{op}). This allows for the assignment of the low-energy LMCT transitions in $[MoO(bdt)_2]^-$ as S_{ip}(nb)→Mo d_{xy} (band 1) and S_{ip}(b)→Mo d_{xy} (band 2),[14] and the analogous spectroscopic features of the model and $DMSOR_{ox}$ imply the same assignments for the enzyme. The energies and intensities of bands 1 and 2 are only consistent with a mono-oxo Mo center in which all four S donors are coordinated to Mo.[24] This directly supports recent XAS[10] and resonance Raman[8] studies that refute the hypothesis that certain Mo−S bonds are labile during the course of catalysis, and the spectral similarity of $[MoO(bdt)_2]^-$ and $DMSOR_{ox}$ suggests very similar coordination geometries, allowing the role of Mo−S bonding in catalysis to be evaluated.

The high oscillator strength of the S_{ip}(nb)→Mo d_{xy} transition reflects a considerable amount of S_{ip}−Mo d_{xy} orbital mixing, and this covalent bonding scheme has been postulated to play a vital role in modulating the Mo reduction potential and facilitating electron transfer regeneration of the active site.[1,22] Inspection of the S_{ip}(b)−Mo d_{xy} molecular orbital (Figure 2) reveals that the S_{ip} orbitals are rotated off the Mo−S bond axes and toward one another, localizing a large amount of S electron density between the two S atoms of each dithiolate. This results in a pseudo-σ bonding interaction that effectively couples the Mo d_{xy} redox orbital into the S_{ip} orbitals of the coordinated dithiolate. This bonding interaction has previously been observed in oxo−Mo monodithiolates,[22] including the "very rapid" intermediate in xanthine oxidase,[25] where it has been proposed to couple the Mo d_{xy} redox orbital into effective ET pathways involving the σ system of the pyranopterin. Therefore, the presence of a *single* axial oxo group in $DMSOR_{ox}$ is essential to orient the Mo d_{xy} redox orbital for maximal interaction with the S_{ip} orbitals of the pyranopterin ene-1,2-dithiolate during ET regeneration of the reduced enzyme active site following formal OAT. The S_{ip}−Mo d_{xy} interaction is maximized when the Mo≡O bond is orthogonal to an ene−dithiolate plane, and this mechanistic prerequisite for facile ET regeneration in pyranopterin-containing enzymes is referred to as the *oxo-gate hypothesis*.[22] Interestingly, it has previously been suggested that the two pyranopterins may function independently in catalysis,[7] with one being an ET conduit or reduction potential modulator while the other drives the OAT reaction. Although this study does not specifically address the OAT half-reaction, it is clear that one pyranopterin could be involved in ET processes[22,25] while the other controls or buffers the Mo reduction potential.[26] This is easily accommodated within the confines of the oxo-gate hypothesis if the Mo≡O bond is canted toward a single dithiolate, maximizing overlap between Mo d_{xy} and S_{ip} orbitals for ET regeneration, while the second dithiolate modulates the reduction potential of the active site during the course of catalysis via the π-donor ability of the S_{op} orbitals.[27] Since this covalent bonding interaction has recently been shown to be present in the xanthine oxidase "very rapid" intermediate,[25] it appears that a pseudo-σ bonding interaction may be a common electronic structure theme of oxo−Mo and oxo−W dithiolate centers in enzymes, providing an efficient way to couple the metal redox orbital into ET pathways involving the pyranopterin.

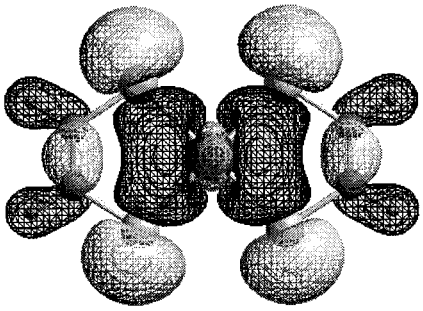

Figure 2. Molecular orbital contour depicting the covalent pseudo-σ interaction between Mo d_{xy} and S_{ip} orbitals.

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Note Added in Proof. A recent 1.3 Å crystal structure of DMSOR supports our assertion that all four S donors remain coordinated to Mo during catalysis (Schindelin et al. *J. Am. Chem. Soc.* **2000**, *122*, 7673).

Supporting Information Available: Electronic absorption/MCD (S1) and Gaussian-resolved absorption/rR enhancement profiles (S2) of $(PPh_4)[MoO(bdt)_2]$. This material is available free of charge via the Internet at http://pubs.acs.org.

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