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Insights into the Nature of Mo(V) Species in Solution: Modeling Catalytic Cycles for Molybdenum Enzymes†

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Abstract

The tris(pyrazolyl)borate and related tripodal N-donor ligands originally developed by Trofimenko stabilize mononuclear compounds containing Mo^{VI}O₂, Mo^{VI}O, Mo^{VO}, and Mo^{IV}O units and effectively inhibit their polynucleation in organic solvents. Dioxo-Mo(VI) complexes of the type LMoO₂(SPh), where L = hydrotris(3,5-dimethylpyrazol-1-yl)borate (Tp*), hydrotris(3-isopropylpyrazol-1-yl)borate (Tp^{iPr}), and hydrotris(3,5-dimethyl-1,2,4-triazol-1-yl)borate (Tz) and related derivatives are the only model systems that mimic the complete reaction sequence of sulfite oxidase, in which oxygen from water is ultimately incorporated into product. The quasi-reversible, one-electron reduction of Tp*MoO₂(SPh) in acetonitrile exhibits a positive potential shift upon addition of a hydroxylic proton donor, and the magnitude of the shift correlates with the acidity of the proton donor. These reductions produce two Mo(V) species, [Tp*Mo^{VO}O₂(SPh)][−] and Tp*Mo^{VO}O(OH)(SPh), that are related by protonation. Measurement of the relative amounts of these two Mo(V) species by EPR spectroscopy enabled the pK_a of the Mo^{VO}(OH) unit in acetonitrile to be determined and showed it to be several pK_a units smaller than that for water in acetonitrile. Similar electrochemical-EPR experiments for Tp^{iPr}MoO₂(SPh) indicated that the pK_a for its Mo^{VO}(OH) unit was ~1.7 units smaller than that for Tp*Mo^{VO}O(OH)(SPh). Density functional theory calculations also predict a smaller pK_a for ^{iPr}Mo^{VO}O(OH)(SPh) compared to Tp*Mo^{VO}O(OH)(SPh). Analysis of these results indicates that coupled electron-proton transfer (CEPT) is thermodynamically favored over the indirect process of metal reduction followed by protonation. The crystal structure of Tp^{iPr}MoO₂(SPh) is also presented.

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SUPPLEMENTARY MATERIAL: Electrochemical-EPR data (Table S1) can be found in the online version. File CCDC 721839 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Introduction

Molybdenum and tungsten enzymes catalyze a wide range of two-electron oxidation and reduction reactions that generally involve oxygen atom transfer reactions.¹⁻⁴ The catalytic cycles for all of the molybdenum enzyme families involve the Mo^{VI}, Mo^V and Mo^{IV} oxidation states of the active site. During catalysis, the ligand environment may change, in both coordination number and ligand oxidation state. The commonly proposed catalytic cycle for sulfite oxidase (SO) shown in Scheme 13· 5 involves an oxygen atom transfer (OAT) reaction followed by two sequential one-electron processes that are proposed to be coupled electron-proton transfer (CEPT) processes. These CEPT steps are mediated by the integral *b*-type heme, and generate the transient Mo(V) forms of SO that have been extensively studied by CW- and pulsed-EPR spectroscopy.^{5· 6} Recently, alternative catalytic pathways in which electron transfer from the Mo center precedes release of product (sulfate) have also been considered.⁷

Essential to all of the mechanisms that have been proposed for SO is an understanding of the chemistry of the transient Mo(V) centers that are produced in the catalytic cycle. Of particular interest is the solution chemistry of the Mo^V(OH)_{*n*} species of Scheme 1. The p*K*_a values of the postulated Mo(OH) and Mo(OH)₂ groups would be expected to play an important role in the proposed CEPT steps and would likely depend upon the oxidation state of the Mo center and the nature of other nearby ligands. The dependence of the p*K*_a of coordinated aquo ligands has been investigated in detail for some Ru(III/II) compounds. For example, oxidation of Ru^{II} in *cis*-[Ru^{II}(bpy)₂(py)(H₂O)]²⁺ (bpy = 2,2'-bipyridine, py = pyridine) to Ru^{III} decreases the p*K*_a for bound H₂O by 9.7 p*K*_a units.⁸ However, similar p*K*_a data are not available for Mo systems because of the strong propensity of Mo^V(OH) compounds to form oxo-bridged dimers in aqueous solution.⁹

The tris(pyrazolyl)borate and related tripodal N₃-donor ligands originally developed by Trofimenko¹⁰⁻¹² stabilize mononuclear compounds containing Mo^{VI}O₂, Mo^{VI}O, Mo^{VO}, and Mo^{IV}O units and effectively inhibit their polynucleation in organic solvents. Spectroscopic, structural, theoretical and reactivity studies of well-characterized Mo and W compounds of these ligands have provided significant insight into the enzyme active sites.¹³⁻¹⁵ The complexes of the type LMoO₂(SPh), where L = hydrotris(3,5-dimethylpyrazol-1-yl)borate (Tp*), hydrotris(3-isopropylpyrazol-1-yl)borate (Tp^{iPr}), and hydrotris(3,5-dimethyl-1,2,4-triazol-1-yl)borate (Tz) and related derivatives^{16, 17} are the only model systems that mimic the complete reaction sequence of SO's (Scheme 1) in which oxygen from water is ultimately incorporated into product.¹⁸ The overall six-coordinate *fac* stereochemistry at the molybdenum center enables the chemical and electronic effects of a wide range of co-ligands to be systematically explored in a controlled environment. The dioxo-Mo(VI) compounds can be reduced chemically or electrochemically (Eq. 1) to two types of Mo(V) centers that are related to one another by transfer of a proton according to Eq. 2.

For example, it has been observed that when phosphines are reacted with Tp*MoO₂(SPh) in the presence of small amounts of water, the formation of Tp*Mo^VO(OH)(SPh) can be detected by Electron Paramagnetic Resonance (EPR).^{16, 17} Also, electrochemical reduction of LMo^{VI}O₂(SPh) compounds generates [LMo^VO₂(SR)]⁻ anions, which are protonated to give LMo^VO(OH)(SR), as shown by previous EPR experiments.^{16· 17} Here, we use

electrochemical and EPR techniques along with density functional theory (DFT) to obtain insights into the nature of the $\text{Mo}^{\text{V}}(\text{OH})$ species that are generated in solution upon reduction of $\text{Tp}^*\text{MoO}_2(\text{SPh})$, $\text{Tp}^{\text{iPr}}\text{MoO}_2(\text{SPh})$, and $\text{TzMoO}_2(\text{SPh})$ compounds.

During this work diffraction quality crystals of $\text{Tp}^{\text{iPr}}\text{MoO}_2(\text{SPh})$ were obtained, and its structure is reported to complement structural reports for other dioxo-Mo(VI) thiolato complexes containing scorpionate ligands. These include $\text{Tp}^*\text{MoO}_2(\text{SPh})$ and $\text{TzMoO}_2(\text{SPh})$ ¹⁷ and the direct analogues $\text{Tp}^{\text{iPr}}\text{MoO}_2(\text{SR})$ ($\text{R} = \text{iPr}, p\text{-tol}, \text{Bz}$).¹⁹

Experimental

Materials

For the electrochemical experiments, all the solvents (anhydrous) were of commercial origin and degassed with three cycles of freeze-pump-thaw. Tetrahydrofuran (THF) and acetonitrile were purified with a MBraun Solvent Purification System. Water was purified through Barnstead Nanopure Purification Systems. Supporting electrolyte employed was tetra-*n*-butylammonium tetrafluoroborate ($\text{Bu}^{\text{n}}_4\text{NBF}_4$), electrochemical grade, from Fluka Chemical Co.). Metal complexes were synthesized according to published procedures.^{16, 17, 20}

Methods

Electrochemistry: The electrochemical experiments were performed at a glassy carbon working electrode, using 0.1M $\text{Bu}^{\text{n}}_4\text{NBF}_4$ as supporting electrolyte. The reference electrode used consisted of an Ag/AgNO_3 (0.1M in acetonitrile) electrode incorporated into a salt bridge containing supporting electrolyte to minimize Ag^+ contamination of the solution. The counter electrode was a platinum wire. Cyclic voltammetry was performed using a BASi CV-50W instrument. Analyte concentrations ranged from 0.4 to 0.6 mM. The proton donating reagents were added gradually with a microsyringe. Sweeps commenced in the reducing direction. Experiments were performed at room temperature ($\sim 298\text{ K}$).

Electron Paramagnetic Resonance: The EPR experiments were performed on a Bruker ESP-300 X-band EPR spectrometer. A standard flat electrochemical cell (Wilma) was used for generating the Mo(V) complexes inside the TE_{102} resonator, and the measurement temperature was about 300 K. The flat cell utilized platinum working and counter electrodes and an Ag/AgNO_3 non-aqueous reference electrode. After generating the EPR-active $[\text{LMo}^{\text{V}}\text{O}_2(\text{SPh})]^-$ species, a known amount of water (the proton donor) was added to generate $\text{LMo}^{\text{V}}\text{O}(\text{OH})(\text{SPh})$ in solution. Experimental conditions for the EPR spectra are given in the legend for Figure 1. The presence of an exchangeable proton in $\text{LMo}^{\text{V}}\text{O}(\text{OH})(\text{SPh})$ was verified by repeating the experiment using D_2O (spectra not shown). The concentrations of $[\text{LMo}^{\text{V}}\text{O}_2(\text{SPh})]^-$ and $\text{LMo}^{\text{V}}\text{O}(\text{OH})(\text{SPh})$ were determined from comparison of their well-separated double-integrated EPR signals with that of the stable Mo(V) complex, $\text{Tp}^*\text{MoOCl}_2$, under the same experimental conditions.

Density Functional Theory

All computations were performed with the ADF2006.01b computational package.²¹⁻²³ Geometry optimizations and frequency calculations were carried out using the VWN functional with the Stoll correction implemented.²⁴ A triple- ζ STO basis set with one polarization function (TZP), available in the ADF package, was used in all calculations. Relativistic effects were taken into account in all calculations by using the scalar ZORA formalism²⁵ implemented as part of the ADF2006.01b program. All electronic structures with unpaired spins were calculated using an unrestricted framework. The theoretical stretching frequencies for all species were calculated analytically with the same DFT model as that for the geometry optimization. Solvation effects on the complexes were modeled through the Conductor-like

Screening Model (COSMO) of solvation.²⁶ The solvent parameters implemented were those defined by the ADF2006.01b program to simulate an acetonitrile solvated environment. Free energy (G) values were calculated from the electronic self-consistent-field (SCF) energies considering the translational, rotational, and vibrational contributions.²⁷ Enthalpy and entropy terms were calculated under standard temperature and pressure. When computing K_a values, the proton was treated as having one molecule in the primary solvation sphere, $\text{CH}_3\text{CN}\cdot\text{H}^+$, and this species was considered to participate in the reaction, $\text{HA} + \text{CH}_3\text{CN} \rightleftharpoons \text{A}^- + \text{CH}_3\text{CN}\cdot\text{H}^+$, in a solvated environment.

Crystallography

Red-brown crystals of $\text{Tp}^{\text{iPr}}\text{MoO}_2(\text{SPh})$ were grown by cooling a saturated solution of the complex in methanol/dichloromethane (5:1). Diffraction data were collected at 130(2) K using a Bruker SMART Apex CCD diffractometer using $\text{Cu-K}\alpha$ radiation (graphite crystal monochromator, $\lambda = 1.5418 \text{ \AA}$).²⁸ Accurate cell parameters and crystal orientations were obtained by least-squares refinement of 2399 reflections with $\theta = 2.82 - 73.03^\circ$. Data were reduced using the program SAINT, and the structure was solved by direct methods and difference Fourier syntheses.²⁸ Hydrogen atoms were included in calculated positions (riding model) with the exception of the hydrogen attached to the boron. Full-matrix least-squares refinement on F^2 , using all data, was carried out with anisotropic displacement parameters applied to all non-hydrogen atoms. Crystallographic data and details of the refinement are presented in Table 1. Figure 2 shows the structure, and selected bond distances and angles are given in Table 2.

Results and Discussion

The solution chemistry of Mo(V) species in these model systems was investigated by reducing $\text{LMoO}_2(\text{SPh})$ electrochemically and following the reaction by using EPR. All compounds studied show a single reduction peak in both acetonitrile and THF, corresponding to one-electron reduction to give the monoanionic species, $[\text{LMoO}_2(\text{SPh})]^-$ as seen in Figure 3A. Addition of water to the solution results in a positive shift of the half-wave potential ($E_{1/2}$), as shown in Figure 3B. No additional peaks appear after adding water, and the quasi-reversibility is retained.

In order to determine the nature of the interaction between water and the metal complex, the effect of several other hydroxylic proton donors on the reduction potential of $\text{LMoO}_2(\text{SPh})$ compounds was investigated. These hydroxylic reagents included methanol, phenol, and 2,2,2-trifluoroethanol (TFE). The $\text{p}K_a$ values of water, methanol, 2,2,2-trifluoroethanol, and phenol in DMSO are 31.2, 29.0, 23.45, and 18.0, respectively.²⁹ The relative order of increasing acidity for these reagents in DMSO is water < methanol < 2,2,2-trifluoroethanol < phenol. Since both DMSO and acetonitrile are polar aprotic solvents, the assumption can be made that the acidity of these hydroxylic reagents will follow the same order in acetonitrile as in DMSO. Figure 4 shows the effect of the different reagents on the half-wave potential ($E_{1/2}$) of $\text{Tp}^*\text{MoO}_2(\text{SPh})$. Each reagent produced a positive potential shift of the reduction potential of $\text{Tp}^*\text{MoO}_2(\text{SPh})$, and the total shift depended on the concentration of the reagent. A more acidic species (better proton donor) produced a greater potential shift relative to its concentration. The most acidic proton donor used in the experiment is phenol, and it produces a dramatic potential shift relative to the other reagents. These observations support the conclusion that the ability of these proton donating reagents to shift the potential of $\text{Tp}^*\text{MoO}_2(\text{SPh})$ depends upon their $\text{p}K_a$ values. This variation in half-wave potential could be attributed to the association between a charged metal complex intermediate and the proton donor.³⁰ A similar notable shift in the $E_{1/2}$ potential was observed for the $\text{TzMoO}_2(\text{SPh})$ complex. However, the potential shift for $\text{Tp}^{\text{iPr}}\text{MoO}_2(\text{SPh})$ was minimal (not shown). These observed potential shifts were significantly different from those expected for a single protonation step (Eq. 2). When the Nernst equation is graphed with

the assumption of a single proton transfer, the theoretical values do not fit the experimental data. However, they can be mathematically modeled by assuming additional interactions of the Mo compounds with the acid molecules. If two interactions are assumed then the equation derived by Gómez *et al.*³⁰ can be used to theoretically predict the potential shifts. The solid lines in Figure 4 were calculated using Eq. 3

$$E_{1/2} = E^0 + (RT/F) \ln \left(1 + K_1 [\text{AH}] + K_1 K_2 [\text{AH}]^2 \right) \quad \text{Eq. 3}$$

Where K_1 and K_2 are association constants for the anions interacting with the proton donating reagent, AH. As will be seen later, actual proton transfer from AH to the anion may be occurring in addition to these hydrogen-bonding interactions, and such proton transfer is not considered in Eq. 3. The plots using Eq. 3 give reasonable fits of the theoretical and experimental values (within a few millivolts of one another) for titrations with most of the proton donating reagents. An exception is the TFE titration curve, which might indicate additional interactions between TFE and the molybdenum complex. Continuous shifts in potential with no change in reversibility and no appearance of new waves indicate hydrogen-bonding of the reduction products.³¹

Previous EPR studies on $\text{Tp}^*\text{Mo}^{\text{VO}}(\text{OH})(\text{SPh})$ and related complexes have shown that a single proton is strongly interacting with the metal center as shown in Eq. 2.¹⁷ Electrochemical reduction in an electrochemical-EPR flat cell in dry acetonitrile gives a signal with $g = 1.92$ that is assigned to the anionic species, $[\text{Tp}^*\text{Mo}^{\text{VO}}_2(\text{SPh})]^-$ (see Figure 1). Reduction in the presence of small amounts of water results in a second signal with $g = 1.95$ and a 13 G splitting that collapses upon addition of D_2O ; this second signal is assigned to $\text{Tp}^*\text{Mo}^{\text{VO}}(\text{OH})(\text{SPh})$, in agreement with previous work.¹⁷ Similar behavior was observed for the analogous Tp^{iPr} and Tz molybdenum complexes (not shown).

In principle, the $\text{p}K_{\text{a}}$ for the $\text{Mo}(\text{OH})$ group can be determined experimentally from the relative amounts of the $[\text{LMo}^{\text{VO}}_2(\text{SPh})]^-$ and $\text{LMo}^{\text{VO}}(\text{OH})(\text{SPh})$ species in solution. This was achieved by reduction of $\text{LMo}^{\text{VI}}\text{O}_2(\text{SPh})$ in an electrochemical-EPR flat cell to generate the $[\text{LMo}^{\text{VO}}_2(\text{SPh})]^-$ species (Eq. 1), followed by addition of a known amount of a proton donor (water) to give $\text{LMo}^{\text{VO}}(\text{OH})(\text{SPh})$ (Eq. 2). The relative amounts of these two $\text{Mo}(\text{V})$ species were then calculated by double-integrating their individual EPR signals, which are well-separated from one another (see Figure 1B and Table S1).

The equilibrium constant, K for Eq. 2 given $\text{HA} = \text{H}_2\text{O}$, is described by Eq. 4.

$$K = \frac{[\text{LMo}^{\text{VO}}(\text{OH})(\text{SPh})][\text{OH}^-]}{[\text{LMo}^{\text{VO}}_2(\text{SPh})]^-[\text{H}_2\text{O}]} = \frac{K_{\text{a,H}_2\text{O}}}{K_{\text{a,MoOH}}} \quad \text{Eq. 4}$$

Here $K_{\text{a,H}_2\text{O}}$ is the acid dissociation constant of water in acetonitrile and $K_{\text{a,MoOH}}$ is that of the protonated Mo^{V} complex. The concentration of the OH^- anion in the solution was assumed to be equal to the concentration of the $\text{LMo}^{\text{VO}}(\text{OH})(\text{SPh})$, which in turn was estimated from EPR spectroscopy. The known concentration of H_2O added to the sample enabled K to be calculated, and hence determination of the $\text{p}K_{\text{a}}$ of the $\text{Mo}^{\text{V}}(\text{OH})$ unit in solution (Tables 3 and S1). The $\text{p}K_{\text{a}}$ values obtained from the above method showed that $\text{Tp}^*\text{Mo}^{\text{VO}}(\text{OH})(\text{SPh})$ is a weaker acid than $\text{Tp}^{\text{iPr}}\text{Mo}^{\text{VO}}(\text{OH})(\text{SPh})$ by about 1.7 $\text{p}K_{\text{a}}$ units. Both complexes have $\text{p}K_{\text{a}}$ values that are several units lower than the $\text{p}K_{\text{a}}$ of water in acetonitrile (33–35).³² These results indicate that the $\text{Mo}^{\text{V}}(\text{OH})$ unit in these complexes is much more acidic than water.

A DFT study on $\text{Tp}^*\text{Mo}^{\text{V}}\text{O}(\text{OH})(\text{SPh})$ and $\text{Tp}^{i\text{Pr}}\text{Mo}^{\text{V}}\text{O}(\text{OH})(\text{SPh})$ gives $\text{p}K_{\text{a}}$ values of 19.7 and 18.0, respectively, in acetonitrile. This acidity order from the DFT calculations is consistent with the electrochemical-EPR experiments (Table 3). The DFT results are also consistent with the experimentally observed $E_{1/2}$ shifts for reduction of the $\text{LMo}^{\text{VI}}\text{O}_2(\text{SPh})$ compounds, where the minimal shift in the half-wave potential of $\text{Tp}^{i\text{Pr}}\text{Mo}^{\text{VI}}\text{O}_2(\text{SPh})$ indicates that the $[\text{Tp}^{i\text{Pr}}\text{Mo}^{\text{V}}\text{O}_2(\text{SPh})]^-$ anion is less basic than the Tp^* and Tz analogs. Thus, the more basic the electrochemically generated anion, the greater the positive potential shift with the addition of acid.³¹

The solution $\text{p}K_{\text{a}}$ value for water is larger than the calculated $\text{p}K_{\text{a}}$ values for the monoanionic species, indicating that the protonation of the monoanion by water is thermodynamically unfavorable in these model systems. However, the >1000 fold excess of water (Table S1) can drive the reaction of Eq. 2 to the right. The theoretical $\text{p}K_{\text{a}}$ values calculated by DFT are several $\text{p}K_{\text{a}}$ units smaller than the experimental values. This result may be a consequence of the calculations treating the primary solvation sphere of the proton as $\text{CH}_3\text{CN}\text{-H}^+$. Additionally, the structure of the added water in acetonitrile is complicated, and the exact number of water molecules interacting with the molybdenum complex is unknown.^{33, 34} Therefore, the theoretically predicted $\text{p}K_{\text{a}}$ might not represent the real situation in solution. An improved computational model is needed to better handle the multiple water and H-bonding interactions. This would also allow testing of the hypothesis that extended hydrogen bonding interactions facilitate the proton transfer in these complexes.

The metal reduction and the ligand protonation are illustrated in Scheme 2. The Gibbs free energy change, ΔG° for each step is also indicated. The indirect reduction, i.e. electrochemical reduction of the metal center followed by protonation is probed in this study. Knowing the ΔG° for those two steps, the potential for direct reduction, which involves coupled electron-proton transfer (CEPT), can be calculated. The potential for the CEPT process (E°_3) can be written as;

$$E^\circ_3 = E^\circ_1 - \frac{RT}{F} \ln K_{\text{a}} \quad \text{Eq. 5}$$

The measured midpoint potential for the electrochemical reduction of $\text{Mo}(\text{VI})$ to $\text{Mo}(\text{V})$ in $\text{Tp}^*\text{MoO}_2(\text{SPh})$ in anhydrous acetonitrile (Fig. 3) is -1060 mV vs. the Ag/AgNO_3 electrode, and the value of K_{a} can be extracted from Table 3. Consequently, the potential for the direct reduction (E°_3) is calculated to be about $+709$ mV. This result indicates that CEPT is thermodynamically favored over the indirect pathway of electron transfer followed by protonation.

Structure of $\text{Tp}^{i\text{Pr}}\text{MoO}_2(\text{SPh})$

The molecular structure of $\text{Tp}^{i\text{Pr}}\text{MoO}_2(\text{SPh})$ is shown in Figure 2. The crystal structures of the direct analogues, $\text{Tp}^{i\text{Pr}}\text{MoO}_2(\text{SR})$ ($\text{R} = i\text{Pr}, p\text{-tol}, \text{Bz}$), were recently reported¹⁹ and their averaged structural parameters (indicated as cf.) are included in the structural description below for $\text{Tp}^{i\text{Pr}}\text{MoO}_2(\text{SPh})$.

The six-coordinate $\text{Tp}^{i\text{Pr}}\text{MoO}_2(\text{SPh})$ molecule exhibits a distorted octahedral geometry, with a coordination sphere comprised of *fac*-tridentate $\text{Tp}^{i\text{Pr}}$ and mutually *cis* thiophenolate and terminal oxo ligands. The largest angular deviations from ideal octahedral geometry are the obtuse $\text{O}=\text{Mo}=\text{O}$ angle ($103.43(15)^\circ$, cf. 103.5° for related complexes), $\text{O}=\text{Mo}-\text{S}(1)$ angles ($97.8(1)^\circ$ and $102.8(1)^\circ$, cf. 99.7°), and the acute $\text{N}-\text{Mo}-\text{N}$ angles (av. 79.3° , cf. 79.4°) associated with the $\text{Tp}^{i\text{Pr}}$ ligand. These lead to *trans* $\text{O}/\text{S}-\text{Mo}-\text{N}$ angles that deviate considerably from 180° , exemplified by the $\text{S}(1)-\text{Mo}-\text{N}(31)$ angle of $155.3(1)^\circ$. Accordingly,

the Mo atom lies much closer to the O₂S face of the 'octahedron' (0.839(2) Å) than the N₃-face (1.541(2) Å). The geometrical distortions are consistent with those observed for related complexes^{17, 19} and for dioxo-Mo(VI) complexes in general.^{35, 36}

The Mo–S(1) distance of 2.408(2) Å is consistent with values reported for analogous compounds (cf. 2.400 Å) while the Mo–S(1)–C(39) angle is very slightly larger than in related complexes (106.4(2))°, cf. 105.71°).^{19, 36} The phenylthiolate (SC₆) group is planar subtending a dihedral angle of 58.0(1)° with the O(1),O(2),N(11),N(21) plane and 32.2(2)° with the pseudo-mirror plane S(1),Mo,N(31). The methine protons of the Tp^{iPr} isopropyl groups are directed toward the pseudo-threefold axis through Mo and B. The Mo–N(11) and Mo–N(21) bonds *trans* to the oxo ligands are more than 0.14 Å longer than the Mo–N(31) bond due to the strong *trans* influence of the oxo ligands.^{35, 37} The two Mo=O distances are equivalent, averaging 1.698 Å, as is typical of such species.^{17, 19, 36} Interestingly, examination of another crystal of "Tp^{iPr}MoO₂(SPh)" yielded a slightly different structure for the molecule with apparently different Mo=O distances that have been ascribed to co-crystallization of minor amounts of Tp^{iPr}MoOCl(SPh) (see Supplementary Material and Figure S1).

Conclusions

The physical properties and reactivities of Tp*MoO₂(SPh) and related compounds have been actively investigated for nearly 20 years. Here we show that the well-known quasi-reversible half-wave reduction potential in aprotic solvents undergoes a positive shift upon the addition of a proton donor, and the magnitude of the shift increases with the acidity of the added donor. This behavior is characteristic of hydrogen-bonding between the reduction products and the proton donor. The magnitude of the shift also depends upon the trispyrazolylborate ligand with Tp*~Tz > Tp^{iPr}. Solution electrochemical-EPR experiments show the presence of two distinct Mo(V) species, one of which possesses a single exchangeable proton. Double integration of the two well-separated EPR signals has provided a semi-quantitative experimental determination of the pK_a of the Mo^V(OH) unit in acetonitrile and indicates that its pK_a is several orders of magnitude smaller than that of water in acetonitrile. The pK_a of the Mo^V(OH) unit is also sensitive to the nature of the trispyrazolylborate ligand, with Tp*Mo^VO(OH)(SPh) having a somewhat larger pK_a than its Tp^{iPr} analogue. DFT calculations of the pK_a values support the experimental trends.

Coupled electron-proton transfer (CEPT) steps have long been proposed as important in the mechanisms of molybdenum enzymes such as SO. CEPT involves changes in the oxidation state of the Mo atom with concomitant changes in the degree of protonation of an oxygen ligand, i.e. Mo=O, Mo(OH), Mo(OH₂).³⁸ To date, there have been few quantitative or semi-quantitative studies of the pK_a of any of these groups, or comparison of their pK_a values with those of water or other proton donors that are present in the active sites of molybdenum enzymes. This study, which uses the well-characterized Tp*MoO₂(SPh) model system that mimics the catalytic chemistry of SO, provides the first semi-quantitative data for the pK_a of a Mo^V(OH) group and shows that it is much more acidic than water. Analysis of these results indicates that CEPT is thermodynamically favored over the indirect process of metal reduction followed by protonation. Direct translation of the present results for the Mo^V(OH) units of model compounds in acetonitrile to SO is not possible because the dielectric constant of the partially buried active site of SO is not known. In addition, the effective dielectric constant of the active site may well vary during the catalytic cycle or upon mutation of nearby amino acid residues.

Finally, extensive kinetic studies on Ru(III/II) and Os(III/II) systems have shown that interpreting CEPT reactions can be very complicated when outer sphere electron transfer is coupled to remote promote transfer events.⁸ Such complications may pertain to SO where

electron transfer between the Mo and heme centers must be coupled with proton reactions on the oxygen ligands of the Mo atom.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.

Commonly proposed catalytic cycle of sulfite oxidase.^{3, 5}

Scheme 2.

Thermodynamics of Mo reduction and protonation. The free energy change, ΔG° for each step is shown. E° = Reduction potential, K_a = dissociation constant, R = the universal gas constant, T = absolute temperature, and F = Faraday constant.

Figure 1.

The EPR spectra obtained from electrochemical reduction of $\text{Tp}^*\text{MoO}_2(\text{SPh})$ (0.5 mM) in acetonitrile containing 0.1 M of Bu_4NBF_4 as the electrolyte. An EPR-electrochemical flat cell was used to simultaneously reduce the species and collect the EPR spectrum. (A) no added water; (B) ~ 1180 mM H_2O . The broad signal at $g = 1.921$ is assigned to $\text{Tp}^*\text{Mo}^{\text{VO}}_2(\text{SR})^-$; the signal at $g = 1.951$ is assigned to $\text{Tp}^*\text{Mo}^{\text{VO}}\text{O}(\text{OH})(\text{SPh})$. Experimental conditions: microwave frequency, 9.762 GHz; microwave power, 4 mW; modulation amplitude, 5 G.

Figure 2. ORTEP-3 projection of $\text{Tp}^{i\text{Pr}}\text{MoO}_2(\text{SPh})$. The numbering schemes for the incompletely labelled pyrazolyl rings parallel that shown for the ring containing N(11). Thermal ellipsoids are drawn at the 30% probability level and hydrogen atoms have been omitted for clarity.

Figure 3. Cyclic voltammograms obtained for a solution of $\text{Tp}^*\text{MoO}_2(\text{SPh})$ (0.5 mM) in 0.1 M $\text{Bu}^n_4\text{NBF}_4$ in acetonitrile at different water concentrations, (A) 0 M H_2O , and (B) 7.2 M H_2O . The working electrode was glassy carbon and the scan rate was 0.1 Vs^{-1} . The reference electrode was Ag/AgNO_3 .

Figure 4.

$E_{1/2}$ values for $\text{Tp}^*\text{MoO}_2(\text{SPh})$ (0.5 mM) plotted as a function of $\text{Log}([\text{acid}])$, where $[\text{acid}]$ is the molar concentration of hydroxylic reagent. The solid lines are theoretical values calculated from the equation $E_{1/2} = E^0 - (RT/F)\ln(1 + K_1[\text{AH}] + K_1K_2[\text{AH}]^2)$, where K_1 and K_2 are constants defining hydrogen bonding interactions and $[\text{AH}]$ is the molar concentration of acid. The solvent is acetonitrile, and all solutions contain 0.1 M of $\text{Bu}^n_4\text{NBF}_4$ as the electrolyte. Reference electrode: Ag/AgNO_3 , Working electrode: glassy carbon, Counter electrode: platinum. Estimated error in $E_{1/2}$ values is ± 5 mV.

Table 1Crystallographic data for $\text{Tp}^{i\text{Pr}}\text{MoO}_2(\text{SPh})$.

Formula	$\text{C}_{24}\text{H}_{33}\text{BMoN}_6\text{O}_2\text{S}$
Fw	576.37
Cryst. system	Monoclinic
Space group	$P2_1/c$
a , Å	18.9201(4)
b , Å	8.06666(14)
c , Å	19.0404(4)
β , °	110.746(2)
V , Å ³	2717.6(3)
Z	4
D_{calc} , Mg/m ³	1.409
$F(000)$	1192
μ , mm ⁻¹	0.550
Unique data	5304
Obs. Data, $I \geq$	3084
$2\sigma(I)$	
R	0.0478
R_w	0.0758

Table 2Selected interatomic Distances and Angles for $\text{Tp}^{\text{iPr}}\text{MoO}_2(\text{SPh})$.^a

Bond Distances (Å)			
Mo–O(1)	1.704(3)	Mo–O(2)	1.693(3)
Mo–S(1)	2.4078(13)	Mo–N(11)	2.324(4)
Mo–N(21)	2.318(4)	Mo–N(31)	2.189(4)
Bond Angles (deg)			
O(1)–Mo–O(2)	103.43(15)	O(1)–Mo–S(1)	102.77(11)
O(1)–Mo–N(11)	165.88(14)	O(1)–Mo–N(21)	87.87(14)
O(1)–Mo–N(31)	93.28(13)	O(2)–Mo–S(1)	97.78(11)
O(2)–Mo–N(11)	88.16(15)	O(2)–Mo–N(21)	168.50(14)
O(2)–Mo–N(31)	93.63(15)	S(1)–Mo–N(11)	83.28(10)
S(1)–Mo–N(21)	81.52(10)	S(1)–Mo–N(31)	155.25(11)
N(11)–Mo–N(21)	80.36(13)	N(11)–Mo–N(31)	77.17(14)
N(21)–Mo–N(31)	80.37(14)	Mo–S(1)–C(39)	106.41(16)

^aThroughout the text and tables describing the structure Mo(1) has been abbreviated to Mo.

Table 3

The apparent pK_a of $Mo^V(OH)$ units in acetonitrile.*

Compound	Apparent pK_a of $Mo(OH)$ unit
$Tp^{iPr}MoO_2(SPh)$	28.2 ± 0.2
$Tp^*MoO_2(SPh)$	29.9 ± 0.3

Calculated from Eq. 4 using the data in Table S1.