Inorg. Chem. **2007**, *46*, 3804–3806

[Mo(Tm^{Me})(O)$_2$Cl]: An Alternative Functional Model of Sulfite Oxidase†

Dawn Wallace,* Lorraine T. Gibson, John Reglinski,* and Mark D. Spicer*

Department of Pure & Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow G1 1XL, U.K.

Received December 8, 2006

The hydrotris(methimazolyl)borate anion (Tm^{Me}) has been used to synthesize an alternative functional model ([Mo(Tm^{Me})(O)$_2$Cl]) of the metalloenzyme sulfite oxidase. It has been shown that the complex undergoes oxygen atom transfer chemistry and that it performs the primary function of the enzyme, sulfite oxidation. A method using ion chromatography has been developed to definitively prove that sulfite is oxidized to sulfate. Employment of a soft tripodal ligand has allowed us to tune the redox potentials of our complex so that they are significantly closer to those reported for sulfite oxidase.

The metalloenzyme sulfite oxidase is termed an oxotransferase, with its primary function being the catalytic oxidation of sulfite to sulfate. The Mo center is square-pyramidal with three S donors in the equatorial plane, one from a cysteinyl side chain and two from a pyranopterindithiolate entity (Figure 1).[1] There are also two cis Mo═O moieties, with the equatorial O atom being transferred during the catalytic cycle.

The proposed enzymatic cycle for the oxidation of sulfite to sulfate involves the interaction of the sulfite anion with the Mo^{VI}═O moiety. Subsequently, an O atom is transferred to form the sulfate anion, which is then displaced by hydroxide from the solvent, giving a Mo^{IV} product.[2] Finally, deprotonation and electron transfer regenerates a Mo^{VI} center.

Many complexes have been synthesized as potential mimics of the active site of sulfite oxidase.[1] A number of factors must be addressed. The complex must be able to undergo O atom transfer (OAT). Also, the ligand should preclude the irreversible μ-O dimerization that produces the Mo^{V} species, [Mo$^{V}_2$(L)(O)$_3$].[1] Finally, the ligand must provide an appropriate coordination sphere for the Mo.

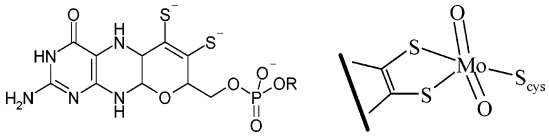

Figure 1. Pyranopterin dithiolate anion (left) and a representation of the active center of sulfite oxidase (right).

Tripodal N$_3$ donor ligands of the tris(pyrazolyl)borate (Tp) family have frequently been used in the synthesis of models for a number of different enzymes, including sulfite oxidase.[3,4] Although the use of an N$_3$ donor system would seem a poor substitute for the three S donors, the models synthesized are currently unmatched by any other system. Nevertheless, these systems are not able to catalytically function in the manner of the enzyme and have redox potentials (−0.4 to −1.67 V vs Ag/AgCl in various solvents)[4,5] that are still vastly different from those of sulfite oxidase (0.13−0.29 V vs Ag/AgCl);[6] hence, there is still room for improvement. We were interested in using the softer S analogue of the Tp ligands, hydrotris(methimazolyl)borate (Tm^{Me}),[7] to synthesize a functional model of sulfite oxidase. Utilization of this ligand allows us to simply replicate all three S donors of the active center.

The dark-red, air-stable [Mo(Tm^{Me})(O)$_2$Cl] (Figure 2) was synthesized from MoO$_2$Cl$_2$(OPPh$_3$)$_2$ and NaTm^{Me} in CHCl$_3$ by adaptation of the method used in the synthesis of [Mo(Tp)(O)$_2$Cl].[8,9] The complex has a distorted octahedral geometry, with the ligand adopting a κ^3 (S,S,S) motif, with two short and one long S−Mo bonds. The two Mo═O moieties adopt a cis conformation, which is consistent with

† Dedicated in memory of Swiatoslaw (Jerry) Trofimenko, the father and guiding light of Scorpionate Chemistry.

* To whom correspondence should be addressed. E-mail: dawn.wallace.100@strath.ac.uk (D.W.), j.reglinski@strath.ac.uk (J.R.), m.d.spicer@strath.ac.uk (M.D.S.). Tel: +44(0) 141 548 4748 (D.W.), +44(0) 141 548 2349 (J.R.), +44(0) 141 548 2800 (M.D.S.). Fax: +44(0) 552 0876.

(1) Enemark, J. H.; Cooney, J. J. A. *Chem. Rev.* **2004**, *104*, 1175 and references cited therein.
(2) (a) Pietsch, M. A.; Couty, M.; Hall, M. B. *J. Phys. Chem.* **1995**, *99*, 16315. (b) Pietsch, M. A.; Hall, M. B. *Inorg. Chem.* **1996**, *35*, 1273. (c) Thomson, L. M.; Hall, M. B. *J. Am. Chem. Soc.* **2001**, *123*, 3995. (d) Kail, B. W.; Perez, L. M.; Zaric, S. D.; Millar, A. J.; Young, C. G.; Hall, M. B.; Basu. P. *Chem.—Eur. J.* **2006**, *12*, 7501.
(3) Inscore, F. E.; Joshi, H. K.; McElhaney, A. E.; Enemark, J. H. *Inorg. Chim. Acta* **2002**, *331*, 246.
(4) Xiao, Z.; Bruck, M. A.; Doyle, C.; Enemark, J. H.; Grittini, C.; Gable, R. W.; Webb, A. G.; Young, C. G. *Inorg. Chem.* **1995**, *34*, 5950.
(5) Subramanian, P.; Spence, J. T.; Ortega, R.; Enemark, J. H. *Inorg. Chem.* **1984**, *23*, 2564.
(6) (a) Spence, J. T.; Kipke, C. A.; Enemark, J. H.; Sunde, R. H. *Inorg. Chem.* **1991**, *30*, 3011. (b) McMaster, J.; Enemark, J. H. *Curr. Opin. Chem. Biol.* **1998**, *2*, 201.
(7) Garner, M.; Reglinski, J.; Cassidy, I.; Spicer, M. D.; Kennedy, A. R. *Chem. Commun.* **1996**, 1975.
(8) Santos, A. M.; Kühn, F. E.; Bruus-Jensen, K.; Lucas, I.; Romão, Herdtweck, E. *J. Chem. Soc., Dalton Trans.* **2001**, 1332.

10.1021/ic700468m CCC: $37.00
Published on Web 04/10/2007

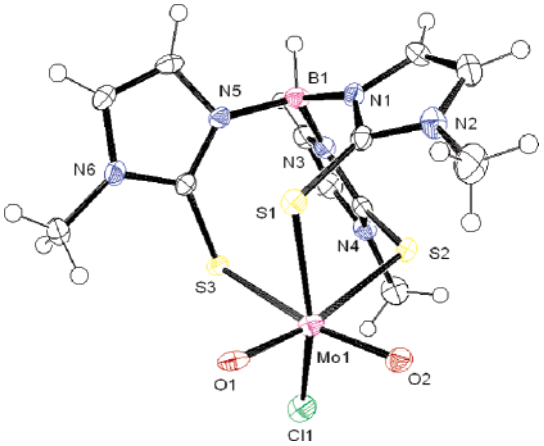

Figure 2. X-ray crystal structure of [Mo(Tm^{Me})(O)$_2$Cl]. Selected bond lengths (Å) and angles (deg): Mo−S1 2.4964(10), Mo−S2 2.6426(10), Mo−S3 2.6607(10), Mo−O1 1.813(3), Mo−O2 1.776(3), Mo−Cl1 2.3459(11); S1−Mo−S2 86.06(3), S1−Mo−S3 85.84(3), S2−Mo−S3 83.16(3), Cl1−Mo−O1 100.33(9), Cl1−Mo−O2 98.43(9), O1−Mo−O2 104.78(12).[9]

the active center of the enzyme complex. While one of the M═O groups in the enzyme is trans to the S donors, the other is apically opposite to the vacant position in the square-pyramidal coordination sphere. In comparison, our complex has both of the M═O groups trans to the S donors. The IR spectrum exhibits two strong ν(Mo═O) bands (948 and 902 cm^{-1}) that correspond to the symmetric and asymmetric vibrations, respectively. To date, this complex is the highest-oxidation-state Tm^{Me} complex reported.

The reaction of [Mo(Tm^{Me})(O)$_2$Cl] with aqueous sodium sulfite produces a color change from red/orange (λ_{max} = 460 nm) to blue/green (λ_{max} = 676 nm), and there is evidence that this is essentially a two-step process. The addition of water alone results in a small change in the color and electronic spectrum of the solution, suggesting hydrolysis to form a new Mo^{VI} species, possibly [Mo(Tm^{Me})(O)$_2$(OH$_2$)]$^+$, with the release of the chloride ion. Subsequent addition of sulfite results in conversion, to a blue reduced (probably Mo^{IV}) species. However, UV−visible studies suggest further subtleties that remain to be investigated. Sulfate ions are detected in the resulting solutions (vide infra), implying that the sulfite ions are oxidized at the metal center with OAT and are then displaced, most probably by water (eqs 1−3).

$$[Mo^{VI}(Tm^{Me})(O)_2Cl] + H_2O \rightarrow [Mo^{VI}(Tm^{Me})(O)_2(OH_2)]^+ + Cl^- \quad (1)$$

$$[Mo^{VI}(Tm^{Me})(O)_2(OH_2)]^+ + SO_3^{2-} \rightarrow [Mo^{IV}(Tm^{Me})(O)(OSO_3)(OH_2)]^- \quad (2)$$

$$[Mo^{IV}(Tm^{Me})(O)(OSO_3)(OH_2)]^- + H_2O \rightarrow [Mo^{IV}(Tm^{Me})(O)(OH_2)_2]^+ + SO_4^{2-} \quad (3)$$

The oxidation of sulfite by sulfite oxidase models incorporating dithiolene ligands has been previously reported by Sarkar et al.[10] This reaction has been characterized by the gravimetric analysis of $BaSO_4$. However, the production of

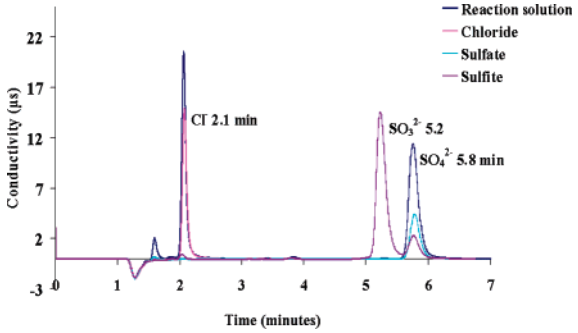

Figure 3. IC chromatogram of chloride (2.1 min), sulfite (5.2 min), sulfate (5.8 min), and the reaction solution.

two anionic species (eqs 1 and 3) allows the proposed mechanism of the reaction to be probed by ion chromatography (IC). The identification of chloride and sulfate ions, and the absence of sulfite ions, in the chromatogram of the reaction solution is consistent with the proposed reaction sequence (Figure 3). Oxidation of sulfite to sulfate in an aqueous solution is not uncommon. Thus, to ensure that the observed sulfate peak was generated by the action of the OAT reaction rather than by the oxidation of sulfite in air, the reaction was performed in dried, degassed solvents. In addition, controls carried out in the absence of the Mo complex do not show appreciable formation of sulfate.

In other studies, reoxidation of Mo^{IV} enzyme models to the parent Mo^{VI} species has been carried by using suitable O donors such as dimethyl sulfoxide (DMSO) or water. To this point, we have not been able to reoxidize our complex. The use of DMSO as an O donor is not successful because of the formation of degradation products. However, the use of RTm^{Me} in future models is expected to eliminate this degradation pathway.

The catalytic oxidation of sulfite to sulfate causes a reduction from Mo^{VI} to Mo^{IV}. The $E_{1/2}$ potential for the enzyme is reported as 0.29 V (Mo^{VI}/Mo^{V} couple) and 0.13 V (Mo^{V}/Mo^{IV} couple, vs Ag/AgCl).[6] The electrochemistry of previously reported Mo^{VI} complexes is wide-ranging and is dependent on the ligand employed. Enemark et al. have synthesized a series of Schiff base complexes incorporating N, O, and S donor atoms.[5] Few of these complexes exhibit reversible electrochemical behavior, with the lowest reduction potential being at −0.48 V (vs Ag/AgCl).

Many tripodal ligands have also been utilized in the endeavor to produce redox-active Mo^{VI} species. Of particular interest to us are the analogous family of Tp complexes. A variety of complexes of the general formula [Mo(L)(O)$_2$X][4] (where L = Tp^R and X = Cl, Br, NCS, OMe, OEt, OPh,

(9) Anal. Calcd for $C_{12}H_{16}N_6S_3BMoO_2Cl$: C, 28.00; H, 3.13; N, 16.33; S, 18.69. Found: C, 27.90; H, 3.11; N, 15.95; S, 18.76. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.02 (br m, 2H, CH), 6.87 (br m, 3H, CH), 6.67 (br m, 1H, CH), 3.86 (s, 3H, CH$_3$), 3.72 (s, 3H, CH$_3$), 3.68 (s, 3H, CH$_3$). IR (cm^{-1}, KBr): 2475 (BH), 950, 905 (Mo═O), 732 (C═S). Crystal data for [Mo(Tm^{Me})(O)$_2$Cl]: $C_{12}H_{16}N_6S_3BMoO_2Cl$, M = 1029.4, monoclinic, space group Cc, a = 14.7805(5) Å, b = 10.6162(4) Å, c = 12.1971(6) Å, β = 93.912(3)°, U = 1909.42(13) Å^3, Z = 4, D_c = 1.79 g cm^{-3}, λ(Mo Kα) = 0.710 73 Å, F(000) = 1032.

(10) (a) Das, S. K.; Chaudhury, P. K.; Biswas, D.; Sarkar, S. *J. Am. Chem. Soc.* **1994**, *116*, 9061. (b) Nagarajan, K.; Chaudhury, P. K.; Srinivasan, B. R.; Sarkar, S. *Chem. Commun.* **2001**, 1786.

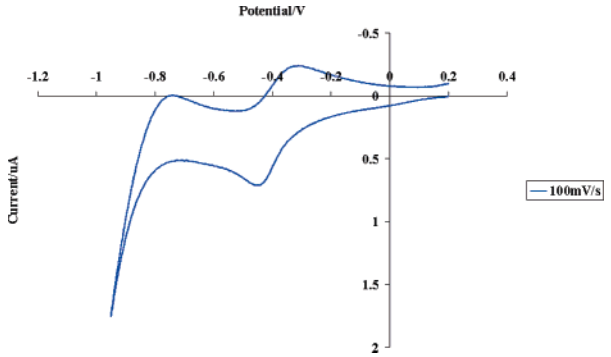

Figure 4. Voltammogram of $[Mo(Tm^{Me})(O)_2Cl]$ in DCM at 100 mV s^{-1}. Electrochemically quasi-reversible reduction wave at −0.39 V (Mo^{VI}/Mo^{V} couple) and an irreversible reduction wave at −0.71 V (Mo^{V}/Mo^{IV} couple vs Ag/AgCl).

$S^{i}Pr$, SPh, SCH_2Ph) have been investigated. Again, complexes show reversible and irreversible electrochemical processes depending on the ligand used. Nevertheless, many complexes reported have reversible behavior (−0.54 to −1.17 V vs Ag/AgCl in MeCN). Through the extensive studies of these compounds, it has been shown that the major requirements for reversible electrochemical reduction are (i) there must be a minimal conformational change upon reduction, restricting substitution trans to the O group, and (ii) a steric or electrostatic barrier must be present in order to eliminate dimerization of the reduced species. More recently, Young et al. have reacted $[Mo(Tp^{iPr})(O)_2Cl]$ with phenols or thiols in the presence of a base[11] to yield complexes of the form $[Mo(Tp^{iPr})(O)_2X]$ (X = OAr, OSR). All complexes give one electrochemically reversible reduction wave at potentials of less than −0.73 V (vs Ag/AgCl in MeCN). Interestingly, there have been no electrochemical data reported for the direct Tp analogue of our complex, $[Mo(Tp)(O)_2Cl]$.

The voltammogram for $[Mo(Tm^{Me})(O)_2Cl]$ (Figure 4) shows an electrochemically reversible reduction wave at −0.39 V (Mo^{VI}/Mo^{V} couple) and an irreversible reduction wave at −0.71 V (Mo^{V}/Mo^{IV} couple vs Ag/AgCl in dichloromethane, DCM). In comparison to the complexes previously used as sulfite oxidase models, the reduction potential of $[Mo(Tm^{Me})(O)_2Cl]$ is more positive and closer to the redox potential of the enzyme.

Dioxomolybdenum(VI) species containing S donor ligands have been used extensively in the study of OAT chemistry.[12] It has been shown that Mo^{VI} Schiff base complexes with O and N donor sets undergo OAT reactions, although these reactions often produce binuclear complexes.[13] The use of S donor atoms, however, facilitates OAT reactions, yielding mononuclear Mo^{IV} complexes.[14]

Tertiary phosphines are commonly used substrates in OAT reactions, with many studies being carried out on systems analogous to ours. Again, there is no published literature on the use of the direct analogoue $[Mo(Tp)(O)_2Cl]$; however, extensive work has been carried out using $[Mo(Tp^*)(O)_2X]$[13] and $[Mo(Tp^{iPr})(O)_2X]$.[15] The utilization of the S-containing, facially capping Tm^{Me} ligand should alter the electronics of the Mo complex, facilitating OAT. The ^{31}P NMR spectrum of the reaction of $[Mo(Tm^{Me})(O)_2Cl]$ with PPh_3 shows unreacted PPh_3 at δ −4.71 and the $OPPh_3$ product at δ 28.16. The NMR shows a modest 10% conversion of PPh_3 to $OPPh_3$.

By utilizing the tripodal ligand Tm^{Me} in our endeavor to model sulfite oxidase, we have successfully synthesized a complex that provides an appropriate coordination sphere for the active Mo center. In comparison to previous systems, our complex displays electrochemical behavior with redox potentials closer to those of the metalloenzyme. Direct reaction of $[Mo(Tm^{Me})(O)_2Cl]$ with sulfite produces sulfate, which is the key reaction of sulfite oxidase. The tentative steps into OAT reactions have demonstrated the viability of this complex as an OAT substrate, although the reaction is slow and will require further optimization.

Acknowledgment. D.W. thanks the EPSRC and University of Strathclyde Doctoral Training Account for financial support.

Note Added after ASAP Publication. This paper was released ASAP on April 10, 2007, with minor errors in eqs 2 and 3. The correct version was posted on April 19, 2007.

Supporting Information Available: Synthesis, characterization, and crystallographic data for $[Mo(Tm^{Me})(O)_2Cl]$ and UV−visible spectroscopic, electrochemistry, and OAT data. This material is available free of charge via the Internet at http://pubs.acs.org.

IC700468M

(11) Millar, A. J.; Doonan, C. J.; Laughlin, L. J.; Tiekink, E. R. T.; Young, C. G. *Inorg. Chim. Acta* **2002**, *337*, 393.
(12) Holm, R. H. *Chem. Rev.* **1987**, *87*, 1401.
(13) (a) Chen, G. J. J.; McDonald, J. W.; Newton, W. E. *Inorg. Chem.* **1976**, *15*, 2612. (b) Boyd, I. W.; Spence, J. T. *Inorg. Chem.* **1982**, *21*, 1602. (c) Craig, J. A.; Harlan, E. W.; Snyder, B. S.; Whitener, M. A.; Holm, R. H. *Inorg. Chem.* **1989**, *28*, 2082. (d) Rao, S. N.; Munshi, K. N.; Rao, N. N. N.; Bhadbhade, M. M.; Suresh, E. *Polyhedron* **1999**, *18*, 2491. (e) Dinda, R.; Sengupta, P.; Ghosh, S.; Sheldrick, W. S. *Eur. J. Inorg. Chem.* **2003**, 363.
(14) (a) Nakamura, A.; Nakayama, M.; Sugihashi, K.; Otsuka, S. *Inorg. Chem.* **1979**, *18*, 394. (b) Topich, J.; Lyon, J. T. *Inorg. Chem.* **1984**, *23*, 3203.
(15) Millar, A. J.; Doonan, C. J.; Smith, P. D.; Nemykin, V. N.; Basu, P.; Young, C. J. *Chem.—Eur. J.* **2005**, *11*, 3255.