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Detection, Isolation, and Characterization of Intermediates in Oxygen Atom Transfer Reactions in Molybdoenzyme Model Systems

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The pterin-containing molybdoenzymes catalyze the net exchange of an oxygen atom between water and substrate and there is evidence to support the involvement of oxygen atom transfer (OAT or oxo transfer) in the reactions of dimethyl sulfoxide reductases (DMSOR), sulfite oxidase, and nitrate reductase.[2−7] Accordingly, many model studies have focused on OAT reactions, most notably the reduction of dimethyl sulfoxide by oxo−Mo(IV) complexes and the oxidation of tertiary phosphines by dioxo−Mo(VI) complexes.[3] Indeed, Schultz et al.[4] have shown that the DMSOR from *Rhodobacter sphaeroides* couples these reactions during catalysis of OAT from Me_2SO to the water-soluble tertiary phosphine 1,3,5-triaza-7-phosphatricyclo[3.3.1.1^{3,7}]-decane. As well, the crystal structure of Me_2S-soaked crystals of oxidized DMSOR from *R. capsulatus* has revealed the presence of an Mo-bound Me_2SO molecule formed upon incomplete OAT from Mo to Me_2S.[5] Putative oxo(phosphine oxide) intermediates formed during the oxidation of phosphines by enzyme or model systems have never been detected or isolated.

The reactions of $LMo^{VI}O_2X$ (L = hydrotris(3,5-dimethylpyrazol-1-yl)borate) with PPh_3 are second-order and produce $OPPh_3$ and "$LMo^{IV}OX$", which may be trapped, e.g., as $LMo^{IV}OX$(solvent) or $LMo^{IV}OX$ (as monodentate X becomes bidentate).[8−10] An associative mechanism has been proposed for the overall OAT reaction.[9] However, it is not clear whether the intermediate, $LMoOX(OPPh_3)$, gives the product by an associative or dissociative mechanism. The reaction of $LMoO_2(SPh)$ with phosphines led Hall and co-workers[6] to examine the reaction of $MoO_2(NH_3)_2(SH)_2$ with PMe_3 by computational methods. In the first step of the reaction, nucleophilic attack of PMe_3 on a π^* Mo=O orbital perpendicular to the MoO_2 unit and at an Mo−O···P angle of ca. 130° takes place. This results in a transition state with a weakened Mo−O bond (1.83 Å), an O−P interaction (2.43 Å), and a P−O−Mo=O torsion angle of 89.7°; the remaining Mo=O bond becomes stronger consistent with a "spectator oxo" function.[11] The $OPMe_3$ ligand then rotates about the Mo−O bond, breaking the Mo−O π interaction to generate an intermediate with Mo=O = 1.67 Å, Mo−O = 2.18 Å, O−P = 1.53 Å, and P−O−Mo=$O_{torsion}$ = 0.5°. The intermediate was 68.9 kcal·mol^{-1} lower in energy than the reactants. At this stage replacement of $OPMe_3$ by water is predicted to take place by an *associative* mechanism.

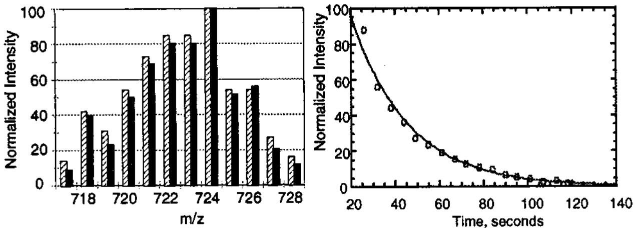

Figure 1. Left panel: The FABMS parent ion of $LMoOCl(OPPh_3)$, formed in the reaction of $LMoO_2Cl$ and PPh_3 in *m*NBA. The experimental and calculated spectra are plotted using hatched and solid bars, respectively. Right panel: A plot of the normalized intensity of the *m*/*z* 724 peak of $LMoOCl(OPPh_3)$ vs time.

Here, we report the detection of oxo(phosphine oxide) intermediates in the OAT reactions of $LMoO_2X$ and PPh_3 by fast atom bombardment mass spectrometry (FABMS) and the use of this technique to assess the stability of the intermediates and examine the kinetics of decay for unstable species.[12] Also, we report the isolation and characterization of $L^{Pr}Mo^{IV}O(OPh)(OPEt_3)$ (L^{Pr} = hydrotris(3-isopropylpyrazol-1-yl)borate), the first stable oxo(phosphine oxide) complex to be synthesized by incomplete OAT in a molybdoenzyme model system.

Intermediates in the reactions of $LMoO_2X$ (X = Cl^-, Br^-, OPh^-, and SPh^-)[8,13] with PPh_3 were detected by FABMS.[14] In each case, a peak cluster indicative of the initial formation of $LMoOX(OPPh_3)$ was observed. Figure 1 shows the parent ion of the intermediate formed in the reaction of $LMoO_2Cl$ and PPh_3; the base peak at *m*/*z* 724 and isotope pattern match those expected for $[LMoOCl(OPPh_3)]^+$ ($[M]^+$). Related species were detected for X = Br^- ($[M]^+$) and X = SPh^- and OPh^- ($[M + H]^+$) complexes. In blanks containing no added PPh_3 only a strong parent ion due to $[LMoO_2X]^+$ was detected. The $[M]^+$ peak intensity of the intermediate $LMoOCl(OPPh_3)$ monitored as a function of time showed an exponential decay with a first-order constant of 0.038 s^{-1} (Figure 1). Under similar experimental conditions, $[LMoO(SPh)(OPPh_3) + H]^+$ decayed with a rate constant of 0.0097 s^{-1} while no decay of $[LMoO(OPh)(OPPh_3) + H]^+$ was observed. These observations are consistent with the

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(14) In a typical experiment, a dichloromethane solution of the complex (1−2 μM) was mixed with an excess (>10 equiv) of PPh_3 directly on an FAB probe containing ca. 1 μL of *m*-nitrobenzoic acid (*m*NBA). The final volume of the reaction mixtures was less that 3 μL. Once mixed, the probe was quickly placed inside a Micromass Autospec-EQ spectrometer (OPUS operating system) and bombarded with a CsI gun; the average time for this operation was ca. 30−45 s. The probe was maintained at a constant temperature of ca. 65 °C. Positive ion FABMS were collected.

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rates of OAT reactions in solution, viz. $Cl^- > SPh^- > OPh^-$.[8] Mass spectrometric evidence that hard co-ligands (e.g., OPh^-) moderate OAT and stabilize oxo(phosphine oxide) intermediates was corroborated by the isolation and characterization of $L^{Pr}MoO(OPh)(OPEt_3)$, described below.

Reaction of orange $L^{Pr}MoO_2(OPh)$[13] with a variety of phosphines in benzene (or acetonitrile) results in golden/green solutions which upon evaporation and trituration with hexanes yield green powders characterized as $L^{Pr}MoO(OPh)(OPR_3)$. The triethylphosphine oxide derivative, $L^{Pr}MoO(OPh)(OPEt_3)$, has been extensively characterized and microanalytical, spectroscopic, and mass spectrometric data are in accord with the formulation given.[15] The complex decomposes in the presence of chlorinated solvents to yield $L^{Pr}Mo^VO(OPh)Cl$ and reacts with propylene sulfide (a sulfur atom transfer reagent) to produce $L^{Pr}MoOS(OPh)$, the cornerstone of a new model for the molybdenum hydroxylases.[16] The phosphine oxide ligand is displaced by coordinating solvents such as acetonitrile.

Six-coordinate, mononuclear $L^{Pr}MoO(OPh)(OPEt_3)$ exhibits a distorted octahedral coordination geometry (Figure 2).[17] The central molybdenum atom is coordinated by terminal oxo, phenoxide, and triethylphosphine oxide ligands, which are mutually *cis*, and a *facial* tridentate L^{Pr} ligand. The Mo−O(1), Mo−O(2), and Mo−O(3) distances of 1.684(3), 2.031(3), and 2.157(3) Å are typical of the ligands involved.[18] The O(1)−Mo−O(2), O(1)−Mo−O(3), and O(1)−Mo−N(11) angles of 108.0(2)°, 97.3(1)°, and 164.8(1)°, respectively, constitute the most significant deviations from octahedral geometry. Consideration of the Mo−N(*n*1) distances indicates that the ligand *trans* influences are in the following order: oxo > OPh^- > $OPEt_3$. Metrical parameters for the $OPEt_3$ ligand include the following: O(3)−P = 1.516(3) Å, Mo−O(3)−P = 130.9(2)°, and $P{-}O(3){-}Mo{=}O(1)_{torsion} = 57.5(3)°$.

The structure of $L^{Pr}MoO(OPh)(OPEt_3)$ does not appear to be sterically imposed as there is a soft energy surface associated with changes in the P−O(3)−Mo═O(1) torsion angle, as assessed by preliminary molecular mechanics calculations; torsion angles close to 0° can be achieved but would be expected to enhance dissociation of the phosphine oxide. The role of packing forces in the orientation of the $OPEt_3$ ligand is difficult to assess, but it is more likely that electronic factors dictate the preferred orientation of the phosphine oxide unit and the complex's stability. This possibility is currently under examination by computational methods. A number of mononuclear oxo(phosphine oxide)−Mo(VI) and −Mo(V) complexes have been crystallographically characterized but none was synthesized by reacting phosphine and a dioxo−Mo(VI) species.[19]

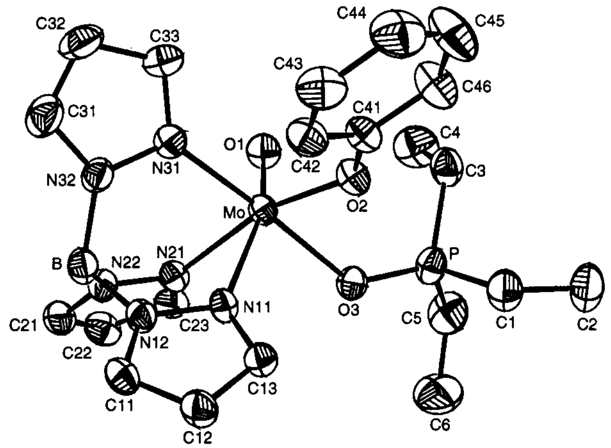

Figure 2. Partial molecular structure of $L^{Pr}MoO(OPh)(OPEt_3)$ (the 3-isopropyl groups and hydrogen atoms have been omitted for clarity). Additional bond distances (Å) and angles (deg) include the following: Mo−N(11) 2.430(4), Mo−N(21) 2.212(4), Mo−N(31) 2.155(4), O(1)−Mo−N(21) 90.0(2), O(1)−Mo−N(31) 91.3(2), O(2)−Mo−O(3) 79.1(1), Mo−O(2)−C(41) 136.5(3).

In summary, we have employed mass spectrometry to detect oxo(phosphine oxide) intermediates in oxygen atom transfer reactions involving $LMoO_2X$ and PPh_3, assess their stability, and monitor the kinetics of decay for unstable species. Intermediates of this type are stabilized by hard O-donor co-ligands which moderate OAT from the $[Mo^{VI}O_2]^{2+}$ center to phosphine. Significantly, the oxo(phosphine oxide) complex, $L^{Pr}MoO(OPh)(OPEt_3)$, has been isolated and characterized by conventional methods, including X-ray crystallography. Experimental evaluation of theoretical and mechanistic proposals[6] and the continued development of trispyrazolylborate molybdoenzyme model systems[2,3,10] are expected outcomes of ongoing work.

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Supporting Information Available: An X-ray crystallographic file in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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[15] **$L^{Pr}MoO(OPh)(OPEt_3)$.** (All under N_2) A solution of $L^{Pr}MoO_2(OPh)$ (562 mg, 1 mmol) in benzene or acetonitrile (30 mL) was treated with triethylphosphine (177 mg, 0.22 mL, 1.5 mmol) and stirred for 15 h at room temperature. The solvent was removed in vacuo yielding a viscous residue that was triturated with hexanes (20 mL) to yield a green powder, which was collected, washed with hexanes (2 × 5 mL), and dried in vacuo. The compound was recrystallized from toluene/hexanes as green chunky crystals. Yield 545 mg, 80%. Anal. Calcd for $C_{30}H_{48}BMoN_6O_3P$: C, 53.13; H, 7.13; N, 12.39. Found: C, 53.38; H, 7.01; N, 12.49. Mass spectra: ESIMS (CH_3CN), *m/z* 680 [M^+], 587 $[M - OPh]^+$, 546 [M − $OPEt_3$]. FABMS (*m*NBA), *m/z* 680 [M^+], 546 [M − $OPEt_3$]. IR (KBr): ν(BH) 2477, 2450 m, ν(OPh) 1590, 1585 m, ν(C═N) 1509 s, $\nu(OPEt_3)$ 1275, 1268 s, ν(Mo═O) 950 s cm^{-1}. ^1H NMR (C_6D_6): δ 0.59, 1.00, 1.08, 1.19, 1.24, 1.68 (each d, 3H, $^3J_{HH} = 6.8$ Hz, CH_3 of Pr); 0.93 (dt, 9H, $^3J_{PH} = 17$ Hz, $^3J_{HH} = 7.5$ Hz, 3 $CH_2C\mathbf{H}_3$); 1.68 and 1.96 (each ddq, 3H, $^2J_{PH} = 15$ Hz, $^2J_{HH} = 15$ Hz, $^3J_{HH} = 7.5$ Hz, 3 $C\mathbf{H}_2CH_3$); 2.65, 4.15, 4.42 (each sept, 1H, $^3J_{HH} = 6.8$ Hz, CH of Pr); 5.86, 5.95, 6.00 (each d, 1H, $^3J_{HH} = 2.4$ Hz, 4-CH); 6.30 (d, 2H, $J = 7.8$ Hz, 2,6-H of OPh); 6.68 (t, 1H, $^3J_{HH} = 7.8$ Hz, 4-H of OPh); 7.07 (t, 2H, $^3J_{HH} = 7.8$ Hz, 3,5-H of OPh); 7.34, 7.42, 7.72 (each d, 1H, $^3J_{HH} = 2.4$ Hz, 5-CH). $^{13}C\{^1H\}$ NMR (C_6D_6): δ 6.14 (d, $J_{CP} = 4.5$ Hz, $CH_2\mathbf{C}H_3$), 18.72 (d, $J_{CP} = 65.5$ Hz, $\mathbf{C}H_2CH_3$), 21.52, 22.72, 24.18, 24.58, 24.80, 26.08 (6 CH_3 of Pr); 26.45, 27.95, 29.16 (3 CH of Pr); 100.69, 101.79, 102.20 (3 4-CH); 116.81 (4-C of OPh); 119.03 (2,6-C of OPh); 128.92 (3,5-C of OPh); 133.95, 136.93, 138.86 (3 5-C); 162.93, 163.24. 164.34 (3 3-C); 173.74 (1-C of OPh).

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[17] **Crystal data:** Green crystals of $L^{Pr}MoO(OPh)(OPEt_3)$ were obtained by slow diffusion of hexane into a toluene solution of the complex. Crystal data for $C_{30}H_{48}BMoN_6O_3P$: $M = 678.47$, orthorhombic, space group $P2_12_12_1$ (no. 19), $a = 14.910(3)$ Å, $b = 24.128(2)$ Å, $c = 10.014(2)$ Å, $U = 3603(1)$ Å^3, $T = 294$ K, $Z = 4$, μ(Mo Kα) = 4.44 cm^{-1}, 5837 reflections measured, 4300 with $I > 3.0\sigma(I)$. Refinement on F^2, using anisotropic thermal parameters for non-hydrogen atoms, converged with $R = 0.039$ and $R_w = 0.047$.

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