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Chemistry of $[Et_4N][Mo^{IV}(SPh)(PPh_3)(mnt)_2]$ as an Analogue of Dissimilatory Nitrate Reductase with Its Inactivation on Substitution of Thiolate by Chloride

Amit Majumdar, Kuntal Pal, and Sabyasachi Sarkar*

Department of Chemistry, IIT Kanpur, Kanpur 208016, India

Received December 20, 2005; E-mail: abya@iitk.ac.in

The DMSO reductase family of molybdenum oxotransferase enzymes conserves two pterin dithiolene (S_2pd) moieties and one protein-derived ligand which normally varies with Ser·O^-, Cys·S^-, or Cys·Se^- residue.[1] In dissimilatory Dd (*Desulfovibrio desulfuricans*) nitrate reductase, the reduced active site has been proposed to contain a desoxo site $[Mo^{IV}(S\cdot Cys)(S_2pd)_2]$, and this active site has been proposed to mediate the reaction 1 through its participation

$$NO_3^- + 2\,H^+ + 2\,e^- \rightleftharpoons NO_2^- + H_2O \qquad (1)$$

in the oxotransfer reaction with its oxidation. The oxidized structure as $[Mo^{VI}(OH_x)(S\cdot Cys)(S_2pd)_2]$ ($x = 1$ or 2) has been established by X-ray crystallography.[2] It has been proposed that nitrate is first bound to the molybdenum of the reduced cofactor with the formation of an enzyme substrate complex followed by the essential oxotransfer and the elimination of nitrite ion with the formation of oxidized oxomolybdenum cofactor, $[Mo^{VI}(O)(S\cdot Cys)(S_2pd)_2]$. This oxo form is believed to be very protophilic, resulting in the formation of protonated oxidized species[2] $[Mo^{VI}(OH_x)(S\cdot Cys)(S_2pd)_2]$ ($x = 1$ or 2). To understand the role of the apo-protein-derived ligand, molybdoenzymes of the DMSO reductase family are studied by site directed mutagenesis.[3] It is known that the replacement of protein ligand serinate by cysteinate or by selenocysteinate changes the properties of the enzyme along with their activity. This protein ligand attached to molybdenum in an active site mainly controls the specificity of the active site toward a substrate. Nitrate reduction by Mo(IV) complex devoid of dithiolene coordination is well-known.[4] Pentacoordinated molybdenum(IV) bis(dithiolene) thiolato complex, having a resemblance to the active site of dissimilatory Dd nitrate reductase, has been reported,[5] but with nitrate, it underwent decomposition[6,7] to $[Mo^V(S_2C_2Me_2)_3]^{1-}$. The hexacoordinated $[Mo^{IV}(CO)(SPh)(S_2C_2Me_2)_2]^{1-}$ on irradiation to remove CO resulted in decomposition[5] to $[Mo^V(S_2C_2Me_2)_3]^{1-}$. Unlike CO, we thought to use PPh_3 as coligand because its bulkiness would help its dissociation, releasing the model cofactor, providing a site for substrate binding with the possible oxotransfer reaction. With the success of 1,2-dicyanoethylenedithiolate (mnt^{2-}) ligand in establishing the functional analogue reaction of sulfite oxidase,[8] we use this ligand for the synthesis of the present model complex in relevance to dissimilatory Dd nitrate reductase. For the stability and also for the availability of the ideal reactive species in solution, we have synthesized[9,10] $[Et_4N][Mo^{IV}(SPh)(PPh_3)(mnt)_2]\cdot CH_2Cl_2$ (**1**). The present communication deals with the chemistry of **1** as a functional analogue of the active site for dissimilatory nitrate reductase of *Desulfovibrio desulfuricans*. It has also been shown that the substitution of thiolate by chloride yielded[9] structurally[10] similar complex $[Et_4N][Mo^{IV}(Cl)(PPh_3)(mnt)_2]\cdot CH_2Cl_2$ (**2**), which under identical conditions and even on standing for hours did not respond to any reductive activity toward nitrate, mimicking inactivation similar to single point mutation.

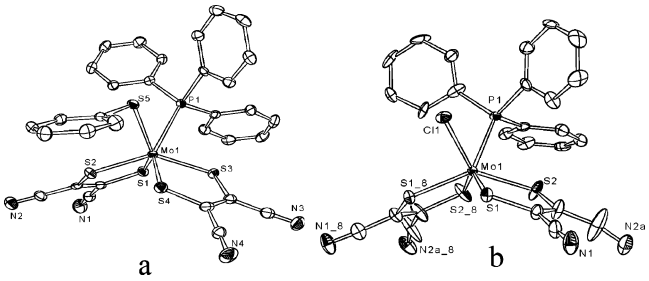


Figure 1. Structure (ORTEP view) of anions of **1** (a) and **2** (b) showing 50% probability thermal ellipsoids. Hydrogen atoms are omitted for clarity. Selected bond distances and angles: For **1**, Mo−$S_{dithiolene}$(average) = 2.362(13) Å, Mo−$S_{thiophenolate}$ = 2.404(13) Å, Mo−P = 2.573(12) Å, Mo−S−P = 76.91(4)°; for **2**, Mo−$S_{dithiolene}$(average) = 2.336(14) Å, Mo−Cl = 2.464(19) Å, Mo−P = 2.540(2) Å, Mo−S−P = 76.36(6)°.

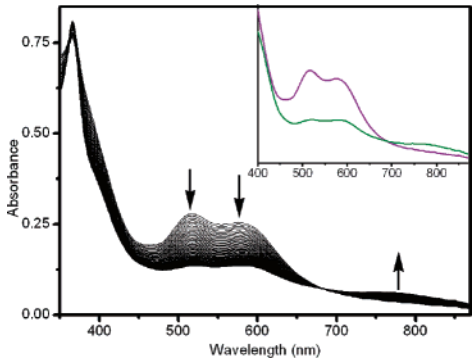


Figure 2. Spectral changes in the reaction of 1×10^{-4} M of **1** with 5×10^{-4} M of $[Bu_4N][NO_3]$ in dichloromethane at 25 °C. Total time = 4 min. Scan rate = 2 s/scan. Inset: spectra of **1** (purple) and its oxidized form (green).

Addition of thiophenol or $[Et_4N][Cl]$ to a solution of $[Et_4N]_2[Mo^{IV}O(mnt)_2]$ and PPh_3 in dichloromethane acidified with methanesulfonic acid at 0 °C led to the isolation of **1** or **2**, respectively, in 90% yield. Complexes **1** and **2** have long Mo−P bonds (Figure 1), and indeed in solution, both dissociate to yield the corresponding pentacoordinated species.[11] Complex **1** reacted readily with $[Bu_4N][NO_3]$ in dichloromethane medium to produce nitrite (confirmed by Griess's reagent).[12] This reaction when monitored by UV−vis spectroscopy showed a clean reaction in the initial phase, with a tight isosbestic point at 680 nm (Figure 2) with the formation of a new absorption at 770 nm assigned to the oxidized species.[13] Addition of PPh_3 into a solution of the oxidized complex immediately bleached the 770 nm band.

A catalytic cycle involving $[Bu_4N][NO_3]$ and PPh_3 as the oxidizing and reducing substrate, respectively, is envisaged as shown in Scheme 1. The initiation of the reaction starts on the dissociation of PPh_3 from **1**. On the basis of the amount of nitrite produced, PPh_3 recovered, and $OPPh_3$ produced,[15,16] the turnover

10.1021/ja0586135 CCC: $33.50

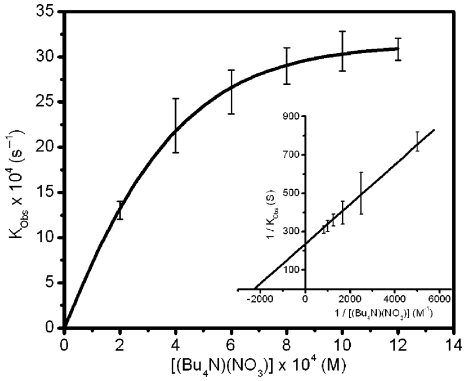
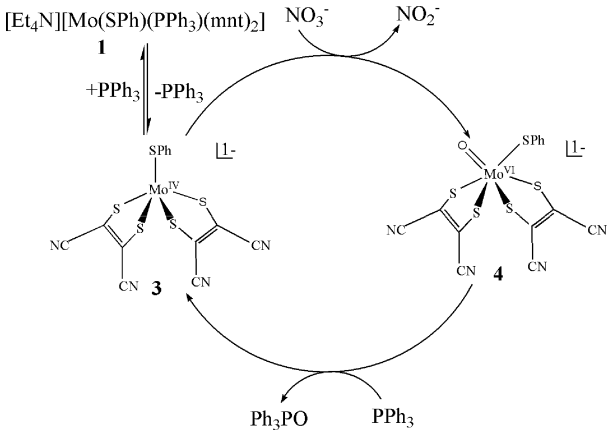


Figure 3. Dependence of the rate of reaction of **1** with 2−12 equiv of $(Bu_4N)(NO_3)$ in dichloromethane at 25 °C on $[(Bu_4N)(NO_3)]$. Inset: the corresponding double reciprocal plot.

Scheme 1. Catalytic Cycle for Nitrate Reduction Using $\{Et_4N][Mo^{IV}(SPh)(mnt)_2\}$ (**3**) as the Active Catalyst and PPh_3 as the Reductant of Its Putative Oxidized Form $\{Et_4N\}\{Mo^{VI}O(SPh)(mnt)_2\}$ (**4**)



number for such a reaction was found to be 50 $mmol^{-1}$ s^{-1}. The kinetic measurements for nitrate reduction were performed using $[Bu_4N][NO_3]$, which showed Michaelis−Menten kinetics.[8] The saturation kinetics and the corresponding Lineweaver−Burk plots are shown in Figure 3. The apparent K_M and the V_{Max} values were found to be 4.3×10^{-4} M and 4.2×10^{-3} s^{-1}, respectively.

It is known that this class of enzymes retains the common $\{Mo^{IV}(X)(S_2pd)_2\}$ moiety with the variation in apo-protein ligation (X) to tune the reactivity toward a specific substrate. For DMSO reductase from *Rhodobacter sphaeroides*, oxygen atom from serinate ligand serves as "X", and this changes to sulfur atom from cysteinate in the case of nitrate reductase in *Desulfovibrio desulfuricans*. Furthermore, this X is selenium ligated selenocysteine residue in the formate dehydrogenase from *Escherichia coli*. We were interested to probe such sincerity in the model reaction with the change of donor ligand. The corresponding phenolate and selenothiolate substituted complexes would have been the best systems to testify such specificity, and these are yet to be fully characterized. Nevertheless, the corresponding chloro complex **2** failed to react with $[Bu_4N][NO_3]$, recognizing the indispensable role of thiolate ligation in **1** in its response to show enzymatic oxotransfer similar to nitrate reductase.

In summary, we demonstrate the reaction of dissimilatory nitrate reductase by model chemistry along with the specificity of the thiolate ligand over chloride for the desired activity. Work is in progress to understand the specificity of the ligand substitution in relevance to other molybdoenzymes.

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Supporting Information Available: Synthetic details, analytical, electrochemical, spectral, and crystallographic data (CIF format) for complexes **1** and **2** and complete ref 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Synthetic details of **1** and **2** are in Supporting Information.
(10) Crystal data for **1**: $C_{41}H_{42}Cl_2MoN_5PS_5$, formula weight 962.96, triclinic, space group $P\bar{1}$, $a = 10.882(5)$ Å, $b = 11.881(5)$ Å, $c = 17.999(5)$ Å, $\alpha = 98.561(5)°$, $\beta = 103.122(5)°$, $\gamma = 97.945(5)°$, $V = 2205.0(15)$ Å^3, $Z = 2$, $T = 100$ K, $D_{calc} = 1.425$ g·cm^{-3}. Of a total of 14 514 reflections collected, 10 381 were independent ($R_{int} = 0.0235$). The structure was solved by direct methods and refined by full-matrix least squares on F^2. Final $R1$ [$I > 2\sigma(I)$] = 0.0576 and $wR2 = 0.1358$ (all data), GOF = 1.068. Crystal data for **2**: $C_{35}H_{37}Cl_3MoN_5PS_4$, formula weight 889.24, orthorhombic, space group *Pnma*, $a = 13.367(5)$ Å, $b = 15.164(5)$ Å, $c = 19.145(5)$ Å, $V = 3881(2)$ Å^3, $Z = 4$, $T = 100$ K, $D_{calc} = 1.522$ g·cm^{-3}. Of a total of 25 187 reflections collected, 4979 were independent ($R_{int} = 0.0820$). The structure was solved by direct methods and refined by full-matrix least squares on F^2. Final $R1$ [$I > 2\sigma(I)$] = $R1 = 0.0721$ and $wR2 = 0.1841$ (all data), GOF = 1.033. Data were collected on a Bruker-AXS smart APEX CCD diffractometer.
(11) The ^{31}P NMR of **1** in dichloromethane showed a multiplet (49−51.5 ppm) due to molybdenum-bound PPh_3, and its intensity diminished concomitant to the appearance of a new singlet (−8.16 ppm) corresponding to the dissociated PPh_3 signal. External addition of PPh_3 restored the intensity of the multiplet. **2** behaved similarly.
(12) Griess, P. Ber. *Dtsch. Chem. Ges*. **1879**, *12*, 427; as cited in Feigl, F. Spot Tests in *Inorganic Analysis*, Elsevier: Amsterdam 1958; p 330. Control system without **1** did not produce nitrite.
(13) The oxidized species after 5 min started to change to another species. The concentration of the nitrite produced steadily increased during the first 5 min and then decreased due to the formation of known[14] $[Et_4N]_2$-$[Mo(NO)_2(mnt)_2]$. Sulfamic acid being insoluble in dichloromethane could not be used to scavange the nitrite ion. Maximum absorbance observed at 770 nm in the reaction between **1** and $[Bu_4N][NO_3]$ within 5 min was considered as the absorbance of the oxidized species at that wavelength. The oxidized product **4** from **1** using Me_3NO could not be isolated in pure form due to its instability under basic byproduct, Me_3N.
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(15) Catalytic system involved 0.01 mmol of **1**, 1 mmol of PPh_3, 1.5 mmol of $[Bu_4N][NO_3]$ in 30 mL of dichloromethane at room temperature. PPh_3 recovered (183 mg) and $OPPh_3$ produced (83 mg) within 1 min of reaction were confirmed by melting point (80 and 152 °C, respectively) and by ^{31}P NMR spectroscopy. A solution of **1** and $[Bu_4N][NO_3]$ in a stoichiometric ratio in dichloromethane showed the presence of only one ^{31}P NMR at 24.97 ppm corresponding to Ph_3PO. Nitrite produced was 80% as measured by standard colorimetric method.
(16) Mono-oxoMo(VI) complexes catalytically oxidize PPh_3 involving desoxo Mo(IV) species. See: (a) Nemykin, V. N.; Davie, S. R.; Mondal, S.; Rubie, N.; Kirk, M. L.; Somogyi, A.; Basu, P. *J. Am. Chem. Soc*. **2002**, *124*, 756−757. (b) Arjoumanian, H.; Corao, C.; Krentzien, H.; Lopez, R.; Teruel, H. *J. Chem. Soc., Chem. Commun*. **1992**, 856−858.

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