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Structural and Functional Analogue of the Active Site of Polysulfide Reductase from *Wolinella succinogenes*

Kowliki Nagarajan,[†] Hemant K. Joshi,[‡] Pradeep K. Chaudhury,[†] Kuntal Pal,[†] J. Jon A. Cooney,[‡] John H. Enemark,*[,‡] and Sabyasachi Sarkar*[,†]

Department of Chemistry, Indian Institute of Technology, Kanpur-208 016, India, and Department of Chemistry, University of Arizona, Tucson, Arizona 85721

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Synthesis of $[PPh_4]_2[Mo(SPh)_2(S_2C_2(CN)_2)_2]$ (**2**) from $[PPh_4]_2[MoO(S_2C_2(CN)_2)_2]$ (**1**) has been achieved to mimic the postulated $\{Mo(S)_6\}$ core of polysulfide reductase with two thiolates and two bis(ene-dithiolate) ligands. Compound **2** reacts with polysulfide to yield H_2S, modeling the function of polysulfide reductase. The facile conversion of **2** back to **1** in moist solvent suggests that the interconversion of the $\{Mo^{IV}{=}O\}$ and $\{Mo^{IV}{-}X\}$ (X = O–Ser, S–Cys, Se–Cys) moieties might occur in the DMSO reductase class of enzymes under appropriate hydrophobic/hydrophilic conditions.

The oxido-reductase class of molybdoenzymes exists in three subclasses known as the sulfite oxidase, xanthine oxidase, and DMSO reductase families.[1] The well-known "spectator oxo group"[2] remains attached to molybdenum in the reduced forms of enzymes of the sulfite oxidase and xanthine oxidase families. However, the reduced forms of enzymes of the DMSO reductase family are now formulated as desoxo-Mo(IV) molybdenum moieties.[3] An additional feature of this diverse family is the wide range of ligands contributed by protein residues, including Mo−OSer, Mo−Scys, and Mo−SeCys.[4−6] Polysulfide reductase (Psr),[7] a member of the DMSO reductase family, catalyses the reduction of polysulfide with evolution of H_2S (eq 1). On the basis of its EPR data and comparison with those of other molybdoenzymes,[4−6] the Mo(V) form of PsrA is proposed to have a $\{Mo(S)_6\}$ core upon reaction with polysulfide.[8] Aside from ligation from a cysteine residue, it has been postulated that the substrate, polysulfide, remains directly ligated to the Mo center during the catalytic cycle (Figure 1).[8]

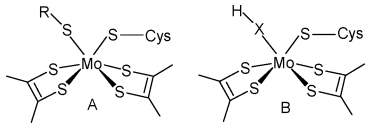

Figure 1. Proposed structures for the observed Mo(V) states in PsrA: (A) polysulfide-bound Mo(V), (B) X = O/S-bound Mo(V).

$$S(S)_nS^{2-} + 2H^+ + 2e^- \rightarrow S(S)_{n-1}S^{2-} + H_2S \qquad (1)$$

In this communication, we report the synthesis of $[PPh_4]_2[Mo^{IV}(SPh)_2(S_2C_2(CN)_2)_2]$[9] (**2**) from $[PPh_4]_2[MoO(S_2C_2(CN)_2)_2]$[10] (**1**) and demonstrate that the former is a structural and functional analogue of PsrA. The structure[11] of the anion of complex **2** is shown in Figure 2 and mimics the postulated $\{Mo(S)_6\}$ core of PsrA with two thiolates and two bis(ene-

* To whom correspondence should be addressed. E-mail abya@iitk.ac.in, fax 91-512-2597265/2597436 (S.S.); e-mail jenemark@u.arizona.edu, fax (520) 626-8065 (J.H.E.).

[†] Indian Institute of Technology.

[‡] University of Arizona.

(1) Hille, R. *Chem. Rev.* **1996**, *96*, 2757−2816.
(2) Rappé, A. K.; Goddard, W. A., III *J. Am. Chem. Soc.* **1982**, *104*, 3287−3294.
(3) Enemark, J. H.; Cosper, M. M. *Met. Ions Biol. Syst*. **2002**, *39*, 621−654.
(4) (a) Schindelin, H.; Kisker, C.; Hilton, J.; Rajagopalan, K. V.; Rees, D. C. *Science* **1996**, *272*, 1615−1621. (b) Schneider, F.; Löwe, J.; Huber, R.; Schindelin, H.; Kisker, D.; Knäblein, J. *J. Mol. Biol.* **1996**, *263*, 53−69. (c) McAlpine, A. S.; McEwan, G.; Shaw, A. L.; Bailey, S. *J. Biol. Inorg. Chem.* **1997**, *2*, 690−701.
(5) Dias, J. M.; Than, M. E.; Humm, A.; Huber, R.; Bourenkov, G. P.; Bartunik, H. D.; Bursakov, S.; Calvete, J.; Caldeira, J.; Carneiro, C.; Moura, J. J. G.; Moura, I.; Ramão, M. J. *Structure* **1999**, *7*, 65−79.
(6) J. C. Boyington, J. C.; Gladyshev, V. N.; Shangulov, S. V.; Stadtman, T. C.; Sun, P. D. *Science* **1997**, *275*, 1305−1308.
(7) (a) Klimmek, O.; Kröger, A.; Steudel, R.; Holdt, G. *Arch. Microbiol.* **1991**, *155*, 177−182. (b) Fauque, G.; Klimmek, O.; Kröger, A. *Methods Enzymol*. **1994**, *243*, 367−383. (c)Hedderich, R.; Klimmek, O.; Kröger, A.; Dirmeier, R.; Keller, M.; Stetter, K. O. *FEMS Microbiol. Rev.* **1999**, *22*, 353−381.
(8) Prisner, T.; Lyubenova, S.; Atabay, Y.; MacMillan, F.; Kröger, A.; Klimmek, O. *J. Biol. Inorg. Chem.* **2003**, *8*, 419−426.
(9) Synthesis of **2**: Treatment of 0.5 mmol of $[PPh_4]_2[MoO(mnt)_2]$[10] (**1**) (0.536 g) with an excess of benzenethiol (0.5 mL) in chloroform at 0 °C with 0.5 mmol (0.1 g) of phosphorous pentachloride (PCl_5) transformed the green color to pink. Addition of 0.5 mL of triethylamine followed by petroleum ether yielded oily pink-colored complex **2**, which was recrystallized from acetonitrile and 2-propanol. Yield: 0.31 g (50%). **2** was characterized by elemental analysis; IR, Raman, and UV−vis spectroscopies; and X-ray crystallography.[11] Spectroscopic data for **2**: IR (KBr pellet, cm^{-1}): υ_{CN}, 2195 (vs); $\upsilon_{C=C}$, 1435 (vs). UV−vis (dichloromethane solution, 0.5×10^{-4}M, nm): 535, 368. Elemental analysis. Calcd for $C_{68}H_{50}MoN_4P_2S_6$: C, 64.15; H, 3.93; N, 4.40. Found: C, 63.84; H, 4.26; N, 4.47. ESMS mass (*m*/*z*): 935 $\{(PPh_4)[Mo(SPh)_2(mnt)_2]\}^-$ (monocationic mononegative ion).
(10) Das, S. K.; Chaudhury, P. K.; Biswas, D.; Sarkar, S. *J. Am. Chem. Soc.* **1994**, *116*, 9061−9070. (b) Maiti, R.; Nagarajan, K.; Sarkar, S. *J. Mol. Str.* **2003**, *656*, 169−176.

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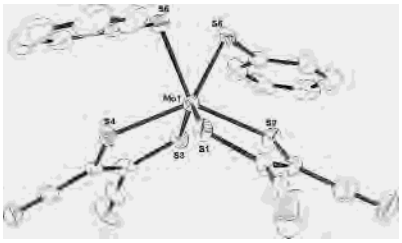


Figure 2. ORTEP view of the anion of **2**. Selected distances (Å) and angles (°): $Mo-S_{dithiolene}$ = 2.3689(12) (mean); Mo−S5 = 2.4505(12); Mo−S6 = 2.4265(12); S6−Mo−S5 = 72.56(4); S2−Mo−S1 = 81.42(4); S3−Mo−S4 = 81.51(4).

dithiolate) ligands. The distance between S_5 and S_6 (2.886 Å) suggests a weak interaction between the two sulfur atoms. This might be the cause for the lability of Mo−SPh bond, which facilitates substrate binding.

In view of the structural similarity of **2** with the proposed cofactor of PsrA (polysulfide bound), its reactivity toward polysulfide[12] was tested. Treatment of **2** with polysulfide in dichloromethane caused the evolution of H_2S.[13] This observation clearly demonstrates the ability of **2** to mimic the reactivity of the enzyme, PsrA, and supports a structure−function relationship with the enzyme active site. However, identification of the complete reaction sequence is complicated by further reactions taking place between the released thiolate and the excess polysulfide. Attempts to isolate and characterize the oxidized product of **2** are in progress.

A proposed mechanism in which **2** converts polysulfide into H_2S in shown in Scheme 1.

A similar mechanism is plausible for the reductive half-reaction of the native enzyme. The cyclic voltammogram of **2** showed a quasireversible oxidative peak (E_{pa}) at 0.17 V vs Ag/AgCl in dichloromethane. The EPR spectrum of the species[14] generated by chemical oxidation of **2** with iodine

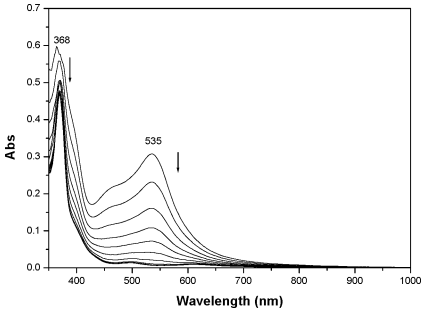
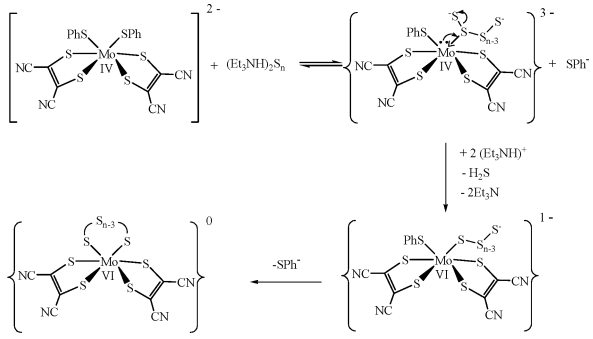


Figure 3. Conversion of $[Mo^{IV}(SPh)_2(mnt)_2]^{2-}$ (**2**) to $[Mo^{IV}O(mnt)_2]^{2-}$ (**1**) in moist dichloromethane. Scans taken at 5-min intervals over 40 min.

Scheme 1



showed the g values $g_{\perp} = 2.01$ and $g_{||} = 1.90$ as compared to the reported $g_{xx} = 1.9874$, $g_{yy} = 2.0025$, $g_{zz} = 2.0165$ of the Mo(V) species PsrA.[8]

Finally, desoxo **2**, which is synthesized from the monoxo (**1**) form,[9] reverts back to **1** in moist dichloromethane (Figure 3), highlighting the interchangeability of the oxo and desoxo Mo(IV) forms, depending on the local environment. This also illustrates the lability of the Mo−SPh bond of **2**. The facile interconversion of **1** and **2** suggests that the stability of desoxo Mo(IV) centers in various members of the DMSO reductase family might be subtly dependent on the hydrophobic conditions near the active site.

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Supporting Information Available: EPR spectrum, cyclic voltammogram, and X-ray crystallographic data in CIF format for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Diffraction-quality single crystals were crystallized by making a layer of 2-propanol over an acetonitrile solution of complex **2** at 0 °C. Crystallographic data for **2**: $C_{68}H_{50}MoN_4P_2S_6 \cdot CH_3CN$, M = 1312.40, monoclinic, space group = $P2(1)/n$, a = 13.3808(11) Å, b = 32.182(3) Å, c = 15.9971(13) Å, α = 90°, β = 111.072(2)°, γ = 90°, V = 6428.1(9) Å^3, Z = 4, ρ_{calcd} = 1.356 Mg/m^3, $F(000)$ = 2696, λ = 0.71073 Å, T = 173(2) K, crystal size = 0.60 × 0.16 × 0.16 mm^3, θ range for utilized data = 1.27−25.58°, reflections utilized = 63421, independent reflections = 12026 [R(int) = 0.1961]; final R indices [$I > 2\sigma(I)$], R1= 0.0454, wR2 = 0.0687; R indices (all data), R1 = 0.1338, wR2 = 0.0859; largest diffraction peak and hole = 0.734 and −0.304 e Å^{-3}, RMS difference density = 0.071 e Å^{-3}.

(12) Treatment of H_2S with elemental sulfur in dichloromethane under basic conditions (with triethylamine) generates polysulfide.

(13) The release of H_2S was confirmed by lead acetate paper, and the experiments were checked with blank tests.

(14) The EPR spectrum as obtained immediately after freezing the reaction mixture changes with time, suggesting the instability of the oxidized product. The native protein displayed varying $\langle g \rangle$ values depending on the use of varied substrates (see ref 8).