A Comparison between the Structures of Tungstopterin Cofactor in Tungsten Oxidoreductase and a Model Complex, *cis*-Dioxobis(2,3-naphthalenedithiolate)tungsten(VI) in Their O-Atom Transfer Reaction

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(Received September 20, 1995)

In our study on an active site model of tungsten oxidoreductase, structural parameters of oxo and thiolate ligands in $(NEt_4)_2[W^{VI}O_2(S_2C_{10}H_6)_2]\cdot H_2O$ (**1**) ($S_2C_{10}H_6$ = 2,3-naphthalenedithiolato) were obtained where *two* dithiolene like coordination exists. A comparison of the structure with the enzyme active site indicated the importance of "least stereochemical movement" of thiolate ligands.

Very recently, a crystallographic report by Rees et al. has revealed the bis(dithiolene) coordination to tungsten ion in aldehyde ferredoxin oxidoreductase.[1] Surprisingly, the reported structure of tungsten cofactor closely resembles our model complexes, $(PPh_4)_2[W^{VI}O_2(S_2C_6H_4)_2]$ ($S_2C_6H_4$ = 1,2-benzenedithiolato) (**2**)[2] and **1** (Figure 1).[3] Both structures have *cis*-dioxobis(dithiolene) coordination on a hexa-coordinated tungsten atom. Because of the uncertainty of the presence of oxo ligands on the tungsten ion of the enzyme, they could not decide the fine geometry. The followings are possible, i.e., trigonal prism (TP), octahedron (Oh) or an intermediate among Oh and TP. The existence of two oxo ligands in the enzyme is not conclusive crystallographically but supported by the EXAFS analysis.[4] Only the coordination structure of thiolates at the W ion can be compared with the model complexes, **1** and **2**. Unfortunately, the other structural parameters of tungsten cofactor, e.g. W-O bond lengths, are not reliable enough. Therefore, our model complex will greatly help to identify the structure, the electronic properties and the reaction mechanism at the tungsten active site.

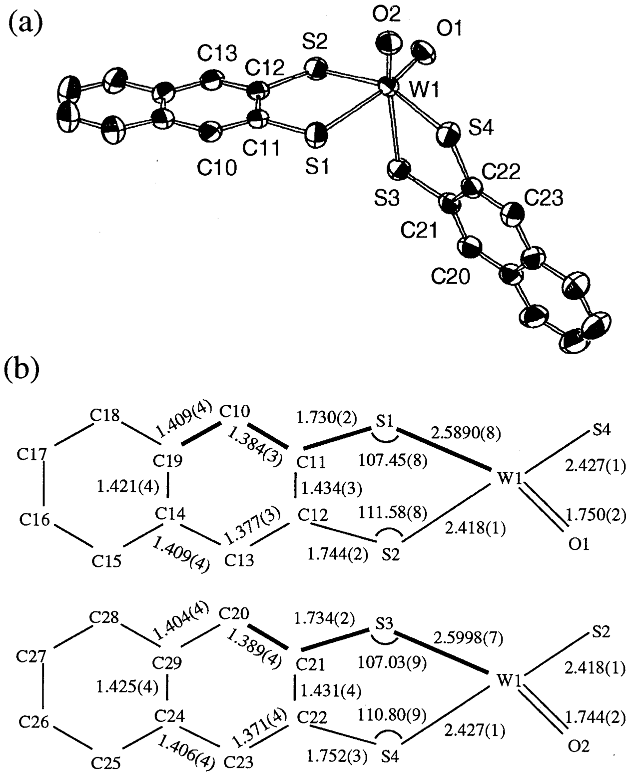

Figure 1. (a) The ORTEP drawing of anion part of $(NEt_4)_2[W^{VI}O_2(S_2C_{10}H_6)_2].H_2O$ (**1**). The 50% probability ellipsoids are shown. (b) Selected bond angles (deg.) and bond lengths (Å) in the structure of **1**.

The most characteristic feature of the model complex, **1**, is the mutual *trans* influence between oxo and thiolate which are both strong π-donors (Figure 2). Thus, remarkably elongated sulfur-tungsten bonds (*trans* to oxo, 2.590(3) and 2.604(3) Å) were observed in the crystal structure, compared with the other (*cis* to oxo) W-S bonds (2.416(3) and 2.433(3) Å). The W-S bond elongation by the mutual *trans* influence between oxo and thiolate ligands is expected to be observed in the tungsten enzyme active site, although the reports of crystallography and EXAFS spectroscopy did not point out such elongation. Even if the tungsten(VI) ion in the active site has TP coordination, the W-S bond elongation is expected from the theoretical analysis.[5] The *trans* influence was also detected in the Raman spectra, where $\nu(W^{VI}{=}O)$ bands were found to be shifted to lower frequencies (883 (ν_s) and 832 (ν_{as}) cm^{-1}) than the ordinary complexes (960 ~ 900 cm^{-1}).[6]

The model studies on the oxidative reactions of the dioxomolybdenum(VI) complexes have revealed that the thiolate ligands are uniquely suitable for the activation of the oxo ligand.[7] Therefore, in the tungsten enzyme, the active site oxo ligands are expected to be activated by the *trans* influence of the

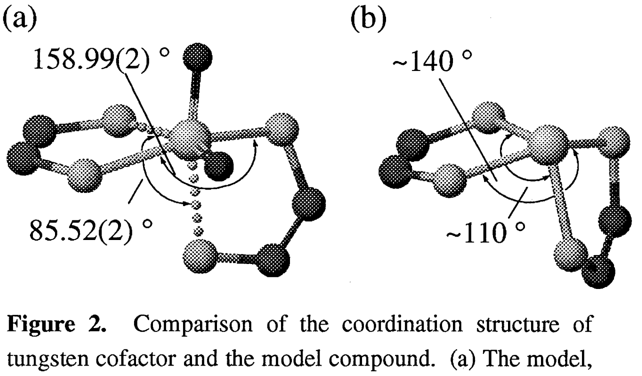

Figure 2. Comparison of the coordination structure of tungsten cofactor and the model compound. (a) The model, $(NEt_4)_2[W^{VI}O_2(S_2C_{10}H_4)_2].H_2O$ (**1**) (octahedron). (b) The tungsten cofactor determined from the crystal structure of aldehyde ferredoxin oxidoreductase from *Pyrococcus furiosus* (an intermediate between trigonal prism and octahedron). The existence and the geometry of oxo ligands are not conclusive crystallographically.

thiolates of the pterin-dithiolene ligand toward the specific oxidation reaction, even if the active site has a trigonal deformation from an Oh geometry.

Chan et al.[8] have recently provided helpful information about the difference between the model complexes (**1** and **2**) and the enzyme active site which was not described in the paper.[1] They have pointed out an important difference in the geometries of the models, **1** and **2**, which make them distinct. While the four sulfurs in the tungsten cofactor have a nearly planar arrangement (an intermediate geometry between Oh and TP), the four sulfurs in **1** and **2** are non-planar with one of sulfurs lying normal to the plane generated by the remaining three (Oh) (Figure 2).

Indeed, in the case of the tungsten cofactor as shown in Figure 2, S-W-S bond angles between dithiolene ligands are ~140° and ~110° for the cofactor.[8] These angles suggest the TP deformation of the geometry of W ion from Oh. On the other hand, our models (**1** and **2**) show the Oh geometry where the corresponding angles are ~160 ° and ~85 °.

The stability of Oh structure is supported by the theoretical calculations compared with other two kinds of geometry, TP and bicapped tetrahedral (BCT) due to the repulsion between thiolates.[5] In general, the preference of a TP geometry has been explained from the S---S bonding interaction between the thiolate ligands of tris(1,2-dithiolato) complexes. If there are repulsive interactions, the coordination will be changed to Oh or BCT from TP.[9] Therefore, the enzyme active site should have some device to reduce the repulsion between thiolate ligands.

Chan et al.[8] also described that "These geometric differences will likely impact the energies of the molecular orbitals of the tungsten center, thus affecting spectroscopic properties, various *trans* effects, and the activation barrier for the oxo transfer process. Thus, while model compounds do exist that approach the structural and catalytic features of the native system, the synthesis of an accurate model for the native site remains to be achieved. With the recent structure determination of the tungsten cofactor, the design and isolation of the more reliable model complexes should be more feasible." We think that the structural difference between the model, **1**, and the tungsten cofactor provides an idea of "least stereochemical movement" in the oxo transfer reaction to realize an efficient reactivity of the enzyme as shown in Figure 3.

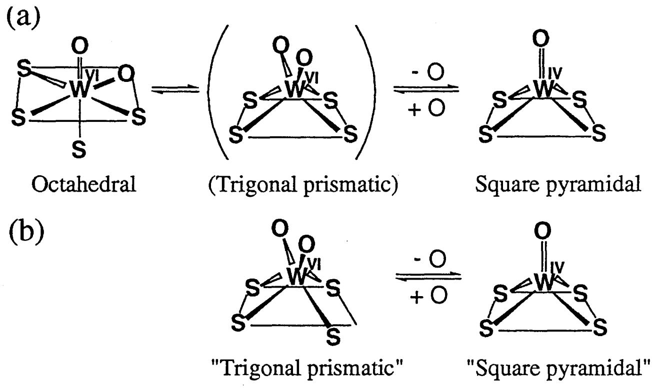

Figure 3. The stereochemical changes at the tungsten-thiolate coordination in the oxo transfer reaction. (a) Small model compounds, $[W^{VI}O_2(S_2C_6H_4)_2]^{2-}$ (**2**) and $[W^{IV}O(S_2C_6H_4)_2]^{2-}$ (**3**). (b) The enzymatic active site where the "least stereochemical movement" of tungstopterin cofactor is proposed. The oxidized and the reduced states have W(VI) and W(IV) ions, respectively.

By the kinetic study[10] of the oxo-accepting reaction of $(NEt_4)_2[Mo^{IV}O(3\text{-triphenylsilyl-3,4-toluenedithiolato})_2]$ with Me_3NO, we have estimated the existence of the TP structure of dioxobis(dithiolato)molybdenum(VI) as a reaction intermediate (Figure 3a) during the formation of a *cis*-$(Mo^{VI}O_2)^{2+}$ structure from an oxo donor adduct of $(Mo^{IV}O)^{2+}$ (*trans*-dioxo coordination). The tungsten ion in the enzyme active site is thus proposed to be situated in an intermediate TP state. There will be little or no movement of the ligating sulfur atoms in the oxo-transfer reaction process (Figure 3b), if the reduced state has square pyramidal geometry like a W(IV) model complex, $(NEt_4)_2[W^{IV}O(S_2C_6H_4)_2]$ (**3**).[2] "Least stereochemical movement" of the ligating sulfur atoms facilitates oxo-transfer without requiring considerable reorganization of the tungsten cofactor. A specific device of the protein active site probably realizes such an unusual geometry. Similar small geometrical change in the electron transfer redox reaction was discussed for an electron transfer enzyme, plastocyanin.[11]

We are grateful for financial supports by JSPS Fellowships (Hiroyuki Oku.; No. 2947, 1995-1997) for Japanese Junior Scientists, and by a Grant-in-Aid for Specially Promoted Research from the Ministry of Education, Science and Culture (Akira Nakamura; No. 06101004).

References and Notes

1 M. K. Chan, S. Mukund, A. Kletzin, M. W. W. Adams, and D. C. Rees, *Science,* **267**, 1463 (1995).
2 N. Ueyama, H. Oku, and A. Nakamura, *J. Am. Chem. Soc.*, **114**, 7310 (1992).
3 The synthesis of **1** was done by the similar procedure to the synthesis of **2**.[8] Anal. Found: C, 49.58; H, 6.26; N, 3.31%. Calcd for $C_{36}H_{54}O_3N_2WS_4$: C, 49.42; H, 6.22; N, 3.20%. Crystal and refinement data for **1**: $P2_1/n$ (monoclinic), a = 7.396(2), b = 17.581(2), c = 28.839(2), β = 95.35(2), V= 3734(3) Å^3, Z = 2, d_{calc} = 1.556 gcm^{-3}; unique reflections = 11311, used reflections = 8863 ($I_o > 3\sigma(I)$), R = 0.023, R_w = 0.033.
4 G. N. George, R. C. Prince, S. Mukund, and M. W. W. Adams, *J. Am. Chem. Soc.*, **114**, 3521 (1992).
5 H. Oku, N. Ueyama, and A. Nakamura, manuscript in preparation.
6 a) H. Oku, thesis, Osaka University (1995). b) H. Oku, N. Ueyama, and A. Nakamura, *Inorg. Chem.*, in press.
7 a) J. Topich and J. T. Lyon III, *Inorg. Chem.*, **23**, 3202 (1984). b) R. H. Holm, *Chem. Rev.*, **87**, 1401 (1987).
8 M. K. Chan, M. W. W. Adams, and D. C. Rees, private communication.
9 K. Tatsumi, I. Matsubara, Y. Inoue, A. Nakamura, and K. Miki, N. Kasai, *J. Am. Chem. Soc.*, **111**, 7766 (1989).
10 H. Oku, N. Ueyama, M. Kondo, and A. Nakamura, *Inorg. Chem.*, **33**, 209 (1994).
11 J. E. Huheey, in *Inorganic Chemistry,* 3rd ed; Harper & Row, Cambridge (1983), p. 867.